



# Investigating synergism within multimodular glycoside hydrolases during wheat straw cell wall deconstruction

Cédric Montanier, Louise Badruna, Thierry Vernet, Anne-Marie Di Guilmi,  
Vincent Burlat, Michael O'Donohue

## ► To cite this version:

Cédric Montanier, Louise Badruna, Thierry Vernet, Anne-Marie Di Guilmi, Vincent Burlat, et al.. Investigating synergism within multimodular glycoside hydrolases during wheat straw cell wall deconstruction. The CBM11 - 11. Carbohydrate Bioengineering Meeting, May 2015, Espoo, Finland. 223 p., 2015, 11. Carbohydrate Bioengineering Meeting. hal-01269244

**HAL Id: hal-01269244**

**<https://hal.science/hal-01269244>**

Submitted on 3 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

11<sup>th</sup>

Carbohydrate

Bioengineering Meeting



10-13 May 2015, Espoo, Finland



ABSTRACTS

## Welcome to CBM11

The eleventh Carbohydrate Bioengineering Meeting (CBM11) focuses on various aspects of carbohydrate-acting enzymes and biomolecules, their mode of action, structure and structure function relationships, engineering and applications. The applications spread from health and nutrition to material sciences. The subjects are highly topical in the present world, where sustainable use of renewables is a key interest, not just for scientists, but for the whole society, governments and industries. Thanks to the contributions of the participants we have been able to design a programme which covers the recent developments in the focus areas of the meeting. The present programme in Espoo includes 55 talks and flash presentations and 133 poster presentations.

The roots of CBM11 go back to Helsingør, Denmark and 1995, when the first CBM meeting was organized twenty years ago. The major aim of the meeting is again to bring together colleagues and scientists active in various fields of carbohydrates, biomolecules and carbohydrate-active enzymes. Our target has been to generate a pleasant environment for knowledge sharing, generating collaborations, and hopefully also for constituting an incubator for many future project ideas.

The meeting takes place in Otaniemi, in a beautiful natural cape of the Baltic Sea in Espoo, the home of Aalto University engineering schools and VTT. The programme runs in Dipoli, a famous conference building designed in early 1960's by Raili and Reima Pietilä showing the interplay of light, Finnish pine wood, copper, and natural rocks. If you are interested in architecture, you may want to take a walk in the campus area, which is designed by the most famous Finnish architect Alvar Aalto and to stop by in the main building of Aalto University and the main library, both from 1960's.

On the behalf of the local Organizing Committee we would like to express our gratitude to all our sponsor companies and to all persons who have contributed to organizing this meeting.

We wish you a fruitful meeting of scientific excellence and enjoyable stay in spring-like Espoo and Finland.

Kristiina Hilden  
Anu Koivula  
Kristiina Kruus  
Markus Linder  
Maija Tenkanen

## **Carbohydrate Bioengineering Meeting History**

CBM1	1995	Elsinore, Denmark
CBM2	1997	La Rochelle, France
CBM3	1999	Newcastle, United Kingdom
CBM4	2001	Stockholm, Sweden
CBM5	2003	Groningen, The Netherlands
CBM6	2005	Barcelona, Spain
CBM7	2007	Braunschweig, Germany
CBM8	2009	Ischia, Naples, Italy
CBM9	2011	Lisbon, Portugal
CBM10	2013	Prague, Czech Republic

## **International Programme Committee**

Birte Svensson (chair)	Technical University of Denmark, Denmark
Vincent Bulone	Royal Institute of Technology, Stockholm, Sweden
Pedro Coutinho	CNRS, Aix-Marseille Université, France
Gideon J. Davies	University of York, United Kingdom
Lubbert Dijkhuizen	University of Groningham, the Netherlands
Ten Feizi	Imperial College, London, United Kingdom
Carlos Fontes	Technical University of Lisbon, Portugal
Vladimir Kren	Academy of Sciences of the Czech Republic, Czech Republic
Takashi Kuriki	Ezaki Glico Co. Ltd., Osaka, Japan
Marco Moracci	CNR, Naples, Italy
Carsten Andersen	Novozymes A/S, Copenhagen, Denmark
Antoni Planas	Universitat Ramon Lull, Barcelona, Spain
Magali Remaud-Simeon	INSA, Toulouse, France
Steve G. Withers	University of British Columbia, Vancouver, Canada

## **Local Organizing Committee**

Maija Tenkanen (chair)	University of Helsinki, Finland
Kristiina Hilden	University of Helsinki, Finland
Anu Koivula	VTT, Finland
Kristiina Kruus	VTT, Finland
Markus Linder	Aalto University, Finland



# 11<sup>th</sup> Carbohydrate Bioengineering Meeting

10-13 May, 2015

Espoo, Finland



## Programme

### Sunday 10.5.2015

**16.00      *Opening of CBM11***

*Chair: Birte Svensson*

**16.05      Welcome speech**

President Tuula Teeri  
Aalto University, Finland

**16.20      Opening lecture**

**T1: From the first CBHI to biorefineries**

Merja Penttilä  
VTT, Finland

***Glycomics, systems glycobiology and bioinformatics***

*Chair: Markus Linder*

**17.00      T2: CAZyChip: a bioChip for bacterial glycoside hydrolases detection and dynamic exploration of microbial diversity for plant cell wall hydrolysis**

Claire Dumon  
Université de Toulouse, France

**17.20      T3: A new generation of chromogenic substrates for high-throughput screening of glycosyl hydrolases, LPMOs and proteases**

Julia Schückel  
University of Copenhagen, Denmark

**17.40      T4: Mining fungal diversity for novel carbohydrate acting enzymes**

Ronald P. de Vries  
Utrecht University, The Netherlands

**18.00      End of the day**

**19 – 21      Get-together, Design Factory**

## Monday 11.5.2015

### ***Mechanisms of carbohydrate-acting enzymes I***

*Chair: Takashi Kuriki*

- 9.00 Key-note lecture**  
**T5: The increasing diversity of lytic polysaccharide monooxygenases**  
Gideon Davies  
University of York, UK
- 9.40 T6: Neutron and high-resolution X-ray structural studies of glycoside hydrolase family 45 endoglucanase from the basidiomycete *Phanerochaete chrysosporium***  
Kiyohiko Igarashi  
University of Tokyo, Japan
- 10.00 T7: New insight into substrate specificity and activity determinants of a starch debranching enzyme gained from substrate: enzyme crystal structures**  
Marie S. Møller  
Carlsberg Laboratory, Denmark
- 10.20 T8: Crystal structures of N-acetylhexosamine 1-kinase and UDP-glucose 4-epimerase in the GNB/LNB pathway from infant-gut associated bifidobacteria**  
Shinya Fushinobu  
University of Tokyo, Japan
- 10.40 Coffee break and poster viewing**

### ***Mechanisms of carbohydrate-acting enzymes II***

*Chair: Anu Koivula*

- 11.20 T9: Crystal structure of the GTFB enzyme, the first representative of the 4,6- $\alpha$ -glucanotransferase subfamily within GH70**  
Tjaard Pijning  
University of Groningen, The Netherlands
- 11.40 T10: Catalytic mechanism of retaining glycosyltransferases: Is Arg293 on the  $\beta$ -face of EXTL2 compatible with it? Insights from QM/MM calculations**  
Laura Masgrau  
Universitat Autònoma de Barcelona, Spain
- 12.00 T11: Structure-function studies of enzymes in the oxidative D-galacturonate pathway**  
Helena Taberman  
University of Eastern Finland, Finland
- 12.20 Flash presentations (5 min each)**  
*Chair: Antoni Planas*
- P15: A single point mutation near the active center is responsible for high efficiency of the *Thermotoga maritima*  $\alpha$ -galactosynthase in the synthesis of known amylase substrate**  
Kirill Bobrov  
B.P.Konstantinov Petersburg Nuclear Physics Institute, Russia



**P16: Insights into LPMO diversity from structural and functional characterization of NcLPMO9C, a broad-specificity lytic polysaccharide monooxygenase**

Anna S. Borisova

Swedish University of Agricultural Sciences, Sweden.

**P32: Assisting effect of a carbohydrate binding module on glycosynthase-catalyzed polymerization**

Magda Faijes

Universitat Ramon Llull, Spain

**P43: Rational design of a novel cyclodextrin glucanotransferase from *Carboxydocella* to improve alkyl glycoside synthesis**

Kazi Zubaida Gulshan Ara

Lund University, Sweden

**P44: Development and application of a synthetic cellulosome-based screening platform for enhanced enzyme discovery**

Johnnie Hahm

Novozymes, Inc. USA

**P49: Neopullulanase subfamily and related specificities of the family GH13 - *in silico* study focused on domain evolution**

Stefan Janecek

Slovak Academy of Sciences, Slovakia

**P50: Characterization of a GH30 glucuronoxylan specific xylanase from *Streptomyces turgidiscabies* C56**

Satoshi Kaneko

University of the Ryukyus, Japan

**P53: Solution structures of glycosaminoglycans and their complexes with complement Factor H: implications for disease**

Sanaullah Khan

University College London, UK

**13.00 Lunch**

***Carbohydrates in health***

*Chair: Vladimir Kren*

**14.20 Key-note lecture**

**T12: Polysaccharide engineering: towards carbohydrate drugs and drug carriers**

Takeshi Takaha

Ezaki Glico Co., Ltd. Japan

**15.00 T13: Structure and mechanism of action of O-acetyltransferase (Oat) A**

Anthony J. Clarke

University of Guelph, Canada

**15.20 T14: Complete switch from  $\alpha$ 2,3- to  $\alpha$ 2,6-regioselectivity in *Pasteurella dagmatis*  $\beta$ -D-galactoside sialyltransferase by active-site redesign**

Katharina Schmölzer

Austrian Centre of Industrial Biotechnology, Austria

**15.40 Poster session and coffee**

***Carbohydrate and enzyme engineering I***

*Chair: Pedro Coutinho*

**16.40 T15: Structure and function in the GH53  $\beta$ -1,4-galactanase family**

Leila Lo Leggio

University of Copenhagen, Denmark

**17.00 T16: Determinants of substrate specificity in chitin oligosaccharide deacetylases: How loops define the de-N-acetylation pattern**

Antoni Planas

Universitat Ramon Llull, Spain

**17.20 T17: Molecular basis for the epimerization of oligosaccharides by cellobiose 2-epimerase**

Wataru Saburi

Hokkaido University, Japan

**17.40 End of the day**

**Tuesday 12.5.2015**

***Structure-function relationships of carbohydrate-acting enzymes I***

*Chair: Carsten Andersen*

**9.00 Key-note lecture**

**T18: Sugar oxidoreductions at the crossroads of mechanistic enzymology and biotechnological application**

Bernd Nidetzky

Technische Universität Graz, Austria

**9.40 T19: Functional characterization of a set of fungal lytic polysaccharide monooxygenase secreted by *Podospira anserina***

Chloé Bennati-Granier

INRA, France

**10.00 T20: Glucooligosaccharide oxidases: Determinants of activity and use in carbohydrate modification**

Emma R. Master

University of Toronto, Canada

**10.20 T21: Engineering of pyranose oxidoreductases for bio-fuelcell applications**

Clemens Peterbauer,

University of Natural Resources and Life Sciences Vienna, Austria

**10.40 Coffee break and poster viewing**

## **Structure-function relationships of carbohydrate-acting enzymes II**

Chair: Kristiina Hilden

- 11.20 T22: The role of carbon starvation in the induction of enzymes that degrade plant-derived carbohydrates in *Aspergillus niger***  
Jolanda van Munster  
University of Nottingham, UK
- 11.40 T23: Esterases of *Myceliophthora thermophila* C1 help in the degradation and modification of lignocellulosic material**  
Laura Leonov  
Dyadic Nederland BV, The Netherlands
- 12.00 T24: Processive action of *Rasamsonia emersonii* cellobiohydrolase Cel7A**  
Anu Koivula  
VTT, Finland
- 12.20 T25: Hydrolysis of arabinoxylo-oligosaccharides and wheat flour arabinoxylan by  $\alpha$ -L-arabinofuranosidases**  
Vincent McKie  
Megazymes, Ireland
- 12.30 Flash presentations (5 min each)**  
Chair: Kristiina Kruus
- P60: Variations in the substrate specificity of cellobiose dehydrogenase**  
Daniel Kracher  
University of Natural Resources and Life Sciences, Vienna, Austria
- P71: Structural and functional insights on the glycoside hydrolases involved in the metabolism of xylooligo- and arabinooligosaccharides in lactic acid bacteria**  
Javier A. Linares-Pastén  
Lund University, Sweden
- P93: Diversity of xylan deacetylases of family CE16: action on acetylated aldotaurouronic acid and glucuronoxylan**  
Vladimir Puchart  
Slovak Academy of Sciences, Slovakia
- P94: Conformational studies on trivalent acetylated mannanose clusters**  
Jani Rahkila  
Åbo Akademi University, Finland
- P130: Expression a hyperthermostable *Thermotoga maritima* xylanase 10B in *Pichia pastoris* GS115 and its tolerance to ionic liquids**  
Hairong Xiong  
College of Life Science, China
- P132: Reconstruction of genome-scale metabolic model of *Brevibacillus thermoruber* 423 for design of improved EPS production strategies**  
Songul Yasar Yildiz  
Marmara University, Turkey
- 13.00 Lunch**

***Synthesis, structure and function of carbohydrates and glycoconjugates***

*Chair: Vincent Bulone*

- 14.20 Key-note lecture**  
**T26: Exploring plant cell wall xylan biosynthesis, structure and function**  
Paul Dupree  
University of Cambridge, UK
- 15.00 T27: Understanding the effect of overexpression of fungal acetyl xylan esterase (AXE1) in hybrid aspen**  
Prashant Mohan-Anupama Pawar  
Swedish University of Agricultural Sciences, Sweden
- 15.20 T28: Bioinspired model assemblies of plant cell walls as sensors for unravelling interaction features of CAZymes**  
Gabriel Paës  
INRA and University of Reims Champagne-Ardenne, France
- 15.40 Poster session and coffee**
- Materials from renewable carbohydrates***  
*Chair: Maija Tenkanen*
- 16.40 T29: Discovery of original  $\alpha$  -transglucosylases from *Leuconostoc citreum* NRRL B-1299 and NRRL B-742 for the synthesis of tailor-made  $\alpha$ -glucans**  
Claire Moulis  
Université de Toulouse, France
- 17.00 T30: Marine-derived bacterial polysaccharides are valuable sources of glycosaminoglycans**  
Lou Lebellenger  
Centre Atlantique, rue de l'Île d'Yeu, France
- 17.20 T31: Spider silk mimicking assembly of nanocellulose**  
Sanni Voutilainen  
Aalto University, Finland
- 17.40 End of the day**
- 19.30 Conference dinner, Restaurant Pörssi (downtown Helsinki)**

**Wednesday 13.5.2015**

***Carbohydrate and enzyme engineering II***

*Chair: Magali Remaud-Simeon*

- 9.00 Key-note lecture**  
**T32: Multiple CBMs enhance starch degradation by members of the human gut microbiota**  
Nicole Koropatkin  
University of Michigan, USA
- 9.40 T33: Functionality of granule-bound starch synthase from the waxy barley cultivar CDC Alamo**  
Kim H. Hebelstrup  
Aarhus University, Denmark
- 10.00 T34: Glucan phosphatases utilize different mechanisms to bind starch and glycogen**  
Matthew S. Gentry  
University of Kentucky, USA
- 10.20 T35: Secondary structure reshuffling modulates the enzymatic activity of a GT-B glycosyltransferase at the membrane interface**  
Natalia Comino  
Universidad del País Vasco / Euskal Herriko Unibertsitatea, Spain
- 10.40 T36: Degrading sulfated sugars from the sea: novel insights into the evolution, dimerization plasticity and catalytic mechanism of the GH117s**  
Elizabeth Ficko-Blean  
Sorbonne Universités, France

**11.00 Coffee break**

***Carbohydrates in nutrition***

*Chair: Marco Moracci*

- 11.40 T37: Functional metagenomics reveals novel pathways of mannoside metabolism by human gut bacteria**  
Gabrielle Potocki-Veronese  
Université de Toulouse, France
- 12.00 T38: Structural basis for arabinoxylo-oligosaccharide capture by probiotic bifidobacteria**  
Maher Abou Hachem  
Technical University of Denmark, Denmark
- 12.20 T39: The modular intramolecular trans-sialidase from *Ruminococcus gnavus* ATCC 29149 suggests a novel mechanism of mucosal adaptation in the human gut microbiota**  
Louise E Tailford  
Institute of Food Research, UK
- 12.40 T40: Galactomannan degradation by *Bifidobacterium***  
Evelina Kulcinskaja  
Lund University, Sweden

*Chair: Lubbert Dijkhuizen*

- 13.00**      **Closing lecture**  
**T41: Glycan utilization by human gut Bacteroides**  
Harry Gilbert  
University of Newcastle upon Tyne, UK
- 13.40**      **Poster awards, closing and invitation to CBM12**
- 14.00**      ***End of CBM11***
- Lunch**

# Table of Contents

## Oral Presentations

<b>T1</b>	<b>From the first CBHI to biorefineries</b> Merja Penttilä	33
<b>T2</b>	<b>CAZyChip: a bioChip for bacterial glycoside hydrolases detection and dynamic exploration of microbial diversity for plant cell wall hydrolysis</b> Anne Abot, Delphine Labourdette, Lidwine Trouilh, Sophie Lamarre, Gabrielle Potocki-Veronese, Lucas Auer, Adèle Lazuka, Guillermina Hernandez-Raquet, Bernard Henrissat, Michael O'Donohue, Claire Dumon and Véronique Anton Leberre	34
<b>T3</b>	<b>A new generation of chromogenic substrates for high-throughput screening of glycosyl hydrolases, LPMOs and proteases</b> Julia Schückel, Stjepan K. Kračun and William G. T. Willats	35
<b>T4</b>	<b>Mining fungal diversity for novel carbohydrate acting enzymes</b> Ronald P. de Vries	36
<b>T5</b>	<b>The increasing diversity of lytic polysaccharide monooxygenases</b> Gideon Davies and the CESBIC consortium	37
<b>T6</b>	<b>Neutron and high-resolution X-ray structural studies of glycoside hydrolase family 45 endoglucanase from the basidiomycete <i>Phanerochaete chrysosporium</i></b> Akihiko Nakamura, Takuya Ishida, Masahiro Samejima, and Kiyohiko Igarashi	38
<b>T7</b>	<b>New insight into substrate specificity and activity determinants of a starch debranching enzyme gained from substrate:enzyme crystal structures</b> Marie S. Møller, Michael S. Windahl, Lyann Sim, Marie Bøjstrup, Maher Abou Hachem, Ole Hindsgaul, Monica Palcic, Birte Svensson, Anette Henriksen	39
<b>T8</b>	<b>Crystal structures of N-acetylhexosamine 1-kinase and UDP-glucose 4-epimerase in the GNB/LNB pathway from infant-gut associated bifidobacteria</b> Young-Woo Nam, Mayo Sato, Takatoshi Arakawa, Mamoru Nishimoto, Motomitsu Kitaoka and Shinya Fushinobu	40
<b>T9</b>	<b>Crystal structure of the GTFB enzyme, the first representative of the 4,6-<math>\alpha</math>-glucanotransferase subfamily within GH70</b> Tjaard Pijning, Yuxiang Bai and Lubbert Dijkhuizen	41

<b>T10</b>	<b>Catalytic mechanism of retaining glycosyltransferases: Is Arg293 on the <math>\beta</math>-face of EXTL2 compatible with it? Insights from QM/MM calculations</b>	
	Laura Masgrau, María Fernanda Mendoza, Hansel Gómez and José M. Lluch	42
<b>T11</b>	<b>Structure-function studies of enzymes in the oxidative D-galacturonate pathway</b>	
	Helena Taberman, Martina Andberg, Tarja Parkkinen, Nina Hakulinen, Merja Penttilä, Anu Koivula and Juha Rouvinen	43
<b>T12</b>	<b>Polysaccharide engineering: towards carbohydrate drugs and drug carriers</b>	
	Takeshi Takaha, Michiyo Yanase, Akiko Kubo, Ryo Kakutani and Takashi Kuriki	44
<b>T13</b>	<b>Structure and mechanism of action of O-acetyltransferase (Oat) A</b>	
	David Sychantha, Laura Kell and Anthony J. Clarke	45
<b>T14</b>	<b>Complete switch from <math>\alpha</math>2,3- to <math>\alpha</math>2,6-regioselectivity in <i>Pasteurella dagmatis</i> <math>\beta</math>-D-galactoside sialyltransferase by active-site redesign</b>	
	Katharina Schmölzer, Tibor Czabany, Christiane Luley-Goedl, Tea Pavkov-Keller, Doris Ribitsch, Helmut Schwab, Karl Gruber, Hansjörg Weber and Bernd Nidetzky	46
<b>T15</b>	<b>Structure and function in the GH53 <math>\beta</math>-1,4-galactanase family</b>	
	Søs Torpenholt, Leonardo De Maria, Jens-Christian N. Poulsen, Mats H. M. Olsson, Lars H. Christensen, Michael Skjød, Peter Westh, Jan H. Jensen and Leila Lo Leggio	47
<b>T16</b>	<b>Determinants of substrate specificity in chitin oligosaccharide deacetylases: how loops define the de-N-acetylation pattern</b>	
	Xevi Biarnés, Hugo Aragunde, David Albasa-Jové, Marcelo E. Guerin, and Antoni Planas	48
<b>T17</b>	<b>Molecular basis for the epimerization of oligosaccharides by cellobiose 2-epimerase</b>	
	Wataru Saburi, Takaaki Fujiwara, Nongluck Jaito, Hirohiko Muto, Hirokazu Matsui, Min Yao, and Haruhide Mori	49
<b>T18</b>	<b>Sugar oxidoreductions at the crossroads of mechanistic enzymology and biotechnological application</b>	
	Bernd Nidetzky	50
<b>T19</b>	<b>Functional characterization of a set of fungal lytic polysaccharide monooxygenase secreted by <i>Podospora anserina</i></b>	
	Chloé Bennati-Granier, Sona Garajova, Charlotte Champion, Sacha Grisel, Mireille Haon, Hélène Rogniaux, Isabelle Gimbert, Eric Record, Jean-Guy Berrin	51
<b>T20</b>	<b>Glucooligosaccharide oxidases: determinants of activity and use in carbohydrate modification</b>	
	Maryam Foumani, Thu Vuong, Benjamin McCormick, and Emma R. Master	52



- T21 Engineering of pyranose oxidoreductases for bio-fuelcell applications**  
Clemens Peterbauer, Dagmar Brugger, Iris Krondorfer, Christoph Gonaus, Leonard Stoica and Dietmar Haltrich 53
- T22 The role of carbon starvation in the induction of enzymes that degrade plant-derived carbohydrates in *Aspergillus niger***  
Jolanda van Munster, Paul Daly, Stephane Delmas, Steven Pullan, Martin Blythe, Sunir Malla, Matthew Kokolski, Xiaolan Yu, Paul Dupree, David Archer 54
- T23 Esterases of *Myceliophthora thermophila* C1 help in the degradation and modification of lignocellulosic material**  
Laura Leonov, Gabriela Bahrim, Henk Schols, Sanna Koutaniemi, Maija Tenkanen, Jaap Visser, Sandra Hinz 55
- T24 Processive action of *Rasamsonia emersonii* cellobiohydrolase cel7A**  
Anu Koivula, Jenni Rahikainen, Akihiko Nakamura, Taku Uchiyama, Takayaki Uchihashi, Terhi Puranen, Kristiina Kruus, Toshio Ando and Kiyohiko Igarashi 56
- T25 Hydrolysis of arabinoxylo-oligosaccharides and wheat flour arabinoxylan by  $\alpha$ -L-arabinofuranosidases**  
Barry McCleary, Vincent McKie and Jennifer Larkin 57
- T26 Exploring plant cell wall xylan biosynthesis, structure and function**  
Paul Dupree, Marta Busse-Wicher, Thomas J. Simmons, Jenny C. Mortimer, Nino Nikolovski, Thiago Gomes, Ray Dupree, Katherine Stott, Nicholas J. Grantham, Jennifer Bromley, Mathias R. Sorieul, Xiaolan Yu, Kathryn S. Lilley, Steven P. Brown, and Munir Skaf 58
- T27 Understanding the effect of overexpression of fungal acetyl xylan esterase (AXE1) in hybrid aspen**  
Prashant Mohan-Anupama Pawar, Marta Derba-Maceluch, Sun-Li Chong, Maija Tenkanen, Madhavi Latha Gandla, Leif Jönsson, Martin Lawoko and Ewa J. Mellerowicz 59
- T28 Bioinspired model assemblies of plant cell walls as sensors for unravelling interaction features of CAZymes**  
Gabriel Paës and Jean-Guy Berrin 60
- T29 Discovery of original  $\alpha$ -transglucosylases from *Leuconostoc citreum* NRRL B-1299 and NRRL B-742 for the synthesis of tailor-made  $\alpha$ -glucans**  
Marlène Vuillemin, Delphine Passerini, Marion Claverie, Etienne Severac, Florent Grimaud, Pierre Monsan, Sandrine Morel, Magali Remaud-Simeon and Claire Moulis 61
- T30 Marine-derived bacterial polysaccharides are valuable sources of glycosaminoglycans**  
Christine Delbarre-Ladrat, Lou Lebellenger, Jacqueline Ratiskol, Corinne Sinquin, Agata Zykawska, Sylvia Collic-Jouault 62

<b>T31</b>	<b>Spider silk mimicking assembly of nanocellulose</b> Sanni Voutilainen, Arja Paananen, Markus Linder	63
<b>T32</b>	<b>Multiple CBMs enhance starch degradation by members of the human gut microbiota</b> Nicole Koropatkin	64
<b>T33</b>	<b>Functionality of granule-bound starch synthase from the waxy barley cultivar CDC Alamo</b> Kim H. Hebelstrup, Morten Munch Nielsen, Massimiliano Carciofi, Katarzyna Krucewicz, Shahnoor Sultana Shaik, Andreas Blennow and Monica M. Palcic	65
<b>T34</b>	<b>Glucan phosphatases utilize different mechanisms to bind starch and glycogen</b> Matthew S. Gentry, Madushi Raththagala, M. Kathryn Brewer, David A Meekins, Satrio Husodo, Vikas Dukhande, and Craig W. Vander Kooi	66
<b>T35</b>	<b>Secondary structure reshuffling modulates the enzymatic activity of a GT-B glycosyltransferase at the membrane interface</b> Natalia Comino and Marcelo Guerin	67
<b>T36</b>	<b>Degrading sulfated sugars from the sea: novel insights into the evolution, dimerization plasticity and catalytic mechanism of the GH117s</b> Elizabeth Ficko-Blean, Delphine Duffieux, Étienne Rebuffet, Robert Larocque, Agnes Groisillier, Gurvan Michel, Mirjam Czjzek	68
<b>T37</b>	<b>Functional metagenomics reveals novel pathways of mannoside metabolism by human gut bacteria</b> Simon Ladevèze, Gianluca Giocci, Laurence Tarquis, Elisabeth Laville, Bernard Henrissat, Samuel Tranier, and Gabrielle Potocki-Veronese	69
<b>T38</b>	<b>Structural basis for arabinoxylo-oligosaccharide capture by probiotic bifidobacteria</b> Morten Ejby, Folmer Fredslund, Andreja Vujcic-Zagar, Birte Svensson, Dirk Jan Slotboom, and Maher Abou Hachem	70
<b>T39</b>	<b>The modular intramolecular <i>trans</i>-sialidase from <i>Ruminococcus gnavus</i> ATCC 29149 suggests a novel mechanism of mucosal adaptation in the human gut microbiota</b> Louise E Tailford, C David Owen, John Walshaw, Emmanuelle H Crost, Jemma Hardy-Goddard, Gwenaëlle Le Gall, Willem M de Vos, Garry L Taylor and Nathalie Juge	71
<b>T40</b>	<b>Galactomannan degradation by <i>Bifidobacterium</i></b> Evelina Kulcinskaja, Frida Fåk, Greta Jakobsdottir, Nittaya Marungruang, Sumitha Reddy, Romany Ibrahim, Anna Rosengren, Margareta Nyman, Henrik Stålbrand	72
<b>T41</b>	<b>Understanding complex glycan utilization in the human microbiota</b> Harry J. Gilbert, Artur Rogowski, Dider Ndeh, Fiona Cuskin, Elisabeth Lowe, Eric C. Martens and David Bolam	73

## Poster Presentations

- P1 Anticoagulant activity of sulfated polysaccharide-rich macroalgae extracts**  
Amandine Adrien, Nicolas Bidiau, Thierry Maugard 77
- P2 Elucidating the impact of N-glycosylation on the ability of recombinant CBM3 from *Clostridium thermocellum* to modify pulp and paper properties**  
Carla Oliveira, Goreti Sepúlveda, Tatiana Q. Aguiar, Francisco M. Gama and Lucília Domingues 78
- P3 Discovery and characterization of novel carbohydrate esterases**  
Pablo Alvira, Gregory Arnal, Sophie Bozonnet, Régis Fauré, Olga Gherbovet, Claire Dumon and Michael O'Donohue 79
- P4 Hydrolysis of xylan by thermophilic family 10 xylanase in the presence of biomass-dissolving ionic liquids**  
Sasikala Anbarasan, Michael Hummel, Herbert Sixta and Ossi Turunen 80
- P5 Swollenin from *Trichoderma reesei* exhibits hydrolytic activity against cellulosic substrates with features of both endoglucanases and cellobiohydrolases**  
Martina Andberg, Merja Penttilä, and Markku Saloheimo 81
- P6 Characterization of a GH62  $\alpha$ -L-arabinofuranosidase from *Aspergillus nidulans*: Linking functional diversity with phylogenetics**  
Susan Andersen, Casper Wilkens, Bent O. Petersen, Barry McCleary, Ole Hindsgaul, Maher Abou Hachem and Birte Svensson 82
- P7 Efficient chemoenzymatic synthesis of antioxidants using feruloyl esterases in detergentless microemulsions**  
Io Antonopoulou, Evangelos Topakas, Laura Leonov, Ulrika Rova, Paul Christakopoulos 83
- P8 Alkyl mannosides produced by alcoholysis with  $\beta$ -mannanases from the fungi *Trichoderma reesei* and *Aspergillus nidulans***  
Anna Aronsson, Johan Svantesson Sjöberg, Eva Nordberg Karlsson, Patrick Adlercreutz and Henrik Stålbrand 84
- P9 Development of microbial production processes for levan polysaccharide**  
Ozlem Ates and Ebru Toksoy Oner 85
- P10 Roles of starch and sucrose in exopolysaccharide formation by *Lactobacillus reuteri***  
Yuxiang Bai, Justyna M. Dobruchowska, Rachel M. van der Kaaij, Albert Woortman, Johann P. Kamerling, Lubbert Dijkhuizen 86

- P11 Towards the set-up of a recombinant protein production facility for fungal carbohydrate-active enzymes using the yeast *Pichia pastoris***  
Mireille Haon, Sacha Grisel, David Navarro, Antoine Gruet, Jean Guy Berrin, Christophe Bignon 87
- P12 Structural analysis of chitin oligosaccharide deacetylases – the “subsite capping model”**  
Xevi Biarnés, Hugo Aragunde, David Albasa-Jové, Marcelo Guerin, and Antoni Planas 88
- P13** The abstract has been withdrawn
- P14 HEXPIN: Hetero-exopolysaccharide – milk protein interactions**  
Johnny Birch, Hörður Kári Harðarson, Maher Abou Hachem, Richard Ipsen, Marie-Rose Van Calsteren, Christel Garrigues, Kristoffer Almdal, Birte Svensson 90
- P15 A single point mutation near the active center is responsible for high efficiency of the *Thermotoga maritima*  $\alpha$ -galactosynthase in the synthesis of known amylase substrate**  
Kirill Bobrov, Anna Borisova, Elena Eneyskaya, Dina Ivanen, Daria Chervyakova, Konstantin Shabalin, Georgy Rychkov and Anna Kulminskaya 91
- P16 Insights into LPMO diversity from structural and functional characterization of NcLPMO9C, a broad-specificity lytic polysaccharide monooxygenase**  
Anna S. Borisova, Trine Isaksen, Maria Dimarogona, Aniko Varnai, Morten Sørli, Aasmund K. Røhr, Christina M. Payne, Jerry Ståhlberg, Mats Sandgren, Vincent G. H. Eijssink 92
- P17 How to quantify enzyme activity and kinetics in "non-bulk" systems? An example through the enzymatic hydrolysis of hemicellulose thin films**  
Amal Zeidi, Lucie Dianteill, Claire Dumon Cédric Montanier, Régis Fauré, Jérôme Morchain, Noureddine Lebaz, Childéric Séverac, Antoine Bouchoux 93
- P18 The CBMomes of cellulolytic bacteria colonizing different ecological niches present distinct carbohydrate specificities**  
Joana L.A. Brás, Diana Ribeiro, Maria J. Romão, Ana L. Carvalho, Wengang Chai, Yan Liu, Ten Feizi, José A.M. Prates, Luís M.A. Ferreira, Carlos M.G.A. Fontes, Angelina S. Palma 94
- P19 Determination of mammalian sialic acids in infant formula**  
Deanna Hurum, Cees Bruggink, Terri Christison, Jeff Rohrer, and Detlef Jensen 95
- P20 Cellobiohydrolase and endoglucanase respond differently to surfactants during the hydrolysis of cellulose**  
Chia-wen C. Hsieh, David Cannella, Henning Jørgensen, Claus Felby and Lisbeth G. Thygesen 96

- P21 From waste to health care product: Pectic oligosaccharides produced from citrus peels by treatment of endo-pectate lyase (PL1B) inhibiting colon cancer cells**  
Soumyadeep Chakraborty and Arun Goyal 97
- P22 Enzymatic synthesis of lipid II and analogues**  
Linya Huang, Shi-Hsien Huang, Ya-Chih Chang, Wei-Chieh Cheng, Ting-Jen Rachel Cheng, Chi-Huey Wong 98
- P23 Modification of cell wall glucuronoxylans by expressing a GH115  $\alpha$ -glucuronidase in *Arabidopsis thaliana***  
Sun-Li Chong, Marta Derba-Maceluch, Sanna Koutaniemi, Maija Tenkanen, and Ewa Mellerowicz 99
- P24 Biochemical characterization of a new GH-70 enzyme from *Leuconostoc citreum* NRRL B-1299**  
Marion Claverie, Marlène Vuillemin, Etienne Severac, Pierre Monsan, Gianluca Cioci, Claire Moulis, Magali Remaud-Siméon 100
- P25 Discovery of novel carbohydrate active enzymes for plant biomass degradation by metagenomics of hyperthermophilic communities**  
Beatrice Cobucci-Ponzano, Andrea Strazzulli, Rosa Giglio, Roberta Iacono, Federica Bitetti, Corinna Schiano di Cola, Federico M. Lauro, Yizhuang Zhou, Jin Xu, Vincent Lombard, Bernard Henrissat, Vania Cardoso, Carlos MGA Fontes and Marco Moracci 101
- P26 Structural and functional investigation of a lytic polysaccharide monooxygenase (LPMO) by NMR spectroscopy**  
Gaston Courtade, Simone Balzer, Zarah Forsberg, Gustav Vaaje-Kolstad, Vincent G. H. Eijsink, Finn L. Aachmann 102
- P27 A novel carbohydrate esterase isolated from an Arctic environmental metagenome**  
Concetta De Santi, Nils-Peder Willassen, Arne Oskar Smalås, Adele Williamson 103
- P28 Towards monoglycosylation of organic molecules with glucanases: reaction –and enzyme engineering**  
Tim Devlamynck, Evelien te Poele, Xiangfeng Meng, Wim Soetaert, Lubbert Dijkhuizen 104
- P29 The feruloyl esterase gene family of *Aspergillus niger***  
Adiphol Dilokpimol, Miia R. Mäkelä, Olga Belova, Sadegh Mansouri, Ronald P. de Vries and Kristiina Hilden 105
- P30 Structural and functional studies of a *Fusarium oxysporum* cutinase with polyethylene terephthalate modification potential**  
Maria Dimarogona, Efstratios Nikolaivits, Maria Kanelli, Paul Christakopoulos, Mats Sandgren and Evangelos Topakas 106

- P31 The hydrophilic character of cytotoxic payloads affects functional properties of antibody-drug conjugates**  
Tero Satomaa, Anja Vilkinan, Titta Kotiranta, Filip S. Ekholm, Virve Pitkänen, Ritva Niemelä, Annamari Heiskanen, Henna Pynnönen, Jari Helin and Juhani Saarinen 107
- P32 Assisting effect of a carbohydrate binding module on glycosynthase-catalyzed polymerization**  
Victoria Codera, Magda Faijes, and Antoni Planas 108
- P33 Crystallographic studies of a member of the lytic polysaccharide monooxygenase family AA13**  
Kristian E.H. Frandsen, Jens-Christian N. Poulsen, Maria A. Stringer, Morten Tovborg, Katja S. Johansen, Leonardo De Maria, Gideon J. Davies, Paul H. Walton, P. Dupree, Bernard Henrissat and Leila Lo Leggio 109
- P34 Endogenous degradation activity for slimy extracellular polysaccharide produced by *Lactobacillus fermentum* TDS030603**  
Shinpei Matsumoto, Kenji Fukuda, and Tadasu Urashima 110
- P35 Activity studies on lytic polysaccharide monooxygenases**  
Aline L. Gaenssle, David Canella, Claus Felby and Morten J. Bjerrum 111
- P36 Characterization of a broad substrate specificity AA9 lytic polysaccharide monooxygenases from *Podospora anserina***  
Soňa Garajová, Chloe Bennati-Granier, Maria Rosa Beccia, Charlotte Champion, Sacha Grisel, Mireille Haon, Simeng Zhou, Bruno Guigliarelli, Isabelle Gimbert, Eric Record and Jean-Guy Berrin 112
- P37 Molecular cloning, expression and characterization of novel endo- $\beta$ -1, 4-mannanase of a family 10 glycoside hydrolase from *Pedobacter saltans* DSM12145**  
Kedar Sharma, Anil Kumar Verma and Arun Goyal 113
- P38 Insights into the mechanism of glucuronoxylan hydrolysis revealed by the 3-dimensional crystal structures of glucuronoxylan-xylanohydrolase (CtXyn30A) from *Clostridium thermocellum***  
Anil Kumar Verma, Arun Goyal, Filipe Freire, Carlos M.G.A. Fontes and Shabir Najmudin 114
- P39 Enhanced saccharification and effective pretreatment of corn cob by utilizing recombinant cellulase and hemicellulase from *Clostridium thermocellum* for bioethanol production**  
Ashutosh Gupta, Debasish Das and Arun Goyal 115
- P40 Structural and functional studies of a copper-dependent lytic polysaccharide monooxygenase from *Bacillus Amyloliquefaciens***  
Rebecca Gregory, Gideon Davies and Paul Walton 116

<b>P41</b>	<b>Metagenome mining of novel enzymes for the bioethanol industry</b> Noam Grimberg and Yuval Shoham	117
<b>P42</b>	<b>Thioglycoligases : innovative biocatalytic tools for S-glycosylated proteins synthesis</b> Laure Guillotin, Pierre Lafite and Richard Daniellou	118
<b>P43</b>	<b>Rational design of a novel cyclodextrin glucanotransferase from <i>Carboxydocella</i> to improve alkyl glycoside synthesis</b> Kazi Zubaida Gulshan Ara, Jonas Jönsson , Pontus Lundemo, Javier A. Linares-Pastén , Patrick Adlercreutz and Eva Nordberg-Karlsson	119
<b>P44</b>	<b>Development and application of a synthetic cellulosome-based screening platform for enhanced enzyme discovery</b> Johnnie Hahm, Elizabeth Znameroski, Fang Liu, Tia Heu, Ian Haydon, Sumati Hasani, Michael Lamsa, Aubrey Jones, William Widner, Ronald Mullikin, Paul Harris, Sarah Teter, Janine Lin	120
<b>P45</b>	<b>Identification of the catalytic residues of glycosidases from <i>Paenibacillus thiaminolyticus</i> as a key into engineering new glycosynthases</b> Katarína Hlat-Glembová, Vojtěch Spiwok, Eva Benešová, Blanka Králová	121
<b>P46</b>	<b>Identification and characterization of a novel unclassified de-N-acetylase from <i>Sulfolobus solfataricus</i></b> Roberta Iacono, Beatrice Cobucci-Ponzano, Andrea Strazzulli and Marco Moracci	122
<b>P47</b>	<b>Development of novel enzymatic tools for the production of xylose-based products within a lignocellulosic biorefinery concept.</b> Eleni Ioannou, Claire Dumon, David Bryant, Narcis Fernandez-Fuentes and Michael O'Donohue	123
<b>P48</b>	<b>Biochemical characterization of a novel aldose-ketose isomerase, mannose isomerase from <i>Marinomonas mediterranea</i></b> Nongluck Jaito, Wataru Saburi, Yuka Tanaka, and Haruhide Mori	124
<b>P49</b>	<b>Neopullulanase subfamily and related specificities of the family GH13 - <i>in silico</i> study focused on domain evolution</b> Stefan Janecek and Andrea Kuchtova	125
<b>P50</b>	<b>Characterization of a GH30 glucuronoxylan specific xylanase from <i>Streptomyces turgidiscabies</i> C56</b> Tomoko Maehara, Zui Fujimoto, Kei Kamino, Yoshiaki Kitamura, and Satoshi Kaneko	126
<b>P51</b>	<b>Chitinases in a lignin-producing cell culture of Norway spruce</b> Kaija Porkka, Silvia Vidal-Melgosa, Julia Schückel, Sanna Koutaniemi, William G. T. Willats and Anna Kärkönen	127

<b>P52</b>	<b>Enzyme properties affecting enzyme adsorption onto lignin in high solid environments</b> Miriam Kellock, Jenni Rahikainen and Kristiina Kruus	128
<b>P53</b>	<b>Solution structures of glycosaminoglycans and their complexes with complement Factor H: implications for disease</b> Sanaullah Khan, Jayesh Gor, Barbara Mulloy and Stephen J. Perkins	129
<b>P54</b>	<b>A novel sialic acid-specific lectin from the mushroom <i>Hericium erinaceum</i></b> Seonghun Kim	130
<b>P55</b>	<b>Enzymatic production of a natural solubilizer rubusoside using a thermostable lactase from <i>Thermus thermophilus</i></b> Doman Kim, Thi Thanh Hanh Nguyen, Jaeyoung Cho, Ye-seul Suh, Eunbae An, Jiyoun Kim, and Shin-Hye Yu	131
<b>P56</b>	<b>Practical preparation of sugar 1-phosphates</b> Motomitsu Kitaoka, Yuan Liu, and Mamoru Nishimoto	132
<b>P57</b>	<b>Structural and functional insights into the CBM50s of two plant GH18 chitinases</b> Yoshihito Kitaoku, Toki Taira, Tomoyuki Numata, Tamo Fukamizo, Takayuki Ohnuma	133
<b>P58</b>	<b>New glucuronoyl esterases for wood processing</b> Sylvia Klaubauf, Silvia Hüttner, Hampus Sunner and Lisbeth Olsson	134
<b>P59</b>	<b>Comparison of transglycosylation abilities of two <math>\alpha</math>-L-fucosidase isozymes from <i>Paenibacillus thiaminolyticus</i></b> Terézia Koval'ová, Patricie Buchtová, Eva Benešová, Tomáš Koval', Petra Lipovová	135
<b>P60</b>	<b>Variations in the substrate specificity of cellobiose dehydrogenase</b> Daniel Kracher, Marita Preims, Alfons Felice, Dietmar Haltrich and Roland Ludwig	136
<b>P61</b>	<b>The first transglycosidase derived from a GH20 <math>\beta</math>-N-acetylhexosaminidase</b> Kristýna Slámová, Jana Krejzová, Natalia Kulik and Vladimír Křen	137
<b>P62</b>	<b>Carbohydrate composition in spruce bark</b> Katariina Kemppainen, Matti Siika-aho and Kristiina Kruus	138
<b>P63</b>	<b>Enzymatic synthesis of functional linear isomaltomegalosaccharide by <i>Gluconobacter oxydans</i> dextran dextrinase</b> Yuya Kumagai, Weeranuch Lang, Juri Sadahiro, Masayuki Okuyama, Haruhide Mori, and Atsuo Kimura	139



- P64 Genomics, systematics and proteomics of the wood-decomposing white rot Basidiomycota Polypore species *Phlebia radiata***  
Jaana Kuuskeri, Olli-Pekka Smolander, Heikki Salavirta, Pia Laine, Ilona Oksanen, Miia R. Mäkelä, Kristiina Hildén, Petri Auvinen, Markku Varjosalo, Lars Paulin and Taina Lundell 140
- P65 A unique multi-domain extracellular GH43 arabinanase determined in different conformational states**  
Shifra Lansky, Rachel Salama, Omer Shwartzstien, Yuval Shoham and Gil Shoham 141
- P66 Structural analysis of Abp, a GH27  $\beta$ -L-arabinopyranosidase from *Geobacillus stearothermophilus***  
Shifra Lansky, Rachel Salama, Hodaya V. Solomon, Yuval Shoham and Gil Shoham 142
- P67 A unique octameric structure of an acetyl-xylan esterase**  
Shifra Lansky, Onit Alalouf, Hodaya V. Solomon, Yuval Shoham and Gil Shoham 143
- P68 Characterization of a Chitin Utilization Locus from *Flavobacterium johnsoniae***  
Johan Larsbrink, Sampada S. Kharade, Kurt J. Kwiatkowski, Alasdair MacKenzie, Yongtao Zhu, Nicole Koropatkin, Mark J. McBride, Vincent G. H. Eijsink, Phil B. Pope 144
- P69 Recombinant production of an exopolysaccharide of interest for health industry**  
L. Lebellenger, J. Ratiskol, C. Siquin, A. Zykwinska, S. Collic-Jouault, M. Dols-Lafargue, C. Delbarre-Ladrat 145
- P70 Exploring complex glycan utilization machinery of *Roseburia* spp. implicated in inflammatory and metabolic disorders**  
Maria Louise Leth, Morten Ejby Hansen and Maher Abou Hachem 146
- P71 Structural and functional insights on the glycoside hydrolases involved in the metabolism of xylooligo- and arabinooligosaccharides in lactic acid bacteria**  
Javier A. Linares-Pastén, Peter Falck, Reza Faryar, Patrick Adlercreutz 147
- P72  $\beta$ -D-galactosidase/fucosidase from *Paenibacillus thiaminolyticus* and its transglycosylation properties and immobilization**  
Petra Lipovová, Miroslav Smola, Veronika Kováčová, Eva Benešová, Šárka Musilová and Vojtěch Spiwok 148
- P73 Cultivation strategies for Chitinasome Expression in *Chitinibacter tainanensis***  
Chao-Hsien Yeh, Jin-Ting Chen, Jeen-Kuan Chen and Chao-Lin Liu 149
- P74 From glycoside hydrolase to transglycosidase through protein and reaction engineering**  
Pontus Lundemo, Eva Nordberg Karlsson and Patrick Adlercreutz 150

<b>P75</b>	<b>The impact of polysaccharide chemistry on the regioselectivity of AnAXE from <i>Aspergillus nidulans</i></b>	
	Galina Mai-Gisondi, Maija Tenkanen and Emma Master	151
<b>P76</b>	<b>Plant biomass degrading potential of a new <i>Penicillium</i> species, <i>Penicillium subrubescens</i></b>	
	Sadegh Mansouri, Miia R. Mäkelä, Ad Wiebenga, Ronald P. de Vries, Kristiina Hildén	152
<b>P77</b>	<b>Genome and in-lab analysis of cold-tolerant xylanolytic <i>Paenibacillus</i> spp isolated from low level radioactive waste repository</b>	
	Kaisa Marjamaa, Minna Vikman, Erna Storgårds, Heikki Salavirta and Merja Itävaara	153
<b>P78</b>	<b>Up-scaling of the synthetic procedure for preparation of oligosaccharide adjuvant for allergen immunotherapy</b>	
	Denys Mavrynsky, Reko Leino	154
<b>P79</b>	<b>Beechwood xylan for the measurement of <i>endo</i>-1,4-<math>\beta</math>-D-xylanase</b>	
	Páraic McGeough, Ida Lazewska and Barry McCleary	155
<b>P80</b>	<b>Novel substrates for the measurement of pullulanase</b>	
	David Mangan, Vincent McKie and Barry McCleary	156
<b>P81</b>	<b>Residue L940 has a crucial role in the specificity of the glucansucrase GTF180 of <i>Lactobacillus reuteri</i> 180</b>	
	Xiangfeng Meng, Justyna M. Dobruchowska, Tjaard Pijning, Cesar A. López, Johannis P. Kamerling and Lubbert Dijkhuizen	157
<b>P82</b>	<b>Truncation of domain V of the multidomain glucansucrase GTF180 of <i>Lactobacillus reuteri</i> 180 heavily impairs its polysaccharide-synthesizing ability</b>	
	Xiangfeng Meng, Justyna M. Dobruchowska, Tjaard Pijning, Gerrit J. Gerwig, Johannis P. Kamerling and Lubbert Dijkhuizen	158
<b>P83</b>	<b>Identification and engineering of new family AA5 galactose oxidases</b>	
	Filip Møllerup, Kirsti Parikka, Maija Tenkanen and Emma Master	159
<b>P84</b>	<b>Investigating synergism within multimodular glycoside hydrolases during wheat straw cell wall deconstruction</b>	
	Thierry Vernet, Anne-Marie DiGuilmi, Michael O'Donohue, Cédric Montanier	160
<b>P85</b>	<b>Development of tailor-made 'oxidative boosted' enzyme mixtures for the bioconversion of targeted feed stocks.</b>	
	Madhu Nair Muraleedharan, Anthi Karnaouri, Maria Dimarogona, Evangelos Topakas, Ulrika Rova, Paul Christakopoulos	161

- P86 Novel GH130  $\beta$ -mannoside phosphorylases**  
 Hiroyuki Nakai, Takanori Nihira, Kazuhiro Chiku, Erika Suzuki, Mamoru Nishimoto, Motomitsu Kitaoka, Ken'ichi Ohtsubo 162
- P87 Discovery of 1,2- $\beta$ -oligoglucan phosphorylase and large scale preparation of 1,2- $\beta$ -glucan**  
 Masahiro Nakajima, Hiroyuki Toyoizumi, Koichi Abe, Yuta Takahashi, Naohisa Sugimoto, Hiroyuki Nakai, Hayao Taguchi and Motomitsu Kitaoka 163
- P88 Exploring the secretomes of starch degrading fungi**  
 Laura Nekiunaite, Gustav Vaaje-Kolstad, Birte Svensson, Magnus Øverlie Arntzen and Maher Abou Hachem 164
- P89 Chemo-enzymatic synthesis of chitoheptaose using a glycosynthase derived from an inverting chitinase with an extended binding cleft**  
 Takayuki Ohnuma, Satoshi Dozen and Tamo Fukamizo 165
- P90 A transglycosylation of catalytic nucleophile mutant of GH97  $\alpha$ -galactosidase with an external nucleophile**  
 Masayuki Okuyama, Kana Matsunaga, Ken-ichi Watanabe, Takayoshi Tagami, Keitaro Yamashita, Haruhide Mori, Min Yao and Atsuo Kimura 166
- P91** The abstract has been withdrawn
- P92 Design of a nano-system targeting the tumor micro-environment for the treatment of tumor by inhibition of a specific  $\beta$ -endoglycosidase responsible for angiogenesis**  
 Nicolas Poupard, Nicolas Bridiau, Jean-Marie Piot, Thierry Maugard, Ingrid Fruitier-Arnaudin 168
- P93 Diversity of xylan deacetylases of family CE16: action on acetylated aldotetrauronic acid and glucuronoxylan**  
 Vladimír Puchart, Jane Agger, Jean-Guy Berrin, Anikó Varnai, Lin-Xiang Li, Alasdair MacKenzie, Vincent G.H. Eijssink, Børge Westereng, Peter Biely 169
- P94 Conformational studies on trivalent acetylated mannobiose clusters**  
 Jani Rahkila, Rajib Panchadhayee, Ana Ardá, Jesús Jiménez-Barbero, and Reko Leino 170
- P95 The conformational free-energy landscape of  $\beta$ -xylose reveals a two-fold catalytic itinerary for  $\beta$ -xylanases**  
 Javier Iglesias-Fernández, Lluís Raich, Albert Ardèvol and Carme Rovira 171
- P96 Structural and biochemical characterization of endo-acting chondroitin AC lyase a family 8 polysaccharide lyase (PsPL8a) from *Pedobacter saltans* DSM 12145**  
 Aruna Rani, Joyeeta Mukherjee, Munishwar N. Gupta and Arun Goyal 172

- P97 Molecular mechanisms of retaining glycosyltransferases. Insight from QM/MM metadynamics simulations**  
Javier Iglesias-Fernández, Albert Ardèvol, Víctor Rojas-Cervellera, Ramón Hurtado-Guerrero, Antoni Planas and Carme Rovira 173
- P98 Comparative analysis of transcriptomes and secretomes of the white-rot fungus *Dichomitus squalens* cultured in lignocellulosic substrates**  
Johanna Rytioja, Miaomiao Zhou, Kristiina Hildén, Marcos Di Falco, Outi-Maaria Sietiö, Adrian Tsang, Ronald P. de Vries and Miia R. Mäkelä 174
- P99 Biochemical characterization and crystal structure of a novel GH127  $\beta$ -L-arabinofuranosidase**  
Rachel Salama, Shifra Lansky, Ruth Goldschmidt, Gil Shoham and Yuval Shoham 175
- P100 Xylooligosaccharides (XOs) from xylan extracted from quinoa (*Chenopodium quinoa*) stalks**  
Daniel Martin Salas-Veizaga; Javier Linares-Pastén; Teresa Álvarez-Aliaga and Eva Nordberg-Karlsson 176
- P101 Conformational study on homoallylic polyol derived from D-mannose**  
Tiina Saloranta, Anssi Peuronen, Johannes Dieterich, Manu Lahtinen, Reko Leino 177
- P102 Protein stability engineering by structure-guided chimeragenesis**  
Mats Sandgren, Nils Mikkelsen, Saeid Karkehadadi, Henrik Hansson, Mikael Gudmundsson, Igor Nikolaev, Sergio Sunux, Amy Liu, Rick Bott, Thijs Kaper 178
- P103 Evaluation of microbial production of exopolysaccharide by *Rhodothermus marinus* strains: potential for industrial biotechnology**  
Roya R.R. Sardari, Evelina Kulcinskaja, and Eva Nordberg Karlsson 179
- P104 Characterization of two trisaccharides produced in the presence of lactose by *Weissella confusa* dextranucrase**  
Qiao Shi, Minna Juvonen, Yaxi Hou, Ilkka Kajala, Antti Nyyssölä, Ndegwa Henry Maina, Hannu Maaheimo, Liisa Virkki, Maija Tenkanen 180
- P105  $\alpha$ -L-Fucosidase from *Fusarium proliferatum* LE1: specificity and transglycosylation abilities**  
Svetlana V. Shvetsova, Kirill S. Bobrov, Konstantin A. Shabalin, Olga L. Vlasova, Elena V. Eneyskaya, Anna A. Kulminskaya 181
- P106 Hemicellulases in total hydrolysis of wood-based substrates**  
Matti Siika-aho, Anikó Várnai, Jaakko Pere, Kaisa Marjamaa and Liisa Viikari 182
- P107 Structure-function relationships in the active site of the blue mussel  $\beta$ -mannanase MeMan5A**  
Johan Svantesson Sjöberg, Viktoria Bågenholm and Henrik Stålbrand 183

- P108 Engineering a thermostable fungal GH10 xylanase, importance of N-terminal amino acids**  
Letian Song, Adrian Tsang and Michel Sylvestre 184
- P109 Development of mannuronan C-5 epimerases to perform *in vitro* tailoring and upgrading of alginates**  
Annalucia Stanisci, Finn Lillelund Aachmann, AnneTøndervik, Håvard Sletta, Gudmund Skjåk-Bræk 185
- P110 Using alginate milk protein complexes for model foods to investigate how food structure affects satiety**  
Emil G. P. Stender, Maher Abou Hachem Per Hägglund, Richard Ipsen and Birte Svensson 186
- P111 Structural enzymology and engineering of  $\beta$ -mannanases and  $\alpha$ -galactosidases for galactomannan modification**  
Anna Rosengren, Evelina Kulcinskaja, Johan Svantesson Sjöberg, Sumitha Reddy, Anna Aronsson, Viktoria Bågenholm, Oskar Aurelius, Derek Logan, and Henrik Stålbrand 187
- P112 Structural and biochemical studies of sugar beet  $\alpha$ -glucosidase exhibiting high specificity for long-chain substrates**  
Takayoshi Tagami, Keitaro Yamashita, Masayuki Okuyama, Haruhide Mori, Min Yao, and Atsuo Kimura 188
- P113 Transcriptional and functional analysis of polysaccharide utilization loci reveals novel mechanisms of carbohydrate foraging by uncultivated gut bacteria**  
Alexandra Tauzin, Elisabeth Laville, Stéphanie Heux, Sébastien Nouaille, Pascal Le Bourgeois, Jean-Charles Portais, Magali Remaud-Simeon, Gabrielle Potocki-Véronèse and Florence Bordes 189
- P114 Is the metabolic preference for specific  $\beta$ -galactosides established by enzymes or by uptake systems in gut adapted bacteria?**  
Mia Christine Theilmann, Morten Ejby, Birte Svensson and Maher Abou Hachem 190
- P115 Chitin hydrolysis by *Chitinbacter tainanensis* enhancing via explosive puffing**  
Min-Lang Tsai, Too Shen Tan and Chao-Lin Liu 191
- P116 Suppressing transglycosylation to improve hydrolysis of cellobiose to glucose**  
Sasikala Anbarasan, Tommi Timoharju, Janice Barthomeuf, Ossi Pastinen, Juha Rouvinen, Matti Leisola and Ossi Turunen. 192
- P117 Evaluation of the secretomes of cellulolytic and chitinolytic microorganisms**  
Tina R. Tuveng, Magnus Ø. Arntzen, Oskar Bengtsson, Gustav Vaaje- Kolstad, Vincent Eijsink 193

- P118 Functional metagenomics boosts enzyme discovery for plant cell wall polymer breakdown**  
 Lisa Ufarté, Elisabeth Laville, Diego Morgavi, Guillermina Hernandez-Raquet, Sophie Bozonnet, Claire Dumon, Patrick Robe, Bernard Henrissat, and Gabrielle Potocki-Veronese 194
- P119 Oligosaccharides production using a glucansucrase from a lactic acid bacteria strain in its free and immobilized form**  
 Simon Johansson, Gilles Bourdin, Charlotte Gancel and Christina Vafeiadi 195
- P120 Factors affecting enzymatic cellulose hydrolysis in ionic liquid solutions**  
 Ronny Wahlström, Jenni Rahikainen, Kristiina Kruus and Anna Suurnäkki 196
- P121 Structural-functional analysis reveals a specific domain organization in family GH20 hexosaminidases**  
 Cristina Val-Cid, Xevi Biarnés, Magda Fajjes and Antoni Planas. 197
- P122 Novel carbohydrate targeting mechanisms by the human gut symbiont *Bacteroides thetaiotaomicron***  
 Alicia Lammerts van Bueren, Eric Martens and Lubbert Dijkhuizen 198
- P123 Insight into structural, biochemical and *in silico* determinants of ligand binding specificity of family 6 carbohydrate binding module (CtCBM6) from *Clostridium thermocellum***  
 Anil Kumar Verma, Pedro Bule, Teresa Ribeiro, Joana L. A. Brás, Joyeeta Mukherjee, Munishwar N. Gupta, Carlos M.G.A. Fontes and Arun Goyal 199
- P124 Diversity in  $\beta$ -galactosidase specificities within *Bifidobacterium*: towards an understanding of  $\beta$ -galactoside metabolism in the gut niche**  
 Alexander Holm Viborg, Maher Abou Hachem, Takane Katayama, Leila Lo Leggio, Motomitsu Kitaoka, Shinya Fushinobu, and Birte Svensson 200
- P125 Mining anaerobic digester consortia metagenomes for secreted carbohydrate active enzymes**  
 Casper Wilkens, Peter Kamp Busk, Bo Pilgaard, Rasmus Kirkegaard, Mads Albertsen, Per Halkjær Nielsen and Lene Lange 201
- P126 Structural and functional characterization of the *Clostridium perfringens* N-acetylmannosamine-6-phosphate 2-epimerase essential for the sialic acid salvage pathway**  
 Marie-Cécile Péliissier, Corinne Sebban-Kreuzer, Françoise Guerlesquin, James A. Brannigan, Yves Bourne and Florence Vincent 202
- P127 Discovering novel glycan utilization loci in probiotic bacteria**  
 Jens Vogensen, Quanhui Wang, Maher Abou Hachem, Siqi Liu, Birte Svensson 203

<b>P128</b>	<b>Activity-based probing of <math>\alpha</math>-L-fucosidase</b> Daniel Wright, Jianbing Jiang, Wouter Kallemijn, Johannes Aerts, Herman Overkleeft, Gideon Davies	204
<b>P129</b>	<b>Gene synthesis, expression and characterization of a thermostable endo-<math>\beta</math>-1, 4-mannanase</b> Yawei Wang, Wei Zhang, Zhengding Su, Ying Zhou, Ossi Turunen, Hairong Xiong	205
<b>P130</b>	<b>Expression a hyperthermostable <i>Thermotoga maritima</i> xylanase 10B in <i>Pichia pastoris</i> GS115 and its tolerance to ionic liquids</b> Yawei Wang, Kubra Telli, Tianyi Yu, Ying Zhou, Sasikala Anbarasan, Baris Binay, Michael Hummel, Herbert Sixta, Ossi Turunen, Hairong Xiong	206
<b>P131</b>	<b>Tailor-made potato starch</b> Xuan Xu, Richard G.F Visser and Luisa M. Trindade	207
<b>P132</b>	<b>Reconstruction of genome-scale metabolic model of <i>Brevibacillus thermoruber</i> 423 for design of improved EPS production strategies</b> Songul Yasar Yildiz	208
<b>P133</b>	<b>NMR spectroscopic methods in engineering of sugar acid pathways in yeast</b> Hannu Maaheimo, Martina Andberg, Yvonne Nygård, Peter Richard, David Thomas, Jonas Excell, Harry Boer, Mervi Toivari, Laura Ruohonen, Anu Koivula and Merja Penttilä	209
	<b>Author index</b>	211





# **Oral Presentations**



**From the first CBHI to biorefineries**

*Merja Penttilä*

merja.penttila@vtt.fi

VTT Technical Research Centre of Finland, Post Office Box 1000, FI-02044 VTT, Finland.

## CAZyChip: a bioChip for bacterial glycoside hydrolases detection and dynamic exploration of microbial diversity for plant cell wall hydrolysis

Anne Abot<sup>1,2,3</sup>, Delphine Labourdette<sup>1,2,3</sup>, Lidwine Trouilh<sup>1,2,3</sup>, Sophie Lamarre<sup>1,2,3</sup>, Gabrielle Potocki-Veronese<sup>1,2,3</sup>, Lucas Auer<sup>1,2,3</sup>, Adèle Lazuka<sup>1,2,3</sup>, Guillermina Hernandez-Raquet<sup>1,2,3</sup>, Bernard Henrissat<sup>4</sup>, Michael O'Donohue<sup>1,2,3</sup>, Claire Dumon<sup>1,2,3</sup> and Véronique Anton Leberre<sup>1,2,3</sup>.

claire.dumon@insa-toulouse.fr and veronique.leberre@insa-toulouse.fr

1. Université de Toulouse, INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France
2. INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
3. CNRS, UMR5504, F-31400 Toulouse, France
4. Architecture et Fonction des Macromolécules Biologiques, UMR7257, Centre National de la Recherche Scientifique (CNRS), Université Aix Marseille, F-13288 Marseille, France.

The development of biocatalysts for the deconstruction of plant cell wall polysaccharides such as cellulose and hemicellulose is currently a major endeavor and will contribute to the development of the bioeconomy.

Micro-organisms play an important role in biotransformation of plant cell walls because they produce large collections of enzymes, including glycoside hydrolases (GHs) that are key enzymes involved in the deconstruction of plant cell wall polysaccharides. In order to explore and elucidate the functional dynamic of microbial communities degrading plant cell wall, we developed a robust and generic tool, the CAZyChip based on DNA microarray containing all the bacterial GH classified in the CAZy database. This chip allows a rapid characterization of GH at transcriptomic level and the characterization of plant cell wall-degrading enzyme systems that act in concert on the different polysaccharide components of lignocellulosic biomass. The custom microarray was tested and validated by the hybridization of GHs RNA extracted from *E. coli* and recombinant *E. coli* strains. Our results suggest that a microarray-based study can detect genes from low-expression in bacteria. In addition, the results of hybridization of complex biological samples such as rumen or termite gut will be presented. The CAZyChip appears to be an effective tool for profiling GH expression in microbial communities that are actively degrading lignocellulosic biomass and could guide the design of enzymatic cocktails.

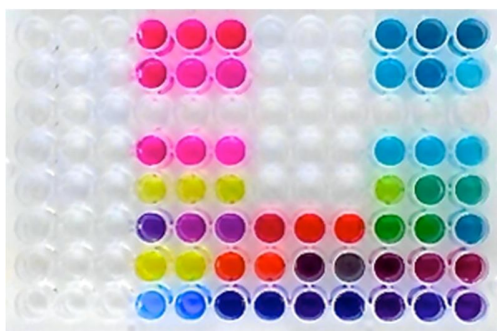
## A new generation of chromogenic substrates for high-throughput screening of glycosyl hydrolases, LPMOs and proteases

*Julia Schückel<sup>1</sup>, Stjepan K. Kračun<sup>1</sup> and William G. T. Willats<sup>1</sup>*

jusch@plen.ku.dk, willats@plen.ku.dk

1. University of Copenhagen, Department of Plant and Environmental Sciences, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

Enzymes that degrade or modify polysaccharides are widespread in pro- and eukaryotes and have multiple biological roles and biotechnological applications. Recent advances in genome and secretome sequencing, together with associated bioinformatic tools have enabled large numbers of putative carbohydrate acting enzymes to be putatively identified. However, there is a paucity of methods for rapidly screening the activities of these enzymes and this is serious bottleneck in the development of enzyme-reliant bio-refining processes. We have developed a new generation of multi-coloured chromogenic polysaccharide and protein substrates that can be used in cheap, convenient and high-throughput multiplexed assays. In addition we have produced substrates of biomass materials in which the complexity of plant cell walls is partially maintained. We show that these substrates can be used to screen the activities of glycosyl hydrolases, lytic polysaccharide monooxygenases (LPMOs) and proteases, and provide insight into substrate availability within biomass. We have validated the technique using microbial enzymes and further show here that these new assays enable the rapid analysis of endogenous enzymes in diverse plant materials.



**Fig 1:** Product plate of a multiplexed assay of different chromogenic polysaccharide hydrogel (CPH) substrates treated with different enzymes.

## **Mining fungal diversity for novel carbohydrate acting enzymes**

Ronald P. de Vries

r.devries@cbs.knaw.nl

Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Uppalalaan 8, 3584 CT Utrecht, The Netherlands

The availability of fungal genome sequences has provided a wealth of new genes and their corresponding enzymes as candidates for novel or better biocatalysts. In particular with respect to enzymes acting on plant biomass, the differences in genome content in the fungal kingdom is enormous and can to a certain extent be related to the natural biotope of the species.

So how do we find the most promising enzymes or enzyme sets from this near limitless pool of candidates? Modern bioinformatics tools can provide the answer to this question, but only in combination with extensive biological data sets that provide insight into the relevance of genomic differences. Extensive transcriptome and proteome datasets on fungi growing on diverse carbon sources, including crude plant biomass as well as pure components thereof allow identification of the enzymes that are required for these different substrates. Comparative genomics and transcriptomics can identify crucial enzymes by selecting those that are present in a large variety of fungi, while phylogeny can pinpoint enzymes that more likely have different properties or substrate specificities.

In this presentation I will provide examples how promising candidate enzymes and enzyme sets can be discovered by combining comparative genomics, transcriptomics and proteomics with growth profiling and data about fungal biotopes and enzymatic function.

## The increasing diversity of lytic polysaccharide monooxygenases

Gideon Davies<sup>1</sup> and the CESBIC consortium<sup>2</sup>

gideon.davies@york.ac.uk

1. University of York,
2. University of York, University of Copenhagen, University of Cambridge, CNRS Marseille, Novozymes A/S

Lytic Polysaccharide Monooxygenases are establishing themselves as important players on biomass conversion (recently reviewed in Refs<sup>1,2</sup>). These mononuclear copper containing enzymes now form four distinct families in the CAZY classification (AA9 and AA10, formally known as GH61 and CBM33) as well as the recently discovered AA11<sup>3</sup> and AA13<sup>4,5</sup> families. Whilst most LPMO families are active on beta-linked polysaccharides, the first starch-active LPMO family has also recently been described and characterised.<sup>4,5</sup> In this lecture I will summarize the LPMO field, highlighting recent work by the CESBIC consortium (University of York, University of Cambridge, University of Copenhagen, CNRS Marseille, and Novozymes A/S) notably in the area of enzyme discovery and characterisation<sup>3,4</sup> of the reactive Cu centre.<sup>6</sup>

### Literature

1. Horn et al *Biotech Biofuels*, **2012**, 5, 45.
2. Hemsworth et al, *Curr Opin Struct Biol*, **2013**, 23, 660-668.
3. Hemsworth et al., *Nature Chemical Biology* **2014**, 10, 122-126.
4. Lo Leggio et al., *Nature Communications* **2015**, 6, Article 5961.
5. Vu et al., *Proc Natl Acad Sci USA*, **2014**, 111, 13822–13827.
6. Kjaergaard et al., *Proc Natl Acad Sci USA* **2014**, 111, 8797-8802.

## Neutron and high-resolution X-ray structural studies of glycoside hydrolase family 45 endoglucanase from the basidiomycete *Phanerochaete chrysosporium*

Akihiko Nakamura, Takuya Ishida, Masahiro Samejima, and Kiyohiko Igarashi

aquarius@mail.ecc.u-tokyo.ac.jp

Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

We employed a neutron diffraction analysis to investigate the catalytic mechanism of the inverting glycoside hydrolase (GH) family 45 cellulase *PcCel45A*, which is an endoglucanase (EG) belonging to subfamily C of this family, isolated from the basidiomycete *Phanerochaete chrysosporium*. The amino acid alignment with other GH family 45 EGs indicates *PcCel45A* lacks putative general base and assisting acidic residues while it has an apparent activity towards cellulose and  $\beta$ -1,3-1,4-glucan (1). To understand the catalytic mechanism of *PcCel45A*, we made a large crystal of 6 mm<sup>3</sup> volume (3 mm x 2 mm x 1 mm) for the neutron protein structural study (2). The results of a joint refinement of the neutron and high-resolution X-ray structures clarified a key role of tautomerization of asparagine 92 to imidic acid as a catalytic base in the inverting cellulase.

### Acknowledgments

We thank Profs. Katsuhiro Kusaka, Taro Yamada, Ichiro Tanaka, Nobuo Niimura in Ibaraki University, Prof. Shinya Fushinobu in the University of Tokyo, Prof. Satoshi Kaneko in the University of Ryukyus, Dr. Kazunori Ohta in Space Environment Utilization Center, Japan Aerospace Exploration Agency, Dr. Hiroaki Tanaka in Confocal Science Inc., Dr. Koji Inaka in Maruwa Foods and Biosciences Inc., and Prof. Yoshiki Higuchi in University of Hyogo for their contributions to this study.

### Literature

1. Igarashi, K., Ishida, T., Hori, C., and Samejima, M., Characterization of endoglucanase belonging to new subfamily of glycoside hydrolase family 45 from the basidiomycete *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol.* 74:5628-5634 (2008)
2. Nakamura, A., Ishida, T., Fushinobu, S., Kusaka, K., Tanaka, I., Inaka, K., Higuchi, Y., Masaki, M., Ohta, K., Kaneko, S., Niimura, N., Igarashi K., and Samejima, M., Phase diagram-guided method for growth of a large crystal of glycoside hydrolase family 45 inverting cellulase suitable for neutron structural analysis, *J. Sync. Rad.* 20: 859-863 (2013)



## New insight into substrate specificity and activity determinants of a starch debranching enzyme gained from substrate:enzyme crystal structures

Marie S. Møller<sup>1,2\*</sup>, Michael S. Windahl<sup>1\*</sup>, Lyann Sim<sup>1\*</sup>, Marie Bøjstrup<sup>1</sup>, Maher Abou Hachem<sup>2</sup>, Ole Hindsgaul<sup>1</sup>, Monica Palcic<sup>1</sup>, Birte Svensson<sup>2</sup>, Anette Henriksen<sup>1</sup>

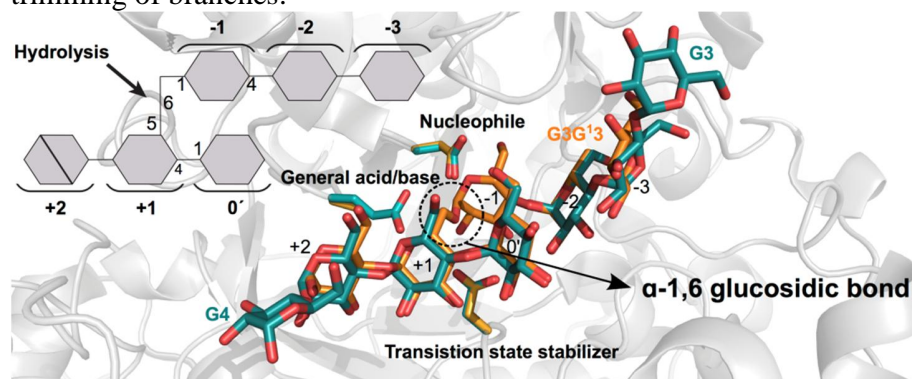
mms@bio.dtu.dk

1. Carlsberg Laboratory, DK-1799 Copenhagen V, Denmark.

2. Department of Systems Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

\*These authors contributed equally to the work

Pullulanases are industrially important starch debranching enzymes and the mechanisms driving their substrate specificities and activities can have a direct influence on the profit ratio in e.g. the industrial manufacturing of glucose and syrups from starch. To date crystal structures of type I pullulanases from 7 different organisms have been solved, including the barley limit dextrinase (LD). Some of these enzyme structures are solved in complex with hydrolysis products or inhibitors, but none of the pullulanases have been structure determined in complex with a natural substrate, i.e. an  $\alpha$ -1,6-branched maltooligosaccharide. Here we present crystal structures of inactive LD in complex with 1) a limit dextrin (PDB 4J3W), and 2) with a pullulan derivative (PDB 4J3X) [1]. These are the first type I pullulanase structures with intact  $\alpha$ -1,6-glucosidic linked substrates spanning the active site. Together with the structures of LD and bacterial pullulanases in complex with hydrolysis products they are used for suggesting both a mechanism for nucleophilicity enhancement in the active site as well as a mechanism for avoidance of dual  $\alpha$ -1,6- and  $\alpha$ -1,4-hydrolytic activity likely to be a biological necessity during starch synthesis, where LD has a role in trimming of branches.



**Fig 1.** Superimposition of barley limit dextrinase in complex with a limit dextrin/branched maltooligosaccharide substrate (G3G'13; colored in orange) and linear products (maltotriose and maltotetraose; coloured in teal), respectively.

**Acknowledgements:** Access to synchrotron beam lines was made possible through the support from DANSCATT. We thank MAX II Laboratory, ESRF and the associated staff for beam time and assistance.

### Literature:

1. Møller, M.S., Windahl, M.S., Sim, L., Bøjstrup, M., Abou Hachem, M., Hindsgaul, O., Palcic, M., Svensson, B. & Henriksen, A., Oligosaccharide and substrate binding in the starch debranching enzyme barley limit dextrinase. *J. Mol. Biol.* (2015). Accepted manuscript.

# Crystal structures of N-acetylhexosamine 1-kinase and UDP-glucose 4-epimerase in the GNB/LNB pathway from infant-gut associated bifidobacteria

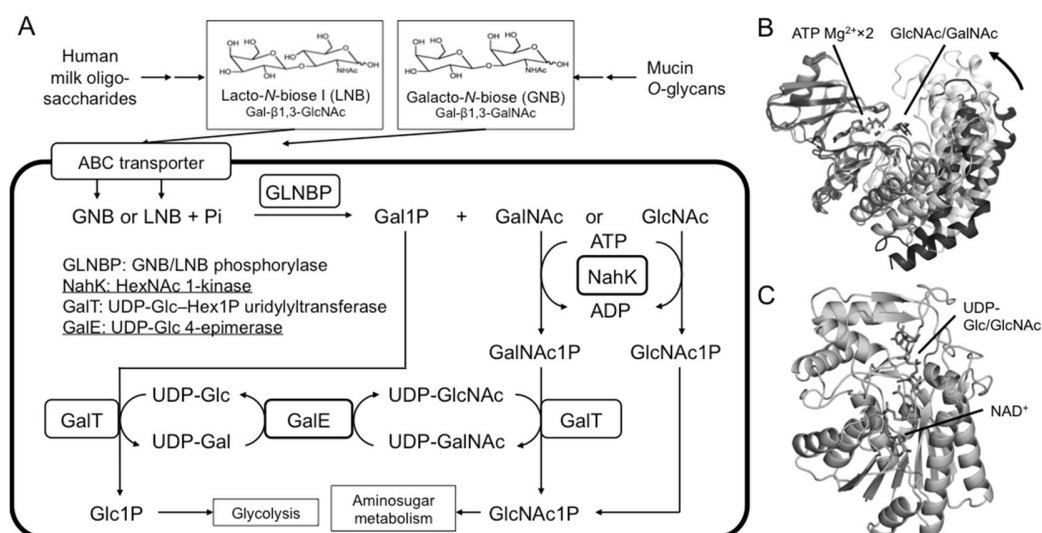
Young-Woo Nam<sup>1</sup>, Mayo Sato<sup>1</sup>, Takatoshi Arakawa<sup>1</sup>, Mamoru Nishimoto<sup>2</sup>, Motomitsu Kitaoka<sup>2</sup> and Shinya Fushinobu<sup>1</sup>

asfushi@mail.ecc.u-tokyo.ac.jp

1. Department of Biotechnology, The University of Tokyo

2. National Food Research Institute, National Agriculture and Food Research Organization

Infant-gut associated Bifidobacteria have a metabolic pathway specific for disaccharides liberated from human milk oligosaccharides (Gal- $\beta$ 1,3-GlcNAc, lacto-*N*-biose I, LNB) and intestinal mucin glycans (Gal- $\beta$ 1,3-GalNAc, galacto-*N*-biose, GNB) (Fig. 1A) [1]. The pathway consists of four intracellular enzymes including *N*-acetylhexosamine 1-kinase (NahK) and UDP-glucose 4-epimerase (GalE) [2]. NahK is an anomeric kinase that can produce various sugar 1-phosphates [3]. GalE has wide substrate specificity and epimerizes both UDP-Glc/Gal and UDP-GlcNAc/GalNAc. We have determined the crystal structures of NahK (Fig. 1B) and GalE (Fig. 1C) from *Bifidobacterium longum* JCM1217. Structural bases for the substrate recognition, catalysis, and ligand-induced movement of these enzymes were revealed.



**Fig 1.** The GNB/LNB pathway (A) and crystal structures of NahK (B) and GalE (C).

## Acknowledgements

This work was supported in part by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

## Literature

1. Kitaoka *et al.* (2005) *Appl. Environ. Microbiol.*, **71**, 3158-3162
2. Nishimoto *et al.* (2007) *Appl. Environ. Microbiol.*, **73**, 6444-6449
3. Liu *et al.* (2015) *Carbohydr. Res.*, **401**, 1-4

## Crystal structure of the GTFB enzyme, the first representative of the 4,6- $\alpha$ -glucanotransferase subfamily within GH70

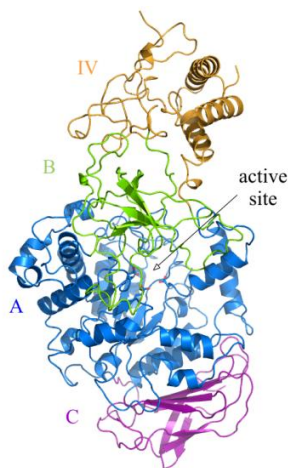
*Tjaard Pijning*<sup>1</sup>, *Yuxiang Bai*<sup>2</sup> and *Lubbert Dijkhuizen*<sup>2</sup>

t.pijning@rug.nl

1. Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
2. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

Within the glycoside hydrolase family GH70, glucansucrases utilize sucrose to synthesize a variety of  $\alpha$ -glucan polymers<sup>1</sup>. Recently a subfamily within GH70 was described<sup>2,3</sup>, containing enzymes highly homologous to glucansucrases but inactive on sucrose. Instead, these enzymes utilize malto-oligosaccharides and starch as glucose donor substrates for  $\alpha$ -glucan synthesis, acting as 4,6- $\alpha$ -glucanotransferases. The linear oligosaccharide products are rich in  $\alpha$ -1,6 glycosidic linkages<sup>4</sup>, and provide an exciting type of carbohydrate for the food industry, acting as prebiotics and providing a soluble fiber.

In this work we determined the 3D atomic structure of GTFB, a GH70 4,6- $\alpha$ -glucanotransferase from *Lactobacillus reuteri* 121, using a construct (GTFB- $\Delta$ N $\Delta$ V) comprising the catalytic domain A as well as domains B, C and IV. The crystal structure of GTFB- $\Delta$ N $\Delta$ V at 1.80 Å (Fig. 1.) allowed us to compare the different specificities within GH70 and to obtain insights in the unique reaction mechanism of 4,6- $\alpha$ -glucanotransferases, which may represent an evolutionary intermediate between the GH13 and GH70 enzyme families.



**Fig 1.** Crystal structure of GTFB- $\Delta$ N $\Delta$ V with the domains and location of the active site indicated.

### Literature

1. Leemhuis et al., J. Biotechnol. 163 (2013), 250-272.
2. Kralj et al., Appl. Environm. Microbiol. 77 (2011), 8154-8163.
3. Leemhuis et al., Appl. Microbiol. Biotechnol. 97 (2012), 181-193.
4. Dobruchowska et al., Glycobiology 22 (2013), 517-528.

# Catalytic mechanism of retaining glycosyltransferases: Is Arg293 on the $\beta$ -face of EXTL2 compatible with it? Insights from QM/MM calculations

Laura Masgrau,<sup>1</sup> María Fernanda Mendoza,<sup>1,2</sup> Hansel Gómez<sup>1,2</sup> and José M. Lluch<sup>1,2</sup>

Laura.Masgrau@uab.cat

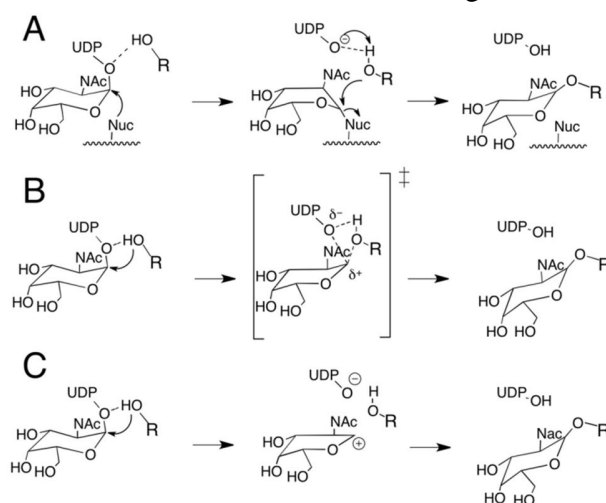
1. Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, Spain

2. Department of Chemistry, Universitat Autònoma de Barcelona, Spain.

The synthesis of pure glycans, and in sufficient quantities, is highly pursued to help the development of glycosciences and related applications. In Nature, glycosyltransferases (GTs) are responsible for their biosynthesis. The catalytic mechanism of GTs, and of retaining GTs in particular, has been under debate for long. In the last years, computational studies have brought light into the discussion. Nevertheless, all the proposed mechanisms involve the formation of oxocarbenium species, either as a short-lived ion-pair intermediate or as a transition state, which hold the development of a positive charge density at the anomeric centre (Fig 1).

In that sense, the active site of retaining  $\alpha$ 1,4-N-acetylhexosaminyltransferase (EXTL2), with a positively charged residue (R293) at close proximity of the anomeric carbon, is puzzling. Does EXTL2 open the door for a new class of mechanism in retaining GTs?

Our goal here has been to evaluate whether the presence of R293 in EXTL2 is compatible with the front-side attack mechanism or whether a different mechanism must be proposed, and to reveal the role of this residue in such position.<sup>1</sup> The results are discussed in the light of what we have learned in the last years about the catalytic mechanism of retaining glycosyltransferases.<sup>2-5</sup>



**Fig 1.** Proposed mechanisms for retaining glycosyltransferases. (A) Double-displacement with formation of a glycosyl-enzyme intermediate, and front-side attack via oxocarbenium (B) transition state or (C) ion pair intermediate.

## Literature

1. Mendoza M.F, Gómez H., Lluch J.M., Masgrau L., *to be submitted*.
2. Gómez H., Polyak I., Thiel W., Lluch J.M., Masgrau L.: *J. Am. Chem. Soc.*, 134, 4743-52 (2012)
3. Gómez H., Lluch J.M., Masgrau L.: *Carbohydr. Res.*, 356, 204-8 (2012)
4. Gómez H., Lluch J.M., Masgrau L.: *J. Am. Chem. Soc.*, 135, 7053-63 (2013)
5. Gómez H., Rojas R., Patel D., Tabak L., Lluch J.M., Masgrau L.: *Org. Biomol. Chem.*, (2014)

## Structure-function studies of enzymes in the oxidative D-galacturonate pathway

*Helena Taberman*<sup>1</sup>, *Martina Andberg*<sup>2</sup>, *Tarja Parkkinen*<sup>1</sup>, *Nina Hakulinen*<sup>1</sup>, *Merja Penttilä*<sup>2</sup>, *Anu Koivula*<sup>2</sup> and *Juha Rouvinen*<sup>1</sup>

helen.taberman@uef.fi

1. University of Eastern Finland, Department of Chemistry, P.O. Box 111, 80101 Joensuu, Finland

2. VTT Technical Research Centre of Finland, P.O. Box 1000, 02044 VTT, Finland

Plant cell wall polysaccharides cellulose, hemicellulose and pectin constitute the major fraction of the lignocellulosic feedstock, and provide the raw material for microbial conversion to fuels and chemicals. Glucose has been the most studied and applied monosaccharide source, but in order to make the biorefining concepts more economically feasible, it is desirable to utilize also the less explored biomass-derived sugars.

Pectin is mainly composed of D-galacturonate, a sugar acid that is used as a carbon and energy source by many bacterial and fungal sources. D-galacturonate has two known catabolic routes in bacteria: the isomerase and the oxidative pathway. The oxidative pathway has been shown to be active in *Agrobacterium tumefaciens* and *Pseudomonas syringae*. In the oxidative pathway in *A. tumefaciens* D-galacturonate is first oxidized by uronate dehydrogenase (*At Udh*) [1] to D-galactaro-1,5-lactone, which is then isomerised to D-galactaro-1,4-lactone either non-enzymatically or by D-galactarolactone isomerase [2]. A novel galactarolactone cycloisomerase (*At Gci*) then catalyses the ring opening into 3-deoxy-2-keto-hexarate [3], which is converted further to  $\alpha$ -ketoglutaric semialdehyde by keto-deoxy-D-galactarate dehydratase (*At KDG*) [4, 5]. Finally,  $\alpha$ -ketoglutaric semialdehyde is oxidized by a dehydrogenase to  $\alpha$ -ketoglutarate, which is a metabolite of the TCA cycle [6].

The structures of *At Udh*, *At Gci* and *At KDG* dehydratase and their complexes have been solved by X-ray crystallography [1, 5, 7]. Structure-function studies are crucial for a comprehensive understanding of the microbial oxidative D-galacturonate pathway and its applications in sustainable chemical production.

**Acknowledgements** The work has been supported by the National Doctoral Programme in Informational and Structural Biology, and the Finnish Centre of Excellence in White Biotechnology-Green Chemistry programme (Academy of Finland decision number 118573).

### Literature

1. Parkkinen, T., Boer, H., Jänis, J., Andberg, M., Penttilä, M., Koivula, A., and Rouvinen, J. (2011) *J. Biol. Chem.* 286, 27294-27300.
2. Bouvier, J. T., Groninger-Poe, F. P., Vetting, M., Almo, S. C., and Gerlt, J. A. (2014) *Biochemistry* 53, 614-616.
3. Andberg, M., Maaheimo, H., Boer, H., Penttilä, M., Koivula, A., and Richard, P. (2012) *J. Biol. Chem.* 287, 17662-17671.
4. Jeffcoat R., Hassal, H., Dagley, S. (1969) *Biochemistry* 115, 977-983.
5. Taberman, H., Andberg, M., Parkkinen, T., Jänis, J., Penttilä, M., Hakulinen, N., Koivula, A., and Rouvinen, J. (2014) *Biochemistry* 53, 8052-8060.
6. Watanabe, S., Yamada, M., Ohtsu, I., and Makino, K. (2007) *J. Biol. Chem.* 282, 6685-6695.
7. Taberman, H., Andberg, M., Parkkinen, T., Richard, P., Hakulinen, N., Koivula, A., and Rouvinen, J. (2014) *Acta Crystallogr. F* 70, 49-52.

## Polysaccharide engineering: towards carbohydrate drugs and drug carriers

Takeshi Takaha<sup>1</sup>, Michiyo Yanase<sup>1</sup>, Akiko Kubo<sup>1</sup>, Ryo Kakutani<sup>1</sup> and Takashi Kuriki<sup>1</sup>

takaha-takeshi@glico.co.jp

1. Institute of Health Sciences, Ezaki Glico Co., Ltd. 4-6-5 Utajima, Nishiyodogawa, Osaka 555-8502, Japan

Bio-macromolecules (e.g. protein, peptide, antibody, DNA or RNA) present in our body is now widely utilized in pharmaceuticals. Carbohydrates, on the other hand, received increasing attention as drug candidates, but carbohydrates used for pharmaceuticals is limited to few glycosaminoglycans (heparin, hyaluronan, chondroitin sulfate).

Ezaki Glico have been working in the field of carbohydrate bioengineering, and aimed to develop new business (products, materials), especially for health and nutrition, from basic findings. These products include several key-enzymes for carbohydrate bioengineering (branching enzyme, amylomaltase, glucan phosphorylases, sucrose phosphorylases and amylases), phosphoryl oligosaccharides of calcium<sup>1)</sup>, cyclic glucans (cycloamylose<sup>2)</sup> and cluster dextrin<sup>3)</sup>), synthetic polysaccharides (amylose and glycogen<sup>4)</sup>) and glycosides<sup>5)</sup>. These materials have been used in food, cosmetic, pharmaceutical, and other industries. We are currently challenging to combine all our resources to develop a versatile platform for carbohydrate drugs and drug carriers.

Glycogen is a predominant polysaccharide in our body with very attractive structure and characteristics. It is a single molecular nano-sized spherical particle with dendritic architecture where numerous non-reducing end glucoses constitute the surface. We have developed enzymatic system to produce artificial glycogen (GD) with strictly controlled particle size. GD are further subjected to non-reducing end specific glycosylation technology where GD surface is modified with various sugar moieties. Surface engineered GD is a novel and versatile platform for carbohydrate drugs and drug carriers.

### Literature

1. Kamasaka, H. *et al. Biosci. Biotechnol. Biochem.* **59**, 1412-1416 (1995)
2. Takaha, T. *et al. J. Biol. Chem.* **271**, 2902-2908 (1996)
3. Takata, H. *et al. J Bacteriol.* **178**, 1600–1606 (1996)
4. Takata, H. *et al. Carbohydr. Res.* **344**, 654-659 (2009)
5. Sugimoto, K. *et al. Biol. Pharm. Bull.* **27**, 510-514 (2004)

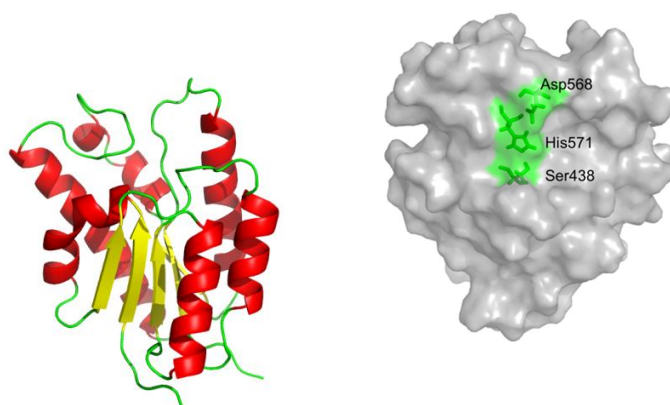
## Structure and mechanism of action of O-acetyltransferase (Oat) A

David Sychantha, Laura Kell and Anthony J. Clarke

a.clarke@exec.uoguelph.ca

Department of Molecular & Cellular Biology, University of Guelph, Guelph, Ontario, Canada

Variations in the chemical structure of peptidoglycan (PG) contribute to resistance to the action of both the innate immune system of host organisms and antibiotics. For example, PG O-acetyltransferase (Oat) A is responsible for the O-acetylation of the C-6 hydroxyl group of N-acetylmuramoyl residues in the PG of Gram-positive bacteria and deletion of the *oatA* (*adr*) gene decreases the inherent minimum inhibitory concentration of penicillin required to kill *Streptococcus pneumoniae* and increases sensitivity of both this human pathogen and *Staphylococcus aureus* to the lysozymes of host immune systems. OatA is predicted to be a bi-modular protein that contains an N-terminal transmembrane domain and a C-terminal extracellular catalytic domain. We have cloned *oatA* from both *S. pneumoniae* and *S. aureus* coding for its C-terminal catalytic domain (OatA<sub>c</sub>) in frame with an N-terminal His<sub>6</sub>-tag. Expression conditions were established for the overproduction of large quantities of soluble proteins which have been purified to apparent homogeneity by a combination of affinity and ion-exchange chromatographies. Both enzymes were demonstrated to function as O-acetyltransferases using the pseudosubstrate acetyl-donor *p*-nitrophenylacetate and chitooligosaccharide acceptors. Kinetic analyses indicated the transferases have specificities for polysaccharides of increasing length and ESI-MS/MS analyses of reaction products suggests they prefer to modify terminal non-reducing residues. The three-dimensional structure of OatA<sub>c</sub> has been solved to 1.1 Å resolution and it is found to have similarity to the SGNH superfamily of hydrolases adopting an  $\alpha/\beta$  hydrolase-like fold. The invariant Asp568, His571 and Ser438 residues are aligned in a shallow pocket on the protein's surface and their respective replacement with Ala confirmed their participation in the catalytic mechanism of the enzyme. Thus, like the PatB paralog of Gram-negative bacteria, OatA is proposed to use a double-displacement mechanism of action similar to that of the serine esterase superfamily of enzymes; however, in the acetyltransfer mechanism water is excluded from the active site and replaced with the C-6 hydroxyl group of the acceptor saccharide residue in PG. Our elucidation of the catalytic pathway of OatA involving a catalytic triad of Ser, His and Asp residues provides valuable insight for the search for, and development of, inhibitors that may serve as leads for the generation of new classes of antibiotics.



**Fig 1.** Structure of *S. pneumoniae* OatA



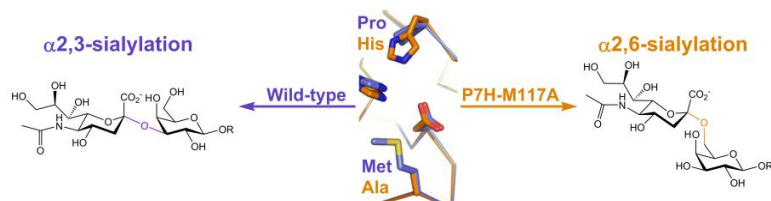
## Complete switch from $\alpha$ 2,3- to $\alpha$ 2,6-regioselectivity in *Pasteurella dagmatis* $\beta$ -D-galactoside sialyltransferase by active-site redesign

Katharina Schmölzer,<sup>1</sup> Tibor Czabany,<sup>2</sup> Christiane Luley-Goedl,<sup>1</sup> Tea Pavkov-Keller,<sup>1</sup> Doris Ribitsch,<sup>1</sup> Helmut Schwab,<sup>3</sup> Karl Gruber,<sup>4</sup> Hansjörg Weber<sup>5</sup> and Bernd Nidetzky<sup>1,2</sup>

katharina.schmoelzer@acib.at

1. Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria.
2. Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12/I, 8010 Graz, Austria.
3. Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria.
4. Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50, 8010 Graz, Austria.
5. Institute of Organic Chemistry, Graz University of Technology, Stremayergasse 9, 8010 Graz, Austria.

$\alpha$ 2,3- and  $\alpha$ 2,6-sialic acid capped oligosaccharides are of high importance for human glycobiology. Currently there is great interest in synthetically generated sialylated human milk oligosaccharides (HMOs), in sialyllactose in particular, as commercial food ingredients with a health promoting effect. Stereo- and regiocontrol are critical problems needing special attention during sialoside synthesis. For selective biocatalytic sialylation sialyltransferases are very useful catalysts that offer high regioselectivity. We present for the first time a structure-guided active-site redesign of a family GT-80  $\beta$ -D-galactoside sialyltransferase (from *Pasteurella dagmatis*)<sup>1,2</sup> to achieve complete switch in enzyme regioselectivity from  $\alpha$ 2,3 in wild type to  $\alpha$ 2,6 in a designed P7H-M117A double mutant.<sup>3</sup> Biochemical data for sialylation of lactose and high-resolution protein crystal structures demonstrate a highly precise active-site enzyme engineering. We show the application of this unique pair of regio-complementary sialyltransferases for the synthesis of  $\alpha$ 2,3/ $\alpha$ 2,6-sialyllactose and  $\alpha$ 2,3/ $\alpha$ 2,6-sialyl-*N*-acetylactosamine. Alternative 3'- or 6'-sialylation of protein asialo-*N*-glycans will also be demonstrated. In this way valuable insight into structure-function relationships of family GT-80 sialyltransferases was obtained.



**Fig 1.** Structurally-guided design of a P7H-M117A double mutant of *P. dagmatis* wild-type  $\alpha$ 2,3-sialyltransferase resulted in a completely regioselective and highly efficient  $\alpha$ 2,6-sialyltransferase.

### Literature

1. K. Schmölzer, D. Ribitsch, T. Czabany, C. Luley-Goedl, D. Kokot, A. Lyskowski, S. Zitzenbacher, H. Schwab, B. Nidetzky, *Glycobiology* **2013**, 23, 1293-1304.
2. K. Schmölzer, C. Luley-Goedl, T. Czabany, D. Ribitsch, H. Schwab, H. Weber, B. Nidetzky, *FEBS Lett.* **2014**, 588, 2978-2984.
3. K. Schmölzer, T. Czabany, C. Luley-Goedl, Tea Pavkov-Keller, D. Ribitsch, H. Schwab, K. Gruber, H. Weber, B. Nidetzky, *Chem. Commun.* **2015**, DOI: 10.1039/c4cc09772f.



## Structure and function in the GH53 $\beta$ -1,4-galactanase family

Søs Torpenholt<sup>1,2</sup>, Leonardo De Maria<sup>2,3</sup>, Jens-Christian N. Poulsen<sup>1</sup>, Mats H. M. Olsson<sup>1</sup>, Lars H. Christensen<sup>2</sup>, Michael Skjød<sup>2,3</sup>, Peter Westh<sup>4</sup>, Jan H. Jensen<sup>1</sup> and Leila Lo Leggio<sup>1</sup>

leila@chem.ku.dk

1. Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark
2. Novozymes A/S, Smørmosevej 25, 2880 Bagsværd, Denmark.
3. Novo Nordisk A/S, Novo Nordisk Park, 2760 Måløv Denmark
4. NSM, Research Unit for Functional Biomaterials, University of Roskilde, Universitetsvej 1, 4000 Roskilde, Denmark

$\beta$ -1,4-galactanases are found in prokaryotic, eukaryotic and archaeal microorganisms, where they are thought to be involved in digestion of galactan side chains of plant pectins. They are found exclusively in the GH53 CAZY family. We have over the years determined a number of structures and studied in detail the structure-function relationships in the family. In this presentation we focus in particular on three aspects, by discussing both own unpublished results and information already available in the literature.

- 1) Substrate specificity: GH53 enzymes belonging to different domains of life show different substrate preferences and different transglycosylation abilities (1-3), which will be discussed in light of structures obtained in complex with oligosaccharides (1,4).
- 2) pH dependence of activity and stability: structures of galactanases with different pH optima are known (5) and efforts to computationally predict and understand the different pH dependences in terms of structure will be illustrated, including data on a variant with shifted pH optimum.
- 3) Stability and thermostability: structural features important for the stability of galactanases will be illustrated by comparison of structures of GH53 enzymes with different stability profiles (5), as well as mutagenesis studies aimed at the generation of thermostable variants (6-7).

### Acknowledgements

We acknowledge gratefully the participation of all coauthors to our publications in this project over the years, and access to MAXLAB and ESRF for synchrotron data collection.

### Literature

1. Ryttersgaard C, Le Nours J, Lo Leggio L, Jørgensen CT et al, Christensen LLH, Bjørnvad M, Larsen S. *J. Mol. Biol.* 2004; 341:107-117.
2. Torpenholt S, Le Nours J, Christensen U, Jahn M, Withers S, Østergaard PR, Borchert TV, Poulsen JC and Lo Leggio L. *Carb. Res.* 2011; 346:2028-2033.
3. Tabachnikov O, Shoham Y. *FEBS J.* 2013;280:950-964.
4. Le Nours J, De Maria L, Welner D, Jørgensen CT, Christensen LLH, Borchert TV, Larsen S and Lo Leggio L *Proteins* 2009;75 :977-989.
5. Le Nours J, Ryttersgaard C, Lo Leggio L, Østergaard PR, Borchert TV, Christensen LLH, Larsen S. *Protein Science* 2003; 12:1195-1204.
6. Larsen DM, Nyffenegger, Swiniarska MM, Thygesen A, Strube ML, Meyer AS, Mikkelsen JD. *Appl. Microbial. Biotechn.* 2014; on line
7. Torpenholt S, De Maria L, Olsson MHM, Christensen LH, Skjød M, Westh P, Jensen JH and Lo Leggio L submitted

## Determinants of substrate specificity in chitin oligosaccharide deacetylases: how loops define the de-*N*-acetylation pattern

Xevi Biarnés<sup>1</sup>, Hugo Aragunde<sup>1</sup>, David Albesa-Jové<sup>2</sup>, Marcelo E. Guerin<sup>2</sup>, and Antoni Planas<sup>1</sup>

antoni.planas@iqs.edu

1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull. 08017 Barcelona, Spain.
2. Unidad de Biofísica, Centro Mixto Consejo Superior de Investigaciones Científicas-Universidad del País Vasco/Euskal Herriko Unibertsitatea, 48940 Bizkaia, Spain

Chitin processing, mainly in the form of depolymerization and de-*N*-acetylation reactions, generates a series of derivatives including chitosan and chitooligosaccharides (COSs), which play remarkable roles in nature. COSs are particularly involved in molecular recognition events, including the modulation of cell signaling and morphogenesis, the immune response, and host-pathogen interactions. Most of the biological activities associated with COSs seem to be largely dependent on the degree of polymerization and the specific acetylation pattern, which define the charge density and the distribution of GlcNAc and GlcNH<sub>2</sub> moieties in chitosan and COS.

Chitin de-*N*-acetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and COS. The deacetylation pattern exhibited by CDAs and related CE4 enzymes active on COS is diverse, some being specific for a single position, others showing multiple attack. *A major challenge is to understand how CDAs specifically define the distribution of GlcNAc and GlcNH<sub>2</sub> moieties in the oligomeric chain.*

To address this question, we here report a structural/functional analysis of CDAs which includes:

- a) The crystal structure of the *Vibrio cholerae* chitin oligosaccharide deacetylase (VcCDA or VcCOD) in four relevant states of its catalytic cycle [1]. Two enzyme complexes with chitobiose and chitotriose represent the first 3D structures of a CDA with its natural substrates in a productive mode for catalysis, thereby unraveling an induced-fit mechanism with a significant conformational change of a loop closing the active site.
- b) Based on these novel structures and structural analyses of CE4 enzymes active on COS, we propose a “Subsite Capping Model” in which the deacetylation pattern exhibited by different CDAs is governed by critical dynamic loops that shape and differentially block accessible subsites in the binding cleft of CE4 enzymes. The model provides the bases for the rational design of CDA enzymes.
- c) Engineered variants to introduce novel deacetylation specificities.

This work is supported by EU grants ChitoBioEngineering and Nano3Bio aimed at the biotechnological production of novel chitosan oligo- and polysaccharides for industrial and medical applications. (<http://www.nano3bio.eu>)

### Literature

1. Andrés E., Albesa-Jové D., Biarnés X., Moerschbacher B.M., Guerin M.E., Planas A. Structural basis of chitin oligosaccharide deacetylation. *Angew. Chem. Int. Ed. Engl.* 53, 6882-6887 (2014).

## Molecular basis for the epimerization of oligosaccharides by cellobiose 2-epimerase

Wataru Saburi<sup>1</sup>, Takaaki Fujiwara<sup>2</sup>, Nongluck Jaito<sup>1</sup>, Hirohiko Muto<sup>1</sup>, Hirokazu Matsui<sup>1</sup>, Min Yao<sup>2</sup>, and Haruhide Mori<sup>1</sup>

saburiw@chem.agr.hokudai.ac.jp

1. Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

2. Faculty of Advanced Life Science, Hokkaido University, Sapporo, Japan

Cellobiose 2-epimerase (CE), which has been first found in a ruminal bacterium, *Ruminococcus albus*, catalyzes the epimerization of 2-OH group of reducing end sugar residue of  $\beta$ 1-4 linked oligosaccharides including cellobiose, lactose, and  $\beta$ 1-4 mannoside. Functional analysis of CE-like proteins revealed that CE is distributed in various bacteria including *Bacteroides fragilis* and *Rhodothermus marinus*. In the genome of *B. fragilis*, the CE gene is a member of a gene cluster including the genes encoding  $\beta$ -mannanase and 4-*O*- $\beta$ -D-mannosyl-D-glucose phosphorylase. Thus CE is predicted to convert  $\beta$ 1-4 mannoside to 4-*O*- $\beta$ -D-mannosyl-D-glucose for further phosphorolysis in the metabolism of  $\beta$ -mannan. As epilactose (4-*O*- $\beta$ -D-galactosyl-D-mannose), produced from lactose by CE, has a prebiotic property, CE is an attractive enzyme for production of a functional foodstuff.

In this study, we have determined the crystal structures of CEs from *R. albus* [1] and *R. marinus* [2]. In *R. marinus* CE (*RmCE*), structures in complex with 4-*O*- $\beta$ -D-glucosyl-D-mannose, epilactose, and cellobiitol (a competitive inhibitor,  $K_i$ , 6.13 mM) were determined. The overall folds of these CEs were  $(\alpha/\alpha)_6$  barrel, similar to those of *N*-acetylglucosamine 2-epimerase (AGE) and aldose ketose isomerase (AKI). In the CEs, two catalytic His residues and amino acid residues surrounding the reducing end sugar part are situated at the positions corresponding to those of AGE and AKI, suggesting that these enzymes share a catalytic mechanism. In the complex of *RmCE* and cellobiitol, cellobiitol took *cis*-enediol like conformation, and C1-C2 bond of the D-glucitol part of cellobiitol was positioned between catalytic His259 and His390. The H2 proton of the D-glucitol part was close to His390, and His390 is the most feasible candidate to abstract the H2 proton from D-glucose residue at the reducing end of substrate. His259 acts as a general acid catalyst to donate a proton to the C2 position of *cis*-enediol intermediate, and the D-glucose residue is converted to D-mannose residue. In contrast to AGE and AKI, which act on monosaccharide substrates, CE has very low activity to a monosaccharide. The  $k_{cat}/K_m$  of *RmCE* for D-mannose was 3,670-fold lower than that of  $\beta$ 1-4 mannoside. Structural analysis showed that Trp385 and Asp188 of *RmCE* have stacking and hydrogen bonding interactions with sugar part at the non-reducing end of substrate, respectively. W385A and D188A mutations more severely decreased activity to disaccharide substrates than to D-mannose, and thus these residues contribute to the disaccharide-selectivity of CE.

### Acknowledgements

Part of this work was supported by JSPS KAKENHI with a Grants-in-Aid for Young Scientists (B) (Grant No. 26850059).

### Literature

1. Fujiwara, T., Saburi, W., et al., *FEBS Lett.*, **587**, 840-846 (2013)
2. Fujiwara, T., Saburi, W., et al., *J. Biol. Chem.*, **289**, 3405-3415 (2014)

## Sugar oxidoreductions at the crossroads of mechanistic enzymology and biotechnological application

Bernd Nidetzky<sup>1,2</sup>

bernd.nidetzky@tugraz.at

1. Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Graz, Austria

2. Austrian Centre of Industrial Biotechnology, Graz, Austria

Simple (single-step) and complex oxidoreductive transformations are widely used in biology to assimilate sugars into cellular metabolism and to generate building blocks for biosynthesis. In biotechnology sugar oxidoreductases have important applications in synthetic bioconversions via catalytic cascade or whole-cell systems. The example of yeast xylose fermentation is used to demonstrate structure- and mechanism-based engineering of sugar oxidoreductases for integration into a recombinant metabolic pathway for xylose-to-ethanol conversion optimized for product yield and fluxional efficiency.<sup>1,2</sup> Enzymes of the biosynthesis of UDP-glucuronic acid,<sup>3</sup> UDP-xylose<sup>4,5</sup> and UDP-apiiose are shown to catalyze multi-step oxidoreductive reactions at their respective active site. Principles used in catalysis are described based on evidence from protein structures and mechanistic studies.

### Literature

1. Petschacher B., Nidetzky B. (2008) *Microb. Cell Fact.* 7, 9
2. Novy V. et al. (2014) *Biotechnol. Biofuels* 7, 49
3. Egger S. et al. (2011) *J. Biol. Chem.* 286, 23877
4. Eixelsberger T. et al. (2012) *J. Biol. Chem.* 287, 31349
5. Eixelsberger T., Nidetzky, B. (2014) *Adv. Synth. Catal.* 356, 3575

## Functional characterization of a set of fungal lytic polysaccharide monooxygenase secreted by *Podospira anserina*

Chloé Bennati-Granier<sup>1,2</sup>, Sona Garajova<sup>1,2</sup>, Charlotte Champion<sup>1,2</sup>, Sacha Grisel<sup>1,2</sup>, Mireille Haon<sup>1,2</sup>, Hélène Rogniaux<sup>3</sup>, Isabelle Gimbert<sup>1,2</sup>, Eric Record<sup>1,2</sup>, Jean-Guy Berrin<sup>1,2</sup>

jean-guy.berrin@univ-amu.fr

1. INRA, UMR1163 BBF, F-13288 Marseille, France

2. Aix Marseille Université, Polytech Marseille, F-13288 Marseille, France

3. INRA, Plateforme BIBS, Unité de Recherche Biopolymères, Interactions, Assemblages, 44316 Nantes, France.

Lignocellulosic biomass is the largest renewable source of carbohydrates for the production of biofuels, biomaterials, and high-value products. The main component of plant cell wall is cellulose, a linear polymer of  $\beta$ -1-4 linked glucose units arranged in linear microfibrils that form very recalcitrant crystalline-like structures. The understanding of enzymatic polysaccharide degradation has progressed intensely in the past few years with the identification of a new class of fungal secreted enzymes, the lytic polysaccharide monooxygenases (LPMOs) that enhance cellulose conversion. The family AA9 comprises about 300 fungal members widely distributed in the genomes of most ascomycetes and basidiomycetes. A striking feature is the extreme expansion in genes encoding AA9s observed in the biomass degrader *Podospira anserina* (i.e. 33 genes) that represents an interesting model to study the oxidative deconstruction of lignocellulose. In this study, we investigated a set of AA9 LPMOs identified in the secretomes of *P. anserina* (Poidevin et al., 2014). Six AA9 LPMOs were expressed heterologously using the methylotrophic yeast *Pichia pastoris*. LPMO activity was assayed on cellulose in synergy with the cellobiose dehydrogenase from the same organism. We showed that the total release of non-oxidized and oxidized oligosaccharides from cellulose was promoted by the presence of a carbohydrate-binding module from the family 1. The investigation of their regioselective mode of action using ionic chromatography and mass spectrometry revealed differences in the position of the oxidative cleavage of cellulose (at the C1 and/or C4 position). This study provides novel insights into the mode of cleavage and substrate specificities of fungal AA9 LPMOs that will facilitate their application for the development of future biorefineries.

### Acknowledgements

This study was carried out in the frame of Futurol and Funcopper projects with financial support from OSEO and the AMIDEX foundation.

### Literature

Poidevin L, Berrin JG, Bennati-Granier C, Levasseur A, Herpoël-Gimbert I, Chevret D, Coutinho PM, Henrissat B, Heiss-Blanquet S, Record E. Comparative analyses of *Podospira anserina* secretomes reveal a large array of lignocellulose-active enzymes. Appl Microbiol Biotechnol. 2014, 98(17):7457-69.

## **Glucooligosaccharide oxidases: determinants of activity and use in carbohydrate modification**

Maryam Foumani<sup>1</sup>, Thu Vuong<sup>1</sup>, Benjamin MacCormick<sup>1</sup>, and Emma R. Master<sup>1</sup>

emma.master@utoronto.ca

1. Department of Chemical Engineering and Applied Chemistry, University of Toronto  
200 College Street, Toronto, Ontario, Canada M5S 3E5

Enzymatic oxidation of polysaccharides can alter the rheology of corresponding polymers and facilitate regioselective modification of complex carbohydrates. Moreover, since enzyme catalyzed reactions can be performed in relatively mild reaction conditions, enzymatically modified polysaccharides generally retain the degree of polymerization and crystallinity of the starting substrate. This presentation will describe our characterization and application of a glucooligosaccharide oxidase from *Sarocladium strictum* (namely, GOOX). GOOX is classified by the CAZy database as a family 7 auxiliary activity, and like other flavin carbohydrate oxidases, targets the hydroxyl group of the anomeric carbon. The comparatively accessible substrate binding site of GOOX enables oxidation of oligomeric and polymeric substrates, while activity on monosaccharides is comparatively low. Catalytic efficiencies on cello-oligosaccharides were similar to those measured using xylo-oligosaccharides with or without arabinose substituents at positions C3 and/or C2 of the non-reducing xylose. By contrast, the presence of uronic acid substituents decreased the catalytic efficiency of GOOX, largely through increasing  $K_m$  values. Mutagenesis studies have identified substrate binding subsites near the substrate binding pocket of GOOX similar to paradigms observed for many glycoside hydrolases; eight additional amino acid substitutions were introduced in an effort to identify determinants of substrate selectivity. Most notably, the W351A substitution at a substrate binding subsite increased  $k_{cat}$  values up to 3-fold on cello- and xylo-oligosaccharides while alleviating substrate inhibition. In addition to oxidizing oligosaccharides, GOOX activity was confirmed using a range of plant polysaccharides. Inspired by the precedence to enhance cellulase performance on insoluble polysaccharides through fusion to cellulose-binding modules, we investigated the impact of carbohydrate-binding modules from different CBM families, on GOOX activity towards high molecular weight and insoluble polysaccharides. In line with trends observed for hydrolytic enzymes, greatest benefits of CBM fusion were observed when using low concentrations of insoluble polysaccharides. Finally, GOOX alone and in combination with other carbohydrate oxidases is being developed for applications in biosensing and valorization of hemicellulose. Recent results towards these ends will be described.

### **Acknowledgements**

Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), the MITACS Elevate program, as well as the Government of Ontario for the project "Forest FAB: Applied Genomics for Functionalized Fibre and Biochemicals" (ORF-RE-05- 005)

## Engineering of pyranose oxidoreductases for bio-fuelcell applications

*Clemens Peterbauer, Dagmar Brugger, Iris Krondorfer, Christoph Gonaus, Leonard Stoica and Dietmar Haltrich*

clemens.peterbauer@boku.ac.at

Food Biotechnology Lab, Department of Food Sciences and Technology, University of Natural Resources and Life Sciences Vienna

Pyranose dehydrogenase (PDH) and pyranose 2-oxidase (POx) are flavoproteins that both catalyze the oxidation of free, non-phosphorylated sugars to the corresponding ketosugars. Pyranose dehydrogenase is limited to a narrow range of electron acceptors and reacts extremely slowly with dioxygen, whereas pyranose 2-oxidase displays high oxidase as well as dehydrogenase activity. For bio-fuelcells, oxygen reactivity is undesirable as it leads to electron "leakage" and the formation of damaging hydrogen peroxide; for biocatalytic applications, oxygen reactivity can be advantageous, as oxygen is ubiquitous and freely available. Site-saturation mutagenesis libraries of twelve (PDH) and eleven (POx) amino acids around the active sites were expressed and screened for oxidase and dehydrogenase activities. Only one PDH variant displayed increased oxygen reactivity to a minor degree. Histidine 103, carrying the covalently attached FAD cofactor, was substituted by tyrosine, phenylalanine, tryptophan and methionine. Variant H103Y displayed a five-fold increase of oxygen reactivity. Stopped flow analysis revealed that the mutation slowed down the reductive half-reaction whereas the oxidative half-reaction was affected to a minor degree. No alterations in the secondary structure were observed. Disruption of the FAD bond also had negative effects on thermal and conformational stability. In POx, variants T166R, Q448H, L545C, L547R and N593C displayed significantly reduced oxidase activities (between 40% and 0.2% of the wildtype) concomitant with unaffected or even increased dehydrogenase activity, dependent on the electron acceptor used (DCPIP, 1,4-benzoquinone or ferricenium ion). Kinetic characterization showed that both affinity and turnover numbers can be affected. The switch from oxidase to dehydrogenase activity was also observed electrochemically, and the effect of the mutations was rationalized based on structural properties. Additionally, we engineered PDH by removing several of the enzyme's N-glycosylation sites, in order to improve electron transfer by reducing the distance of the active site to the electron surface and facilitate denser enzyme packing. We found improved properties including a low level of Direct Electron Transfer with a variant lacking two glycosylation sites close to the active site, with only small negative effects on the variant's expressability and stability, and determined that the bulk of the overglycosylation of the recombinantly expressed enzyme is located on only one glycosylation site.

### Literature

1. Brugger D, Krondorfer I, Shelswell C, Huber-Dittes B, Haltrich D, Peterbauer CK (2014) PLoS ONE 9, 0109242
2. Krondorfer I, Brugger D, Paukner R, Scheiblbrandtner S, Pirker KF, Hofbauer S, Furtmueller PG, Obinger C, Haltrich D, Peterbauer CK (2014) Arch Biochem Biophys 558, 111-119
3. Krondorfer I, Lipp K, Brugger D, Staudigl P, Sygmond C, Haltrich D, Peterbauer CK (2014) PLoS ONE 9, e91145

## The role of carbon starvation in the induction of enzymes that degrade plant-derived carbohydrates in *Aspergillus niger*

*Jolanda van Munster*<sup>1</sup>, *Paul Daly*<sup>1</sup>, *Stephane Delmas*<sup>1</sup>, *Steven Pullan*<sup>1</sup>, *Martin Blythe*<sup>2</sup>, *Sunir Malla*<sup>2</sup>, *Matthew Kokolski*<sup>1</sup>, *Xiaolan Yu*<sup>3</sup>, *Paul Dupree*<sup>3</sup>, *David Archer*<sup>1</sup>

Jolanda.van\_Munster@nottingham.ac.uk

1. School of Life Sciences, University of Nottingham, Nottingham, United Kingdom;
2. Deep Seq, Faculty of Medicine and Health Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom;
3. Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

The saccharification of lignocellulosic biomass for the production of second generation biofuels requires cheaper and more effective enzyme mixtures. Fungi are an important source of such enzymes, but the understanding of the regulation and induction of the encoding genes is still incomplete. To explore the induction mechanism, we analysed the response of the industrially important fungus *Aspergillus niger* to wheat straw, with a focus on events occurring shortly after exposure to the substrate. RNA sequencing showed that the complexity of transcriptional response increased over time.

Importantly, the influence of carbon starvation during lignocellulose degradation was demonstrated by a substantial overlap in CAZyme-encoding transcripts induced during both early carbon starvation and early exposure to straw. The up-regulation of the expression of a high number of genes encoding CAZymes that are active on plant-derived carbohydrates during early carbon starvation suggests that these enzymes could be involved in a scouting role during starvation, releasing inducing sugars from complex plant polysaccharides. We show that carbon-starved cultures indeed release CAZymes with predicted activity on plant polysaccharides. Analysis of the enzymatic activity and the reaction products, indicates that these proteins are enzymes that can degrade various plant polysaccharides to generate both known, as well as potentially new, inducers of CAZymes<sup>1</sup>

### Literature

- 1.van Munster, Daly *et al*, (2014) Fungal Genet. Biol. 72; 34-47.



## **Esterases of *Myceliophthora thermophila* C1 help in the degradation and modification of lignocellulosic material**

Laura Leonov<sup>1</sup>, Gabriela Bahrim<sup>2</sup>, Henk Schols<sup>3</sup>, Sanna Koutaniemi<sup>4</sup>, Maija Tenkanen<sup>4</sup>, Jaap Visser<sup>1</sup>, Sandra Hinz<sup>1</sup>

shinz@dyadic.nl

1. Dyadic Nederland BV, Wageningen, The Netherlands
2. Faculty of Food Science and Engineering, “Dunarea de Jos” University of Galati, Galati, Romania
3. Wageningen University, Laboratory of Food Chemistry, Wageningen, The Netherlands
4. University of Helsinki, Department of Food and Environmental Chemistry, Helsinki, Finland

Lignocellulosic biomass is the most abundant renewable resource on Earth. Its value is based on the carbohydrate polymers that are present in plant cell walls. Here, depending on the type of plant, one finds a cellulose-hemicellulose network embedded in a matrix of pectic or lignin substances. The strength of the network is improved by ester bonds of cinnamic acids such as ferulic acid that cross-link hemicelluloses with lignin or with pectin, hindering the enzymatic degradation of the whole system. Besides ferulic acid esters, also acetic acid esters and glucuronic acid esters can be present in hemicelluloses and crosslink with other cell wall components (glucuronic acid). In the degradation of lignocellulosic materials, a key step in opening up this tight structure is the removal of the esters.

Dyadic develops the filamentous fungus *Myceliophthora thermophila* C1 as a proprietary protein production platform for the efficient production of tailor-made enzyme mixtures for the degradation or modification of lignocellulosic biomass. This fungal strain encodes, along with many cellulases and hemicellulases, various accessory enzymes including eight feruloyl, thirteen acetyl and two glucuronoyl esterases. Five of the feruloyl esterases, six of the acetyl esterases (classified as CE1, CE3, CE4, CE5 and CE16) and two glucuronoyl esterases (CE15) found in the C1 genome have been produced individually in a dedicated C1 host strain, and subsequently purified and characterized. Results show that they all have differences in their mode of action and substrate specificities, even if they belong to the same enzyme family. These differences in mode of action and substrate specificity form the basis for the use of these enzymes in a variety of applications, for instance in processes to make bio-based fuels and chemicals processes.

## Processive action of *Rasamsonia emersonii* cellobiohydrolase Cel7A

Anu Koivula<sup>1</sup>, Jenni Rahikainen<sup>1</sup>, Akihiko Nakamura<sup>2</sup>, Taku Uchiyama<sup>2</sup>, Takayaki Uchihashi<sup>3,4,5</sup>, Terhi Puranen<sup>6</sup>, Kristiina Kruus<sup>1</sup>, Toshio Ando<sup>3,4,5</sup> and Kiyohiko Igarashi<sup>2</sup>

anu.koivula@vtt.fi

1. VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, FI-02044 VTT, Finland
2. Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
3. Department of Physics, Kanazawa University, Kanazawa 920-1192, Japan
4. Bio-AFM Frontier Research Center, College of Science and Engineering, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan
5. Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Sanboncho, Chiyoda-ku, Tokyo 102-0075, Japan
6. ROAL Oy, Tykkimäentie 15, 05200 Rajamäki, Finland

*Rasamsonia emersonii* cellobiohydrolase *ReCel7A*, which was formerly called *Talaromyces emersonii* Cel7A, is a thermostable cellulase that occurs naturally without a carbohydrate-binding module (CBM). The three-dimensional structure of the enzyme has been solved (Grassick et al., 2004). Due to its thermostability (Voutilainen et al., 2010; Voutilainen et al., 2013) and good lignin-tolerance (Rahikainen et al., 2013), *ReCel7A* is a highly relevant enzyme for total hydrolysis applications for the biofuels industry.

In this study, *ReCel7A* enzyme with and without a family-1 CBM was studied using various crystalline and amorphous cellulosic substrates. In addition, the action of *ReCel7A* was monitored with the high-speed atomic force microscopy (HS-AFM) that allows real-time observation of cellobiohydrolase movement on crystalline cellulose surfaces (Igarashi et al., 2011). The paper discusses the processivity, as well as the differences in mode of action between the widely studied *Trichoderma reesei* Cel7A and *ReCel7A*.

### Acknowledgements

This work was supported by the Academy of Finland mobility grant (J.Rahikainen).

### Literature

1. Grassick, A., Murray, P.G., Thompson, R., Collins, C.M., Byrnes, L., Birrane, G., Higgins, T.M. and Tuohy, M.G. (2004) Eur. J. Biochem., 271, 4495–4506.
2. Voutilainen, S., Murray, P., Tuohy, M. and Koivula, A. (2010) Expression of *Talaromyces emersonii* cellobiohydrolase Cel7A in *Saccharomyces cerevisiae* and rational mutagenesis to improve its thermostability and activity. PEDS, 23, 69–79.
3. Voutilainen S.P., Nurmi-Rantala, S., Penttilä M., and Koivula A. (2013) Engineering chimeric thermostable GH7 cellobiohydrolases in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 98, 2991–3001.
4. Rahikainen, J. L., Moilanen, U., Nurmi-Rantala, S., Lappas, A., Koivula, A., Viikari, L., & Kruus, K. (2013). Effect of temperature on lignin-derived inhibition studied with three structurally different cellobiohydrolases. Bioresource Technology, 146, 118–25.
5. Igarashi K., Uchihashi, T., Koivula A., Wada, M., Kimura, S., Okamoto, T., Penttilä M., Ando, T. and Samejima, M. (2011) Traffic jams reduce hydrolytic efficiency of cellulase on cellulose surface. Science, 33, 1279–1282.

## Hydrolysis of arabinoxylo-oligosaccharides and wheat flour arabinoxylan by $\alpha$ -L-arabinofuranosidases

Barry McCleary<sup>1</sup>, Vincent McKie<sup>1</sup> and Jennifer Larkin<sup>1</sup>

barry@megazyme.com

1. Megazyme International Ireland, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.

Arabinofuranosidases which hydrolyse terminal  $\alpha$ -L-arabinofuranose from polymeric arabinoxylans are termed arabinoxylan arabinofuranosidases (AXH) and these have been divided into two groups depending on their substrate specificities. The enzyme AXH-m acts on  $\alpha$ -L-(1-2)- and (1-3)-linked Araf units on monosubstituted Xylp residues<sup>1</sup>, whereas AXH-d3 releases only  $\alpha$ -L-(1-3)-linked Araf units from disubstituted Xylp residues<sup>2</sup> within arabinoxylan. Relatively little is known on the action of specific  $\alpha$ -L-arabinofuranosidase on defined oligosaccharides. In one recent study, Borsenberger *et al.*<sup>3</sup> reported on the action of several  $\alpha$ -L-arabinofuranosidases from *B. adolescentis* and *Thermobacillus xylanilyticus* on A<sup>2+3</sup>XX and three synthetic di-arabinofuranosylated substrates and demonstrated that these substrates can be used to characterize and probe selectivity of the arabinoxylan-active  $\alpha$ -L-arabinofuranosidases. Work on specific native AXOS has been limited by the lack of availability of these substrates. In this paper we report on methods for the preparation of specific AXOS, with both doubly substituted D-Xylp residues and with singly substituted D-Xylp residues (1,3- and 1,2-linked and either terminal or within the 1,4- $\beta$ -D-xylo-oligosaccharide backbone), including A<sup>3</sup>X, A<sup>2</sup>XX, A<sup>3</sup>XX, XA<sup>3</sup>XX, XA<sup>2</sup>XX and A<sup>2+3</sup>XX. Relative rates of hydrolysis of these oligosaccharides by several  $\alpha$ -L-arabinofuranosidases are shown in Table 1. Clearly there are differences in the rates of hydrolysis of AXOS by the different enzymes, even differences between the AXH-m. These oligosaccharides should prove valuable in defining the substrate binding requirements and specificities of the wide spectrum of  $\alpha$ -L-arabinofuranosidases under investigation.

**Table 1.** Relative rates of hydrolysis of arabinoxylo-oligosaccharides by  $\alpha$ -L-arabinofuranosidase enzymes.

Substrate	<i>B. adolescentis</i> Q5JB56 (GH43)	<i>B. ovatus</i> BACOVA_03417 A7LZZ1 (GH43)	<i>B. ovatus</i> BACOVA_03421 A7LZZ4 (GH43)	<i>B. ovatus</i> BACOVA_03425 A7LZZ8 (GH43)	<i>U. maydis</i> Q4P6F4 (GH62)	<i>A. niger</i> B3GQR2 (GH51)
A <sup>3</sup> X*	0	0	2.6	1.9	0.6	133
A <sup>2</sup> XX*	0	<0.02	28.5	24.9	2.7	21
XA <sup>2</sup> XX*	0	0	28.5	10.2	6.3	5
XA <sup>2</sup> XX and XA <sup>3</sup> XX*	0	0	28.5	27.7	26.4	5
A <sup>2+3</sup> XX*	285	208	0	0	0	4
Arabinoxylan (wheat) **	67	650	16	23	30	0.7
Arabinan (sugar beet) **	0.54	0.53	-	0.013	0.003	10.3
Debranched Arabinan (sugar beet) **	0	0	-	0	0	0.31
p-NP- $\alpha$ -L-Araf***	0.031	0.325	-	0.328	0.012	29

### Literature

1. Siguier *et al.*, 2014. J. Biol. Chem. 289, 5261-5273.
2. Van Laere *et al.*, 1997. J. Appl. Microbiol. Biotechnol. 47, 231-235.
3. Borsenberger *et al.*, 1997. Biochim. Biophys. Acta 1840, 3106-3114.

## Exploring plant cell wall xylan biosynthesis, structure and function

*Paul Dupree<sup>1</sup>, Marta Busse-Wicher<sup>1</sup>, Thomas J. Simmons<sup>1</sup>, Jenny C. Mortimer<sup>1</sup>, Nino Nikolovski<sup>1</sup>, Thiago Gomes<sup>3</sup>, Ray Dupree<sup>2</sup>, Katherine Stott<sup>1</sup>, Nicholas J. Grantham<sup>1</sup>, Jennifer Bromley<sup>1</sup>, Mathias R. Sorieul<sup>1</sup>, Xiaolan Yu<sup>1</sup>, Kathryn S. Lilley<sup>1</sup>, Steven P. Brown<sup>2</sup>, and Munir Skaf<sup>3</sup>*

p.dupree@bioc.cam.ac.uk

1. Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK
2. Department of Physics, University of Warwick, Coventry CV4 7AL
3. Institute of Chemistry, University of Campinas-UNICAMP, PO Box 6154, Campinas, SP, 13084-862, Brazil

The two most abundant polysaccharides in plant cell walls are cellulose and xylan. Interactions between these polysaccharides are important for cell wall properties, but the nature of the interactions are not understood. To discover enzymes of cellulose and xylan biosynthesis we generated a catalogue of plant Golgi proteins using a quantitative proteomics approach. Glycosyltransferases of unknown function form a substantial proportion of proteins in the plant Golgi, and a substantial proportion are not yet catalogued in CAZy GT families (1). Using mutant *Arabidopsis* as a model system, we have found enzymes involved in xylan backbone synthesis and substitution with sugars. Surprisingly, digestion of the xylan polysaccharide revealed that the spacing of sugar and acetate decorations follows a precise periodic pattern. We propose this spacing allows some of the cell wall xylan to fold as a flat two-fold helical screw ribbon in order to hydrogen bond with the glucan chains in cellulose microfibrils (2). Multidimensional <sup>13</sup>C solid state NMR of intact plant cell walls suggests that xylan indeed is present in multiple conformations (3). We propose a model for the assembly and molecular architecture of the xylan:cellulose interactions in plant cell walls.

### Acknowledgements

This work was supported by the BBSRC Grant BB/G016240/1 - The BBSRC Sustainable Bioenergy Cell Wall Sugars Programme. The UK 850 MHz solid-state NMR Facility was funded by EPSRC grant number EP/F017901/1 and the BBSRC, as well as the University of Warwick including via part funding through Birmingham Science City Advanced Materials Projects 1 and 2, by Advantage West Midlands (AWM) and the European Regional Development Fund (ERDF).

### Literature

1. Nikolovski et al. (2013) *Plant Physiology*, 160:1037-51
2. Busse-Wicher et al. (2014) *Plant Journal*, 79:492-506
3. Dupree et al. (2015) *Biochemistry*, in press.

## Understanding the effect of overexpression of fungal acetyl xylan esterase (AXE1) in hybrid aspen

*Prashant Mohan-Anupama Pawar<sup>1</sup>, Marta Derba-Maceluch<sup>1</sup>, Sun-Li Chong<sup>2</sup>, Maija Tenkanen<sup>2</sup>, Madhavi Latha Gandla<sup>3</sup>, Leif Jönsson<sup>3</sup>, Martin Lawoko<sup>4</sup> and Ewa J. Mellerowicz<sup>1</sup>*

ewa.mellerowicz@slu.se

1. Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Sweden.
2. Department of Food and Environmental Sciences, University of Helsinki, Finland.
3. Department of Chemistry, Umeå University, Sweden.
4. Wallenberg Wood Science Centre (WWSC), Department of Fiber and Polymer Technology, School of Chemical Science, Royal Institute of Technology, KTH, Stockholm, Sweden

Xylan is one of the most abundant biopolymers on the earth. 60-70% of xylopyranosyl residues in hardwood xylan backbone are acetylated at *O*-2 and/or *O*-3 position. Enzymatic and chemical deacetylation of xylan helps in increasing the saccharification potential of lignocellulosic biomass. Hence, we would like to study the feasibility of *in planta* xylan deacetylation for improved saccharification properties of wood.

Xylan acetyl esterase AXE1 from *Aspergillus niger* was expressed in hybrid aspen and targeted to cell walls. The independent transgenic lines showed decreased in xylan acetylation. Their growth was not affected. The overall composition of cell wall was unchanged but crystallinity of cellulose was increased. Interestingly, the S/G ratio was decreased in the lignin of transgenic lines. To understand the source of this difference, we isolated and we are currently analyzing different fractions of lignin carbohydrate complexes (LCC). Enzymatic saccharification of transgenic lines yielded more glucose without pretreatment and after acid pretreatment as compared to wild type. The results indicate that post synthetic modification in *O*-acetylation of xylan is a promising approach to increase biofuel production and to study complex interactions between different cell wall polymers.

## Bioinspired model assemblies of plant cell walls as sensors for unravelling interaction features of CAZymes

*Gabriel Paës<sup>1</sup> and Jean-Guy Berrin<sup>2</sup>*

gabriel.paes@reims.inra.fr

1. INRA and University of Reims Champagne-Ardenne, UMR0614 FARE, 2 esplanade Roland-Garros, 51100 Reims
2. INRA and Aix-Marseille University, UMR1173 BBF, 13288 Marseille, France

Carbohydrate-active enzymes (CAZymes) play a central role in the deconstruction of plant cell wall biomass. But their activity is often restricted by sterical hindrances that limit their accessibility to substrate and by non-specific interactions that stick enzymes far from their substrate. Generally, the biochemical characterization of CAZymes is performed with pure isolated polymers or synthetic molecules, which do not reflect the chemical and structural complexity of plant cell walls. Thus there is a need to better assess the interactions of CAZymes with relevant materials.

To achieve this purpose, we have first designed bioinspired model assemblies that contain some of the polymers and covalent interactions found in plant cell walls. These assemblies contain feruloylated arabinoxylans (FAXs), cellulose nano-crystals (CNCs) and model lignin (DHP) at various concentrations in solution (free polymers) or in gels (cross-linked polymers) [1]. Then, starting from a set of modulated endoglucanases belonging to glycoside hydrolase family 45, appended or not to one CBM or five CBMs from family 1 [2], we have grafted these proteins to a fluorophore so that they become fluorescent probes. Each probe has been directly embedded into the different assemblies and their mobility and interactions evaluated by confocal microscopy using the FRAP technique [3].

Overall, results show that the different controlled features related to the assemblies (solution or gel, polymer concentration, water content) and to the probes (type, size) have different impact on affinity, varying in this order: probe size > DHP concentration > FAX concentration. More importantly, the GH45 endoglucanase interacts with DHP through some surface motifs that are partially masked when the enzyme is appended to at least one CBM, making the enzyme alone more sensitive to interactions with lignin. Bioinspired model assemblies are therefore relevant for identifying and hierarchizing features influencing CAZymes affinity that restrict their mobility, thus hampering their activity.

### Literature

1. Paës G and Chabbert B (2012) Characterization of arabinoxylan / cellulose nanocrystals gels to investigate fluorescent probes mobility in bio-inspired models of plant secondary cell wall. *Biomacromolecules* 13, 206-214
2. Couturier M, Feliu J, Haon M, Navarro D, Lesage-Meessen L, Coutinho PM and Berrin JG (2011) A thermostable GH45 endoglucanase from yeast: impact of its atypical multimodularity on activity. *Microb. Cell. Fact.* 10
3. Paës G, Burr S, Saab M-B, Molinari M, Aguié-Béghin V and Chabbert B (2013) Modeling progression of fluorescent probes in bioinspired lignocellulosic assemblies. *Biomacromolecules* 14, 2196-2205

## Discovery of original $\alpha$ -transglucosylases from *Leuconostoc citreum* NRRL B-1299 and NRRL B-742 for the synthesis of tailor-made $\alpha$ -glucans

Marlène Vuillemin<sup>1</sup>, Delphine Passerini<sup>1</sup>, Marion Claverie<sup>1</sup>, Etienne Severac<sup>1</sup>, Florent Grimaud<sup>1,2</sup>, Pierre Monsan<sup>1,2</sup>, Sandrine Morel<sup>1</sup>, Magali Remaud-Simeon<sup>1</sup> and Claire Moulis<sup>1</sup>

moulis@insa-toulouse.fr

1. Université de Toulouse, France, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France, CNRS, UMR5504, F-31400 Toulouse, France, INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France.
2. Toulouse White Biotechnology, Parc Technologique du canal, 3 rue des satellites, F-31400 Toulouse, France.

Due to their renewability, biodegradability and carbon-neutrality, bio-sourced polymers represent attractive alternatives to polymers derived from carbon fossil fuels, as revealed by their broad range of applications in food & feed, agriculture, health, or in chemical industries. In this context, the  $\alpha$ -transglucosylases produced by some lactic acid bacteria are of particular interest as they catalyze, from sucrose, the synthesis of high molar mass  $\alpha$ -glucans, glucooligosaccharides or gluco-conjugates which offer access to a large panel of products varying in term of size and linkage specificity<sup>1</sup>. These  $\alpha$ -transglucosylases are classified in GH70 family, which comprises today 258 sequences for only 59 enzymes biochemically characterized<sup>2</sup>. To broaden GH70 applications, deepen structure function relationship studies and identify determinants of linkage and product specificity, a genomic approach focused on two original strains of *L. citreum* sp. was initiated. The two selected strains, *L. citreum* NRRL B-1299 and NRRL B-742, were identified in the fifties as producers of original  $\alpha$ -glucans highly branched with  $\alpha$ -(1→2) or  $\alpha$ -(1→3) linked glucosyl units, respectively. However, strong association to the cell wall of the enzymes involved in polymer synthesis limited their isolation and characterization.

Genome sequencing and analysis of the two strains allowed to draw up the full inventory of their GH70 encoding genes, and the discovery of a battery of GH70 enzymes never described before. Genome comparison and description of the main characteristic of two branching sucrases specialized in dextran grafting through  $\alpha$ -(1→2) or  $\alpha$ -(1→3) linkages, as well as two dextransucrases dedicated to low molar mass dextrans synthesis will be presented and discussed with regard to their specificity and their potential for technical innovation.

### Acknowledgements

This work was supported by the French National Research Agency (ANR-12-CDII-0005, Engel 2012-2015)

### Literature

1. Leemhuis H *et al.* (2013) *J. Biotechnol.* **163**, 250–272
2. Lombard Vet *et al.* (2013) *Nucleic Acids Res.* **42**, 490–495

## Marine-derived bacterial polysaccharides are valuable sources of glycosaminoglycans

Christine Delbarre-Ladrat, Lou Lebellenger, Jacqueline Ratiskol, Corinne Sinquin, Agata Zykwinska, Sylvia Collic-Jouault

Christine.Delbarre.Ladrat@ifremer.fr

Ifremer, Microbial Ecosystems and Marine Molecules for Biotechnology, Centre Atlantique, rue de l'Île d'Yeu, BP 21105, 44311 Nantes Cédex 3, France

The bacteria from marine ecosystems are a highly valuable resource for original biomolecules and biocatalysts. In particular, marine prokaryotes offer a source of safe, biocompatible, biodegradable and renewable products especially polysaccharides. Based on their structural features, specific biological functions have been highlighted; they are of great interest in human health as glycosaminoglycans-like (GAG-like) molecules. The biological activity of glycopolymers derives from their molecular structure including molecular size as well as sulphation pattern. The use of polysaccharides, in particular for human health, requires the control of molecular characteristics and the understanding of the biosynthesis mechanism and regulation can help in controlling the final molecular features. On the other way, to maximize polysaccharide applications as GAG-like molecules and generate new biological functions, these molecules may be suitably chemically or enzymatically engineered (depolymerised and sulphated). The characteristics of enzymatic methods to get oligosaccharides of biological relevance meet well the needs of better control of the modification process and of environmentally safer processing steps. Therefore, new specific carbohydrate active enzymes and sulfotransferases are needed. The bacteria that produce polysaccharides are also a source of key enzymes for the production of tailor-made polysaccharides.

The study of microbial life adapted to deep-sea hydrothermal vents conditions is a way of discovering new biomolecules with innovative properties and potential applications in human health. Marine bacteria able to produce exopolysaccharides having glycosaminoglycan properties belong to *Vibrio* and *Alteromonas* genera; in particular, some of them are naturally sulfated; this is very rare within the microbial world. Data on three exopolysaccharide (EPS) producing bacteria –*Vibrio diabolicus*, *Alteromonas infernus*, and HYD721 strain– isolated from deep sea habitats will be presented. These would include EPS production in bioreactors, molecular mechanisms of the polysaccharide biosynthesis, comparative genomic and phenotypic features as well as data on carbohydrate active enzymes especially sulfotransferases.

These studies provide a better basic knowledge on the biosynthesis of bioactive polysaccharides and would provide means to control the production as well to engineer it.



## Spider silk mimicking assembly of nanocellulose

Sanni Voutilainen<sup>1,2</sup>, Arja Paananen<sup>2</sup>, Markus Linder<sup>1</sup>

sanni.voutilainen@vtt.fi

1. Aalto University, School of Chemical Technology, Department of Biotechnology and Chemical Technology, Biomolecular materials
2. VTT, Technical Research Centre of Finland

Natural materials are known for their strength and toughness. Spider silk and cellulose are both nature's high end fibres having good mechanical properties in the range of man-made high-tech materials<sup>1,2</sup>. Composites made from nanocellulose are attractive for material applications due to the strength but also the low cost and biocompatibility of the raw material. Spider dragline silk produced by orb-weaving spider is a unique fibrous protein material consisting of three clearly different sequence sections: 1) core of the spidroin is highly repetitive sequence of polyalanine stretches interrupted with glycine-rich regions, 2) in the ends of the repetitive core, the spidroin has globular modules called N-terminal module and 3) C-terminal module. Both N- and C- terminal modules have a role when spidroin protein monomers are being assembled to form silk fibres. The N-terminal module forms a homodimer and firmly interconnects the spidroins when the pH in the spider silk gland is lowered from pH 7 to pH 6 – 5.5<sup>3</sup>.

The excellent mechanical properties of both spider silk and cellulose are due to the precise nanoscale organisation of the molecules in nature, which is a grand challenge when nanocellulose or silk materials are produced. We utilized a carbohydrate binding module (CBM) to attach the spider silk N-terminal module on cellulose nanofibers for mimicking nature's way to assemble spider silk spidroin as functional fibres. The biochemical properties of the recombinant fusion proteins were studied and it became clear that the pH dependent dimerization of NT-module is functional in the fusion proteins and not affected by the CBM fusion. In addition, the binding properties of the fusion proteins on nanocellulose were studied and the rheological properties of the nanocellulose/protein mixture revealed clear self-organization of the material when the pH was changed to favour the dimerization of the NT-module.

**Acknowledgements:** This work was supported by the Academy of Finland through its Centre of Excellence Programme "Molecular Engineering of Biosynthetic Hybrid Materials research" (2014-2019) and under project: A genetic engineering approach for a structure function understanding of the adhesive matrix in biomimetic composites.

### Literature

1. Eisoltd L, Smith A, Scheibel T. Decoding the secrets of spider silk. *Materials Today*. 2011;14(3)
2. Lee K, Aitomäki Y, Berglund LA, Oksman K, Bismarck A. On the use of nanocellulose as reinforcement in polymer matrix composites. *Composites Sci Technol*. 2014;105
3. Askarieh G, Hedhammar M, Nordling K, et al. Self-assembly of spider silk proteins is controlled by a pH-sensitive relay. *Nature*. 2010;465(7295)

## Multiple CBMs enhance starch degradation by members of the human gut microbiota

Nicole Koropatkin

nkoropat@umich.edu

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109

Starch is an abundant carbohydrate in our diet and human gut bacteria have evolved many different strategies for scavenging this polysaccharide. We have studied the molecular mechanism of starch degradation and import by two representative members of the major gut bacterial phyla, the Gram-negative Bacteroidetes and the Gram-positive Firmicutes. In both phyla starch is targeted to the surface of the cell but processed by distinctly different amylases and import machinery. The starch utilization system (Sus) of *Bacteroides thetaiotaomicron* is comprised of five cell surface proteins that work together to bind, degrade and import starch. The lipoproteins SusDEFG have a combined total of eight distinct non-catalytic starch-binding sites that have apparently distinct roles in starch sensing and uptake. Among these, SusD, SusE and SusF have unique structures that facilitate the capture and import of maltooligosaccharides [1, 2]. SusG, a surface amylase, features a starch-binding CBM58, inserted within the catalytic domain, but is dispensable in starch utilization[3]. Our single molecule imaging of SusG in live *B. theta* suggests that this protein interacts dynamically with other Sus proteins during starch digestion[4]. In contrast to the Sus employed by members of the Bacteroidetes, members of the Firmicutes, such as *Eubacterium rectale*, encode one or more cell-wall anchored amylases with multiple tandem starch-binding domains. Our recent work with *E. rectale* has demonstrated its ability to digest granular cornstarch to maltotetraose that is then scavenged by two abundant maltooligosaccharide-binding proteins as part of ABC transporters that are specific for maltotriose and larger oligosaccharides[5]. Comparative genomic analysis of this pathway suggests that it is highly conserved among related Firmicutes, and may account for the ability of these bacteria to flourish in individuals that consume a diet high in resistant starch. Through a detailed understanding of how human gut bacteria acquire carbohydrate nutrition in the highly competitive gut ecosystem, we can develop prebiotic and probiotic strategies to manipulate the composition of this community towards improved human health.

### Literature

1. Koropatkin, N.M., et al., *Starch catabolism by a prominent human gut symbiont is directed by the recognition of amylose helices*. Structure, 2008. 16(7): p. 1105-15.
2. Cameron, E.A., et al., *Multidomain Carbohydrate-binding Proteins Involved in Bacteroides thetaiotaomicron Starch Metabolism*. J Biol Chem, 2012. 287(41): p. 34614-25.
3. Koropatkin, N.M. and T.J. Smith, *SusG: A unique cell-membrane-associated alpha-amylase from a prominent human gut symbiont targets complex starch molecules*. Structure, 2010. 18(2): p. 200-215.
4. Karunatilaka, K.S., et al., *Superresolution imaging captures carbohydrate utilization dynamics in human gut symbionts*. MBio, 2014. 5(6): p. e02172.
5. Cockburn, D.W., et al., *Molecular details of a starch utilization pathway in the human gut symbiont Eubacterium rectale*. Mol Microbiol, 2015. 95(2): p. 209-30.

## Functionality of granule-bound starch synthase from the waxy barley cultivar CDC Alamo

*Kim H. Hebelstrup<sup>2</sup>, Morten Munch Nielsen<sup>1</sup>, Massimiliano Carciofi<sup>2</sup>, Katarzyna Krucewicz<sup>1</sup>, Shahnoor Sultana Shaik<sup>3</sup>, Andreas Blennow<sup>3</sup> and Monica M. Palcic<sup>1</sup>*

kim.hebelstrup@mbg.au.dk

1. Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-1799 København V, Denmark
2. Department of Molecular Biology and Genetics, Section of Crop Genetics and Biotechnology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark
3. Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Amylose synthesis in plants is strictly dependent on activity of Granule-Bound Starch Synthase (GBSS) enzyme types. *Near-waxy* or *waxy* cultivars are starch crops containing either little or no amylose. Such cultivars have been identified among maize, potato, wheat, barley and rice. This phenotype has so far been associated with a single locus which has been mapped to GBSS-type genes. Most such waxy varieties are a result of either low or no expression of a single GBSS gene. But there are waxy varieties, where the GBSS enzymes are expressed at a normal level. For such types, non-silent SNPs have been suggested to result in loss of catalytic activity. We here cloned the cDNA and characterized the HvGBSSIa enzyme from the amylose-free barley variety CDC Alamo, and found that the HvGBSSIa from CDC Alamo actively synthesizes amylose both in vitro and in vivo. GFP tagged HvGBSSIa's of both the non-waxy type and the CDC Alamo type were found to localize in concentric circles strictly within starch granules in transgenic barley lines. Finally HvGBSSIa from CDC Alamo was fully able to complement the waxy phenotype when expressed transgenically in other waxy varieties.

In conclusion these observations are not in agreement with HvGBSSIa from the amylose-free barley variety CDC Alamo being catalytically inactive, suggesting that the amylose-free phenotype of CDC Alamo is related to other factors than GBSS.

## Glucan phosphatases utilize different mechanisms to bind starch and glycogen

Matthew S. Gentry, Madushi Raththagala, M. Kathryn Brewer, David A Meekins, Satrio Husodo, Vikas Dukhande, and Craig W. Vander Kooi

matthew.gentry@uky.edu

Department of Molecular and Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, KY 40536, USA

Lafora disease (LD), a fatal neurodegenerative epilepsy, and starch metabolism are linked by a family of enzymes that we discovered called glucan phosphatases. Glucans are the most abundant polymer in plants, with cellulose serving as the structural component and starch as the energy reserve. Instead of starch, humans utilize glycogen as their primary carbohydrate storage molecule. Recent work shows that the metabolism of both starch and glycogen is dependent on the action of glucan phosphatases.

Plants release the energy in starch via a three-step process: starch phosphorylation, degradation, and dephosphorylation. Dikinases phosphorylate outer starch glucose units to make them water-soluble and enzyme accessible so that amylases can release maltose and glucose. Following amylase activity, the phosphate must be removed by glucan phosphatases. In the absence of glucan phosphatases, plants cannot access the energy stored in starch and the starch granules grow in size while plant growth is stunted.

The *EPM2A* gene encodes laforin and recessive mutations in *EPM2A* result in LD. We demonstrated that laforin is a human glucan phosphatase. In the absence of laforin activity, glycogen transforms into a hyper-phosphorylated, water-insoluble, starch-like Lafora body (LB). LBs are the suspected cause of neuronal apoptosis, neurodegeneration, and eventual death of LD patients.

We recently determined crystal structures of the *Arabidopsis* starch phosphatase Starch Excess 4 (SEX4) with phospho-glucan bound at 1.62Å. In addition, we determined the structure of *Like Sex Four2* (LSF2) both with and without phospho-glucan product bound at 2.3Å and 1.65Å, respectively. Most recently, we determined the structure of human laforin bound to phosphoglucan product. Our data demonstrate that each phosphatase utilizes a unique mechanism to bind and dephosphorylate its substrate.

These structures are the first of glucan phosphatases, and they provide new insights into the molecular basis of this medically-, agriculturally-, and industry-relevant enzyme family as well as their unique mechanisms of catalysis, substrate specificity, and interaction with glucans.

## Secondary structure reshuffling modulates the enzymatic activity of a GT-B glycosyltransferase at the membrane interface

*Natalia Comino<sup>1</sup> and Marcelo Guerin<sup>1,2,3</sup>*

mrcguerin@gmail.com

1. Unidad de Biofísica, Centro Mixto Consejo Superior de Investigaciones Científicas-Universidad del País Vasco/Euskal Herriko Unibertsitatea (CSIC, UPV/EHU), Leioa, Bizkaia 48940, Spain.
2. Departamento de Bioquímica, Universidad del País Vasco, Spain.
3. IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

The phosphatidylinositol mannosyltransferase PimA is an essential membrane-associated enzyme that initiates the biosynthetic pathway of key structural elements and virulence factors of *Mycobacterium tuberculosis*, such as phosphatidylinositol mannosides (PIM), lipomannan and lipoarabinomannan (1, 2, 3). PimA is an amphitropic glycosyltransferase that belongs to the GT-B superfamily, composed of two Rossmann-fold domains separated by a central cleft that includes the catalytic center (4). As observed for other GT-B enzymes, binding of the sugar donor substrate GDP-Man to PimA stabilizes a ‘closed’ state, in which crucial residues from the N- and C-terminal domains are brought together to form a functionally competent active site. In contrast, binding of the sugar acceptor substrate phosphatidylinositol (PI) was observed to have a destabilizing effect on PimA, possibly due to the formation of a more relaxed enzyme-ligand complex (5). The structural flexibility of PimA was further stressed by recent single-molecule and small-angle X-ray scattering studies, which demonstrated that PimA displays a highly dynamic N-terminal domain (6).

To advance our understanding of the molecular mechanisms that govern substrate binding and catalysis, we have now determined the crystal structure of the apo form of PimA. The most striking feature of this crystal form is that apo PimA did not exhibit the ‘classical’ Rossmann-fold topology, which was previously observed for the same enzyme in complex with GDP or GDP-Man. We demonstrate that the occurrence of a conformational switch between the apo and GDP-bound crystal structures of PimA must involve both  $\beta$ -strand-to- $\alpha$ -helix and  $\alpha$ -helix-to- $\beta$ -strand transitions. These structural changes seem to modulate catalysis and are promoted by interactions of the protein with anionic phospholipids in the membrane. Although scant structural information is currently available on protein catalysis at the lipid-water interface, our studies demonstrate that protein-membrane interactions might entail unanticipated structural changes in otherwise well conserved protein architectures, and suggests that similar changes may also play a functional role in other membrane-associated GT-B enzymes (7).

### Literature

1. Guerin et al., J. Biol. Chem. 285, 33577-33583 (2010). Review.
2. Albesa-Jové et al., Glycobiology 24, 108-124 (2014). Review.
3. Boldrin et al., J. Bact. doi:10.1128/JB.01346-13 (2014)
4. Guerin et al., J. Biol. Chem. (2007)
5. Guerin et al., J. Biol. Chem. 284, 21613-21625 (2009)
6. Giganti et al., J. Biol. Chem. 288, 29797-29808 (2013)
7. Giganti et al., Nat. Chem. Biol. 11(1), 16-8 (2015).

## Degrading sulfated sugars from the sea: novel insights into the evolution, dimerization plasticity and catalytic mechanism of the GH117s

*Elizabeth Ficko-Blean<sup>1,2</sup>, Delphine Duffieux<sup>1,2</sup>, Étienne Rebuffet<sup>1,2</sup>, Robert Larocque<sup>1,2</sup>, Agnes Groisillier<sup>1,2</sup>, Gurvan Michel<sup>1,2\*</sup>, Mirjam Czjzek<sup>1,2\*</sup>*

efickoblean@sb-roscoff.fr

1. Sorbonne Universités, UPMC Univ Paris 06, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688, Roscoff cedex, Bretagne, France
2. CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688, Roscoff cedex, Bretagne, France

Marine polysaccharides differ from their terrestrial counterparts, notably, all marine algae produce sulfated polysaccharides, which are absent in land plants. The sulfated galactan agar is a key component of some red algal cell walls. Red algae synthesize an unusual carbohydrate moiety from the galactose scaffold, the  $\alpha$ -1,3-linked bicyclic 3,6-anhydrogalactose. The synthesis of 3,6-anhydrogalactose is unique to red macroalgae and contributes to flexibility and hydration, which is important given the extreme environmental forces exerted by the ocean on the organism.

Red macroalgae are important primary producers to the marine ecosystem and a large proportion of their organic biomass is recycled through the food chain. Microbes have developed complex systems designed for the degradation of agar and other marine polysaccharides. The marine bacterium *Zobellia galactanivorans*, which was isolated from a red alga, produces five paralogous family 117 glycoside hydrolase enzymes (GH117s). The GH117s are involved in the final step of agarose degradation which results in the production of a monosaccharide. To date, there are only structure function studies on the Clade A GH117s which demonstrate exo- $\alpha$ -1,3-(3,6-anhydro)-L-galactosidase activity. The Clade C enzymes are predicted to have different substrate specificities. Thus, two Clade C GH117s from *Z. galactanivorans* were chosen for in-depth structural and functional analyses.

An interesting feature of the GH117s is that they form structural homodimers whereby the two monomers participate in domain swapping. The two Clade C GH117s studied exhibit different modes of dimerization as well as active site differences. Crystal soaks with oligosaccharide substrate resulted in a product complex with the  $\beta$ -3,6-anhydro-L-galactose moiety. The  $\beta$ -anomer of 3,6-anhydro-L-galactose has been hypothesized to exist but this is the first time that the structure of this unusual bicyclic sugar has been clearly elucidated. The product complex provides strong crystallographic evidence for an inverting catalytic mechanism in this family as well as insight into the structure of the unique sugar produced by hydrolysis of agarose.

Many new marine bacterial genomes containing GH117 enzymes have been released since the first phylogenetic tree was published in 2011. New phylogenetic analysis reveals novel clades within the family and suggests members from other clades may themselves have distinctive specificities. Thus, there remains much to be understood about this GH family designed for the final step in the degradation of sulfated marine galactans.

## Functional metagenomics reveals novel pathways of mannoside metabolization by human gut bacteria

Simon Ladevèze<sup>1,2,3</sup>, Gianluca Giocci<sup>1,2,3</sup>, Laurence Tarquis<sup>1,2,3</sup>, Elisabeth Laville<sup>1,2,3</sup>, Bernard Henrissat<sup>4</sup>, Samuel Tranier<sup>5,6</sup>, and Gabrielle Potocki-Veronese<sup>1,2,3</sup>

veronese@insa-toulouse.fr

1. Université de Toulouse, INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France
2. INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
3. CNRS, UMR5504, F-31400 Toulouse, France
4. Architecture et Fonction des Macromolécules Biologiques, UMR6098, CNRS, Universités Aix-Marseille I & II, 163 Avenue de Luminy, F-13288 Marseille, France
5. Institut de Pharmacologie et de Biologie Structurale (IPBS), Centre National de la Recherche Scientifique (CNRS), 205, route de Narbonne, BP 64182, Toulouse, 31077, France.
6. Université de Toulouse, Université Paul Sabatier, IPBS, 31077 Toulouse, France.

The human gut hosts a complex bacterial community that plays a major role in nutrition and in maintaining human health. To face the huge structural diversity of carbohydrates which constitute their main carbon sources, intestinal bacteria produce a full repertoire of CAZymes, of which glycoside hydrolases are the main constituents. However, other types of enzymes, like glycoside-phosphorylases, participate in the breakdown of complex carbohydrates, by working in synergy with glycoside hydrolases. The prevalence of glycoside-phosphorylases and their role in carbohydrate metabolism is therefore difficult to evaluate on the basis of sequence data alone, because of their structural and mechanistic similarities with glycoside hydrolases and glycosyltransferases.

Recently, integration of biochemical, metagenomic and genomic data at the level of the entire human gut ecosystem, revealed novel mechanisms of plant and host glycan metabolization, involving mannoside-phosphorylases of different GH130 subfamilies<sup>1,2</sup>. Analysis of the first crystal structure of a N-glycan phosphorylase highlighted the molecular determinants of GH130 functional specificities. Thanks to these data, these fascinating enzymes now appear as new targets to study interactions between host, dietary constituents and gut microbes, in particular in the context of inflammatory bowel diseases.

### Literature

1. Tasse, L. *et al.* Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Res.* 20, 1605-1612 (2010).
2. Ladevèze, D. *et al.* Role of glycoside phosphorylases in mannose foraging by human gut bacteria. *J Biol Chem* 288, 32370–32383 (2013).

## Structural basis for arabinoxylo-oligosaccharide capture by probiotic bifidobacteria

Morten Ejby,<sup>1</sup> Folmer Fredslund,<sup>1,2</sup> Andreja Vujicic-Zagar,<sup>3</sup> Birte Svensson,<sup>1</sup> Dirk Jan Slotboom,<sup>3</sup> and Maher Abou Hachem<sup>1</sup>

maha@bio.dtu.dk.

1. Dept. of Systems Biology, Technical University of Denmark

2. Dept. of Chemistry, University of Copenhagen

3. Institute for Biomolecular Sciences & Biotechnology, Rijksuniversiteit Groningen

Glycan utilization is an important factor modulating the composition of the gut microbiota,<sup>1</sup> but insight into oligosaccharide uptake by this microbial community is scarce. Arabinoxylan is abundant in human diet, and its arabinoxylo-oligosaccharide (AXOS) oligomers are selectively fermented by gut adapted health stimulating bifidobacteria in the lower part of the gastrointestinal tract. The AXOS utilization locus in the probiotic bacterium *Bifidobacterium animalis* subsp. *lactis* B1-04 harbours an ATP-binding cassette (ABC) transport system.<sup>2</sup> The binding protein *BLAXBP*, which is associated to this ABC transporter binds a range of arabinosyl-decorated and undecorated xylo-oligosaccharides, with preference for tri- and tetrasaccharides. Crystal structures of *BLAXBP* in complex with four different ligands revealed the basis for this recognition as the transport protein is able to bind oligosaccharides in two opposite orientations, which facilitates the optimization of interactions with the various ligands. Binding site plasticity is further enhanced by a spacious binding pocket accommodating decorations at different main chain positions and conformational flexibility of a lid-like loop. Phylogenetic and genetic analyses show that *BLAXBP* is highly conserved within *Bifidobacterium*, but is lacking in other gut microbiota taxa. These novel data highlight the role of glycan uptake in establishing metabolic specialization of bifidobacteria and in contributing to syntrophy (cross-feeding) in arabinoxytan metabolism amongst different taxa in the gut niche.

### Acknowledgements

This study was funded by a FøSu grant from the Danish Strategic Research Council to the project ‘Gene discovery and molecular interactions in pre/probiotics systems. Focus on carbohydrate prebiotics’.

### Literature

1. Koropatkin, N. M., et al. (2012) *Nat. Rev. Microbiol.* **10**, 323-335
2. Andersen, J. M., et al. (2013) *BMC Genomics* **14**:312
3. Ejby, M., et al. (2013) *Mol. Microbiol.* **90**, 1100-1112



## The modular intramolecular *trans*-sialidase from *Ruminococcus gnavus* ATCC 29149 suggests a novel mechanism of mucosal adaptation in the human gut microbiota

Louise E Tailford<sup>1,#</sup>, C David Owen<sup>2,#</sup>, John Walshaw<sup>1</sup>, Emmanuelle H Crost<sup>1</sup>, Jemma Hardy-Goddard<sup>1</sup>, Gwenaelle Le Gall<sup>1</sup>, Willem M de Vos<sup>3</sup>, Garry L Taylor<sup>2</sup> and Nathalie Juge<sup>1</sup>

nathalie.juge@ifr.ac.uk

1. Institute of Food Research, The Gut Health and Food Safety Institute Strategic Programme, Norwich Research Park, Norwich, NR4 7UA, UK
2. Biomolecular Sciences Building, University of St Andrews, KY16 9ST
3. Laboratory of Microbiology, Wageningen UR, Building 316, Dreijenplein 10, 6703 HB Wageningen, The Netherlands, and Departments of Bacteriology & Immunology and Veterinary Bioscience, University of Helsinki, P.O. Box 66, FI-00014 Helsinki, Finland

<sup>#</sup>contributed equally to the work

The gastrointestinal (GI) mucus layer is colonized by a dense community of microbes catabolizing dietary and host (mucin) carbohydrates during their expansion within the gut. Alterations in mucosal carbohydrate availability impact on the composition of microbial species including enteric pathogens.

The species *Ruminococcus gnavus* is a commensal anaerobe present in the GI tract of  $\geq 90\%$  of humans and has been implicated in gut-related diseases such as inflammatory bowel diseases (IBD). The ability of *R. gnavus* strains to grow on GI mucin is strain dependent and relies on the expression of a potential GH33 glycoside hydrolase, as shown by comparative genomics and transcriptomics<sup>1</sup>.

Here we show using a combination of enzymology and crystallography, that this enzyme is an intramolecular *trans*-sialidase (IT-sialidase) which allows the bacteria to cleave off terminal sialic acid residues from mucins, releasing 2,7-anhydro-Neu5Ac instead of free sialic acid<sup>2</sup>. The IT-sialidase is specific for  $\alpha$ 2-3 linked sialic acid conjugates although it can accommodate different moieties at the non-reducing end, as determined by High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection (HPAEC) and Nuclear Magnetic Resonance (NMR). The sialidase consists of a GH33 catalytic domain and a carbohydrate binding domain CBM40. The contribution of both domains to the activity and unusual substrate specificity of the enzyme will be discussed in light of X-ray structures.

Using bioinformatics approaches, we showed evidence of IT-sialidases in human metagenome sequence data with a prevalence in samples from IBD metagenomes<sup>2</sup>. Our results uncover a previously unrecognised enzymatic activity in the gut microbiota, which may contribute to the adaptation of intestinal bacteria to the mucosal environment in health and disease.

**Acknowledgements** Bernard Henrissat for help with CAZy domain boundaries.

### Literature

1. Crost EH, Tailford LE, Le Gall G, Fons M, Henrissat B, Juge N. 2013. Utilisation of mucin glycans by the human gut symbiont *Ruminococcus gnavus* is strain-dependent. PLoS One 8:e76341.
2. Tailford, LE, Owen, CD, Walshaw, J, Crost, EH, Hardy-Goddard, J, Le Gall, G, de Vos, WM, Taylor, GL and Nathalie Juge, N. (submitted) Discovery of intramolecular *trans*-sialidases in human gut microbiota suggests novel mechanisms of mucosal adaptation.

## Galactomannan degradation by *Bifidobacterium*

Evelina Kulcinskaja<sup>1</sup>, Frida Fåk<sup>2</sup>, Greta Jakobsdottir<sup>2</sup>, Nittaya Marungruang<sup>2</sup>, Sumitha Reddy<sup>1</sup>, Romany Ibrahim<sup>1</sup>, Anna Rosengren<sup>1</sup>, Margareta Nyman<sup>2</sup>, Henrik Ståhlbrand<sup>1</sup>

evelina.kulcinskaja@biochemistry.lu.se

1. Biochemistry and Structural Biology, Department of Chemistry, Lund University, Sweden

2. Food for Health Science Centre, Lund University, Sweden

The gut microflora is important for the wellbeing of the host. The microflora keeps out pathogenic bacteria, stimulates the immune system and produces nutrients that can be used by the host [1]. It is therefore of interest to study how dietary fibers affects the composition of the gut flora and how gut bacteria metabolize fibers, including enzyme-catalyzed hydrolysis.

We have studied the effect on the caecal bacteria composition and metabolic responses in rats fed with low and high fat diets in combination with guar galactomannan of low, medium and high viscosity. The low viscosity guar galactomannan was prepared by enzymatic hydrolysis of guar galactomannan using a  $\beta$ -mannanase from *Aspergillus niger*. *Bifidobacterium* sp. were upregulated when the rats were fed guar galactomannan regardless of viscosity [2]. Some of the genome sequenced bifidobacteria harbor  $\beta$ -mannanases, the main enzymes responsible for galactomannan degradation. We have cloned and characterized a GH26  $\beta$ -mannanase, BaMan26A, from *Bifidobacterium adolescentis* ATCC15703.

BaMan26A is active on guar and carob galactomannans and crystalline mannan from ivory nut. The enzyme is modular, containing apart from the catalytic module an immunoglobulin-like and two carbohydrate binding modules of family 23 which bind to carob galactomannan (Kd 8.8 mg/l) [3]. The main products from carob galactomannan were found to be mannotriose and digalactosylated mannotriose. These produced mannoooligosaccharides were used as a carbon source for *in vitro* growth studies of *B. adolescentis*.

The growth studies of *B. adolescentis* showed no uptake of the mannoooligosaccharides, but the digalactosylated mannotriose, one of the dominant products from carob hydrolysis, seemed to be degraded into mannotriose and galactose, which could be utilized by *B. adolescentis* to grow.

### Literature

1. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) *Science* 307: 1915-1920.
2. Fåk F, Jakobsdottir G, Kulcinskaja E, Marungruang N, Matziouridou C, Nilsson U, Ståhlbrand H, Nyman M. *Submitted to PLOS ONE*
3. Kulcinskaja E, Rosengren A, Ibrahim R, Kolenová K, Ståhlbrand H (2013) *Appl Environ Microbiol* 79: 133-140.

## Understanding complex glycan utilization in the human microbiota

Harry J. Gilbert<sup>1</sup>, Artur Rogowski<sup>1</sup>, Dider Ndeh<sup>1</sup>, Fiona Cuskin<sup>1</sup>, Elisabeth Lowe<sup>1</sup>, Eric C. Martens<sup>2</sup> and David Bolam<sup>1</sup>

harry.gilbert@ncl.ac.uk

1. Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom.
2. Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

The human large bowel is colonized by a community of microbes, the microbiota, which has a significant impact on human health and nutrition through the production of short chain fatty acids (SCFAs), and by interaction with the host immune system. The major nutrients available to these organisms are dietary glycans, also known as complex carbohydrates. Thus, dietary and nutraceutical strategies, based on complex carbohydrates, can, potentially, be deployed to encourage the dominance of beneficial microbes in the microbiota, particularly those producing health promoting SCFAs such as propionate and butyrate, and bacteria that have an anti-inflammatory impact through its interaction with the human immune system, ensuring that this microbial ecosystem has a positive influence on human health. This approach, however, is greatly restricted by a critical lack of understanding of the mechanisms by which complex glycans are metabolized by the microbiota. Significantly, the wealth of genomic and metagenomic microbiota sequence presents an exciting, but so far unfulfilled, opportunity to make decisive advances in our understanding of glycan metabolism in the human large bowel. This seminar will review our biochemical, genetic and microbiological strategies, in harness with metagenomic and genomic data, to understand the mechanisms of complex glycans utilization by the human microbiota<sup>1,2,3</sup>. The models established, will trigger the development of novel dietary strategies that are designed to maximize human health through manipulation of microbiota structure.

### Literature

- 1.Cuskin et al. (2015) Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. *Nature* **517**, 165-169
- 2.Rogowski et al. (2015) Glycan complexity dictates microbial resource allocation in the large intestine. *Nature Communications* under review.
- 3.Martens et al. (2012) Recognition and degradation of plant cell wall polysaccharides by two human gut symbiont *PLoS Biology* **9**, e1001221 (1-16)



# **Poster Presentations**



## Anticoagulant activity of sulfated polysaccharide-rich macroalgae extracts

Amandine Adrien<sup>1</sup>, Nicolas Bidiau<sup>1</sup>, Thierry Maugard<sup>1</sup>

amandine.adrien@gmail.com

1. LIENSs Laboratory, UMR 7266 CNR S-ULR, Université de La Rochelle, Equipe Approches Moléculaires, Environnement-Santé, Avenue Michel Crépeau, 17042 La Rochelle, France

Macroalgae have been used in traditional medicine for centuries and perceived as a food with great health benefits. Recently, several studies have shown that macroalgae possess a variety of biological activities (Mohamed et al. 2012; Andrade et al. 2013) and are capable of synthesizing a wide range of key nutrients such as proteins, minerals, and fibers (Lahaye 1991; Fleurence 1999). Macroalgae are also an important source of polysaccharides, such as sulfated polysaccharides, which are the source for many of their potential properties that might find relevance in nutraceutical, pharmaceutical and cosmeceutical applications (Jiao et al. 2011). Nowadays, to treat health troubles linked to thrombo-embolic complication, most of the anticoagulant treatments are heparin-based. Despite its major anticoagulant activity, heparin can cause serious adverse events. Moreover, its low bioavailability makes such a treatment really expensive. Given the risks and high costs of these treatments, there is a compelling need for further investigation of new sources of anticoagulants. To study the anticoagulant potential of sulfated polysaccharides from macroalgae, different extracts were prepared using various extraction processes leading to enriched polysaccharides fractions. Extracts from six edible seaweeds, including brown (*Laminaria digitata*, *Fucus vesiculosus*, *Himanthalia elongata*, *Ascophyllum nodosum*), green (*Ulva lactuca*), and red (*Chondrus crispus*) macroalgae, were prepared and the biochemical composition of each extract was determined. The potential anticoagulant activity of each extract was also investigated using different scales, from the specific antithrombin-dependent pathway (anti-Xa and anti-IIa) to the intrinsic and/or common (Activated Partial Thromboplastin Time), extrinsic (Prothrombin Time) or common (Thrombin Time) anticoagulant pathways. Furthermore, the extract anticoagulant properties were compared with those of commercial anticoagulants: heparin and Lovenox®.

### Literature

1. Andrade, Paula B., Mariana Barbosa, Rui Pedro Matos, Graciliana Lopes, Juliana Vinholes, Teresa Mouga, et Patrícia Valentão. 2013. « Valuable compounds in macroalgae extracts ». *Food Chemistry* 138 (2–3): 1819–28. doi:10.1016/j.foodchem.2012.11.0813.
2. Fleurence, Joël. 1999. « Seaweed proteins: biochemical, nutritional aspects and potential uses ». *Trends in Food Science & Technology* 10 (1): 25–28. doi:10.1016/S0924-2244(99)00015-1
3. Jiao, Guangling, Guangli Yu, Junzeng Zhang, et H. Stephen Ewart. 2011. « Chemical Structures and Bioactivities of Sulfated Polysaccharides from Marine Algae ». *Marine Drugs* 9 (2): 196 – 223. doi:10.3390/md9020196.
4. Lahaye, M. 1991. « Marine Algae as Sources of Fibres: Determination of Soluble and Insoluble Dietary Fibre Contents in Some ‘sea Vegetables’ ». *Journal of the Science of Food and Agriculture* 54 (4): 587–94. doi:10.1002/jsfa.2740540410.
5. Mohamed, Suhaila, Siti Nadia Hashim, et Hafeedza Abdul Rahman. 2012. « Seaweeds: A sustainable functional food for complementary and alternative therapy ». *Trends in Food Science & Technology* 23 (2): 83–96. doi:10.1016/j.tifs.2011.09.001

## Elucidating the impact of N-glycosylation on the ability of recombinant CBM3 from *Clostridium thermocellum* to modify pulp and paper properties

Carla Oliveira<sup>1</sup>, Goreti Sepúlveda<sup>1</sup>, Tatiana Q. Aguiar<sup>1</sup>, Francisco M. Gama<sup>1</sup> and Lucília Domingues<sup>1</sup>

tatiana.aguiar@deb.uminho.pt

1. CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

The hydrolytic activity of the enzymes traditionally used in paper industry to modify pulp and paper properties often leads to the reduction of fiber strength [1]. Thus, the use of the carbohydrate-binding modules (CBMs) of these enzymes has emerged as an interesting alternative, as CBMs have been shown to enhance the surface/interface properties of cellulose fibers independently from the catalytic domain [2].

The CBM3 from the *Clostridium thermocellum* CipA scaffolding protein was previously reported to improve pulp drainability when conjugated with polyethyleneglycol (PEG), which was used to mimetize glycosylation [3]. Otherwise, it didn't affect any of the pulp and paper properties studied [3]. Although not glycosylated in nature, this CBM3 has three potential N-glycosylation sites. Therefore, to elucidate the impact of N-glycosylation on its ability to modify the properties of cellulose fibers, we assessed the effect of two versions of this CBM3 recombinantly produced in *Pichia pastoris*, one non-glycosylated and other glycosylated, on the properties of *Eucalyptus globulus* pulps and handsheets.

Although glycosylation reduced the CBM3 adsorption (16%) and affinity (35%) to Avicel (microcrystalline cellulose), as well as the CBM3 ability to promote the hydrophobization of Whatman's paper surface, the two CBM3 versions affected the *E. globulus* pulp and paper properties in a similar way. None of the CBM3s altered the drainability of *E. globulus* pulps, but both improved significantly the burst and tensile strength indexes of *E. globulus* handsheets, by up to 12% and 10%, respectively. This yet unknown capacity of CBM3 to improve paper strength-related properties was dependent on the amount of CBM3 used, but not on its glycosylation. Therefore, our results show that the N-glycans attached to CBM3 did not significantly change its effects over pulp and paper properties.

### Acknowledgements

This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through Project GlycoCBMs (PTDC/AGR-FOR/3090/2012 - FCOMP-01-0124-FEDER-027948) and fellowship SFRH/BDP/63831/2009 to Carla Oliveira.

### Literature

1. Bhat MK. 2000. Biotechnol Adv. 18, 355-383.
2. Shi XR, Zheng F, Pan RH, Wang J, Ding SJ. 2014. Bioresources. 9, 3117-3131.
3. Machado J, Araujo A, Pinto R, Gama FM. 2009. Cellulose. 16, 817-824.



## Discovery and characterization of novel carbohydrate esterases

*Pablo Alvira*<sup>1,2,3</sup>, *Gregory Arnal*<sup>1,2,3</sup>, *Sophie Bozonnet*<sup>1,2,3</sup>, *Régis Fauré*<sup>1,2,3</sup>, *Olga Gherbovet*<sup>1,2,3</sup>, *Claire Dumon*<sup>1,2,3</sup> and *Michael O'Donohue*<sup>1,2,3</sup>

claire.dumon@insa-toulouse.fr and michael.odonohue@insa-toulouse.fr

1. Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France

2. INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France

3. CNRS, UMR5504, F-31400 Toulouse, France

In the context of a biobased economy, valorizing most of sugars contained in the plant cell walls becomes a crucial target. One step towards achieving this goal will be to advance in pentoses extraction, a neglected aspect of biorefining compared to hexoses utilization. Most of pentoses are contained in hemicelluloses, which represents up to 40% of lignocellulosic biomass and therefore a huge sugar reservoir. Many hemicelluloses are highly substituted by esterified phenolic or acetic acids which contribute to the indigestibility of the polysaccharides (1, 2). Removal of these substitutions by carbohydrate-acting esterases (CEs) such as feruloyl esterases and acetyl esterases exposes glycosidic linkages, playing an important role in the biomass deconstruction to enhance enzymatic cocktails.

While many interesting fungal CEs have already been studied, little is known about bacterial CEs. In previous study a functional analysis of metagenomics libraries from invertebrates was performed, revealing numerous bacterial hemicellulose active-enzymes (3). Regarding CEs, 63 sequences encoding novel putative CEs were revealed, with 20 CEs classified in the CAZy database belonging to the families CE1, CE4 and CE7. These enzymes have been cloned and expressed in *E. coli*, and subsequently characterized in order to evaluate their activity on model and complex substrates. Several multimodular enzymes composed of glycosyl hydrolases, carbohydrate binding modules and esterase domains were identified, cloned and expressed. Here we present the potential properties of the most original and promising enzymes.

### Literature

1. Selig MJ, Adney WS, Himmel ME, Decker SR: The impact of cell wall acetylation on corn stover hydrolysis by cellulolytic and xylanolytic enzymes. *Cellulose* 2009, 16:711-722.
2. Faulds CB, What can feruloyl esterases do for us? *Phytochemistry Reviews* 2010, 9:121-132.
3. Bastien G, Arnal G, Bozonnet S, Laguerre S, Ferreira F, Faure R, Henriessat B, Lefevre F, Robe P, Bouchez O, Noirot C, Dumon C, O'Donohue M. Mining for hemicellulases in the fungus-growing termite *Pseudacanthotermes militaris* using functional metagenomics. *Biotechnol Biofuels* 2013, 6:78.

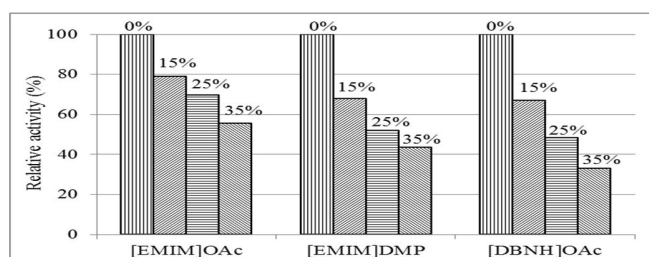
## Hydrolysis of xylan by thermophilic family 10 xylanase in the presence of biomass-dissolving ionic liquids

*Sasikala Anbarasan<sup>1</sup>, Michael Hummel<sup>2</sup>, Herbert Sixta<sup>2</sup> and Ossi Turunen<sup>1</sup>*

sasikala.anbarasan@aalto.fi

1. Department of Biotechnology and Chemical Technology, School of Chemical Technology, Aalto University, P.O. Box 16100, 00076 Aalto, Finland.
2. Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box, 16300, 00076 Aalto, Finland

Xylanases are used in lignocellulose hydrolysis to increase the accessibility of cellulolytic enzymes to cellulose and cleave xylo-oligosaccharides, which can be inhibitors for cellulolytic enzymes. Ionic liquids (ILs) are studied in the pretreatment of lignocellulose hydrolysis. However, the remaining ionic liquids can be detrimental to the enzymes used in the hydrolysis. Therefore, finding cellulases and hemicellulases that remain active in the presence of residual ILs is necessary for efficient processing of lignocellulose with IL pretreatment and enzymatic hydrolysis. We have studied GH11 and GH10 xylanases for their tolerance towards ILs (Li et al., 2013; Chawachart et al., 2014). Thermostable GH10 xylanase from *Thermopolyspora flexuosa* (TfXYN10A) turned out to be one of the most tolerant xylanases. It tolerated partly 15-35% water solutions of the tested ILs (Fig. 1). The presence of ILs reduces the temperature optimum of the enzyme, but at 80°C the half-life was even increased in the presence of 15% and 35% of [EMIM]OAc and 15% [DBNH]OAc (Table 1).



**Fig 1.** Effect of biomass-dissolving ionic liquids on TfXYN10A. Reaction conditions were 2 h at 60 °C, pH 5, with 1% xylan as substrate.

**Table 1.** Half-life (min) of Tf XYN10A at 80°C, pH-5.

IL (%)	[EMIM]OAc	[ EMIM] DMP	[ DBNH]OAc
0 %	64.9 + 2.5	64.9 + 2.5	64.9 + 2.5
15 %	97.8 + 4	50.9 + 3.2	85.6 + 4.3
35 %	94.3 + 2.1	45.7 + 2.8	46.7 + 3.7

### Literature

1. Li, H., Kankaanpää, A., Hummel, M., Sixta, H., Xiong, H. and Turunen, O. (2013) Thermostabilization of extremophilic *Dictyoglomus thermophilum* GH11 xylanase by an N-terminal disulphide bridge and the effect of ionic liquid [emim]OAc on the enzymatic performance. *Enzyme Microb. Technol.* 53: 414-419.
2. Chawachart, N., Anbarasan, S., Turunen, S., Li, H., Khanongnuch, C., Hummel, M., Sixta, H., Granström, T., Lumyong, S. and Turunen, O. (2014) Thermal behavior of GH10 Xylanase from *Thermoascus aurantiacus* SL16W and tolerance to ionic liquid [emim]OAc. *Extremophiles* 18: 1023-1034.

## Swollenin from *Trichoderma reesei* exhibits hydrolytic activity against cellulosic substrates with features of both endoglucanases and cellobiohydrolases

Martina Andberg, Merja Penttilä, and Markku Saloheimo

[martina.andberg@vtt.fi](mailto:martina.andberg@vtt.fi)

VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Espoo, Finland.

The cellulolytic and hemicellulolytic enzymes of *Trichoderma reesei* comprise one of the best characterised enzyme systems involved in lignocellulose degradation. In this work, swollenin (SWOI), a protein recognised based on its sequence similarity with plant expansins, has been characterised [1]. As most of the *T. reesei* cellulases, SWOI is composed of two modules, an N-terminal cellulose binding module (CBM) connected by a linker region to an expansin-like catalytic module [2]. SWOI and its catalytic domain were subjected to analysis of their hydrolytic activity on different soluble carbohydrate polymers. By measuring the production of reducing ends, zymogram-, and viscosity analysis, SWOI was shown to have activity on substrates containing  $\beta$ -1,4 glucosidic bonds, *i.e.* carboxymethyl cellulose, hydroxyethyl cellulose and  $\beta$ -glucan. The formation of oligosaccharides from  $\beta$ -glucan was analysed by HPLC and showed cellobiose as the main reaction product. SWOI was also able to hydrolyse soluble cello-oligosaccharides and the products formed were all consistent with SWOI cleaving a cellobiose unit off the substrate. In conclusion, the *T. reesei* swollenin showed a unique mode of action with similarities with action of both endoglucanases and cellobiohydrolases.

### Acknowledgements

The work was supported by the Academy of Finland (Project 52439).

### Literature

1. Andberg, M., Penttilä, M., Saloheimo M. (2015) *BITE*, in press
2. Saloheimo, M., Paloheimo, M., Hakola, S., Pere, J., Swanson, B., Nyssönen, E., Bhatia, A., Ward, M., Penttilä, M. (2002) *Eur. J. Biochem.* 269, 4202–4211.

## Characterization of a GH62 $\alpha$ -L-arabinofuranosidase from *Aspergillus nidulans*: Linking functional diversity with phylogenetics

*Susan Andersen*<sup>1</sup>, *Casper Wilkens*<sup>1,2</sup>, *Bent O. Petersen*<sup>3</sup>, *Barry McCleary*<sup>4</sup>, *Ole Hindsgaul*<sup>3</sup>, *Maher Abou Hachem*<sup>1</sup> and *Birte Svensson*<sup>1</sup>

su@bio.dtu.dk (SA), cw@bio.aau.dk (CW) or bis@bio.dtu.dk (BS)

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
2. Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Faculty of Engineering and Science, Aalborg University, Copenhagen, Denmark
3. Carbohydrate Chemistry Group, Carlsberg Laboratory, Valby, Denmark
4. Megazyme International, Bray, Co. Wicklow, Ireland

L-arabinofuranose (Araf) residues are abundant in plant cell walls as L-arabinan chains of pectin and side chains in arabinoxylan, arabinogalactan and gum arabic. Removal of Araf residues is important in complete degradation of plant material in various industrial processes such as bioethanol production (1). The present *Aspergillus nidulans* L-arabinofuranosidase (AnAbf62A-m2,3) of glycoside hydrolase family 62 (GH62) displays unusually high activity for low viscosity wheat arabinoxylan and moderate ability to hydrolyse sugar beet L-arabinan and 4-nitrophenyl- $\alpha$ -L-arabinofuranoside. NMR studies revealed that AnAbf62A-m2,3 hydrolyses Araf from singly  $\alpha$ -1,2 and  $\alpha$ -1,3-substituted xylose residues releasing  $\alpha$ -1,3-Araf three times faster than  $\alpha$ -1,2-Araf. It has been shown that arabinoxylans that contain singly  $\alpha$ -1,2 and  $\alpha$ -1,3-Araf have to be oriented oppositely for hydrolysis (2). The slower rate of  $\alpha$ -1,2-Araf release may thus reflect that arabinoxylan binds weaker in the orientation that is compatible with hydrolysis of  $\alpha$ -1,2-Araf from xylose of the backbone. NMR analysis further showed that AnAbf62A-m2,3 releases product with inverted anomeric configuration, as for the GH43 (3), the other clan GH-F member (RW.ERROR - Unable to find reference:33).

Finally, phylogeny and analysis with the Peptide Pattern Recognition (PPR) tool revealed that sequences within the GH62 are divide into tree distinctive subfamilies, which differ from the two subfamilies, which the GH62 family was previously divided into (5). The PPR analysis further revealed sequence motifs that distinguish the three subfamilies, which might be important for the functionality of the GH62s within each subfamily.

### Acknowledgements

This work is supported by the Danish Council for Independent Research | Natural Sciences (FNU) (to BS), a PhD fellowship from the Technical University of Denmark (to SA), and a joint PhD fellowship from the Technical University of Denmark and FNU (to CW).

### Literature

1. Jordan, D. B., *et al.*, Biochem. J. 442 (2012) 241-252
2. Wang, W., *et al.*, Appl. Environ. Microbiol. 80 (2014) 5317-5329
3. Pitson, S. M. *et al.*, FEBS Lett. 398 (1996) 7-11
4. Lombard, V., *et al.*, Nucleic. Acids Res. 42 (2014) D490–D495
5. Siguier B., J. Biol. Chem. 289 (2014) 5261-5273

## Efficient chemoenzymatic synthesis of antioxidants using feruloyl esterases in detergentless microemulsions

*Io Antonopoulou<sup>1</sup>, Evangelos Topakas<sup>2</sup>, Laura Leonov<sup>3</sup>, Ulrika Rova<sup>1</sup>, Paul Christakopoulos<sup>1</sup>*

paul.christakopoulos@ltu.se

1. Biochemical Process Engineering, *Division of Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, SE-97187, Sweden*
2. Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 15700, Greece
3. Dyadic Netherlands, Nieuwe Kanaal 7-S, 6709 PA Wageningen, the Netherlands

Feruloyl esterases (FAEs, E.C. 3.1.1.73) have been proved appealing biocatalysts not only for the degradation of plant biomass but also for the modification of compounds with potential use in food, cosmetic and pharmaceutical industries <sup>[1-4]</sup>. The enzymatic synthesis of biologically active compounds with antioxidant activity using carbohydrate esterases as biocatalysts allows more flexible process configurations such as lower temperatures (50-60°C) comparing to the counterpart chemical process (150°C), one step production of one product instead of mixtures and no need of by-product and catalyst residues removal.

Aim of this work is the optimization of reaction conditions for the efficient synthesis of the following biologically active compounds: prenyl ferulate, prenyl caffeate, glyceryl ferulate and 5-*O*-(trans-feruloyl)-arabinofuranose. The products were obtained by the enzymatic transesterification of a ferulate ester with aliphatic alcohols or sugars and present improved hydrophobicity/hydrophilicity comparing to the starting antioxidant. For boosting the FAEs' synthetic pathway, non-conventional media with low water content are required. For this purpose, a ternary system of n-hexane/alcohol/water forming detergentless microemulsions <sup>[5]</sup> was selected, where reaction conditions were optimized regarding the best temperature, solvent composition and substrate/enzyme ratio. The possibility of implementing alternative reaction media such as ionic liquids, organic solvents, oil-in water microemulsions was also evaluated.

### Acknowledgements

The financial support of FP7 KBBE. 2013.3.3-04 OPTIBIOCAT is gratefully acknowledged. The tested FAEs were supplied by Dyadic, Netherlands. Vinyl ferulate and vinyl caffeate were supplied by Taros Chemicals, Germany.

### Literature

1. Topakas E, Stamatis H, Biely P, Kekos D, Macris BJ, Christakopoulos P. (2003). *J. Biotechnol.* 102:33-44.
2. Topakas E, Vafiadi C, Stamatis H, Christakopoulos P. (2005). *Enzyme Microb. Technol.* 36:729-736
3. Vafiadi C, Topakas E, Alissandratos A, Faulds CB, Christakopoulos P. (2008). *J. Biotechnol.* 133: 497-504
4. Topakas E, Vafiadi C, Christakopoulos P. (2007). *Proc Biochem.* 42:497-509
5. Khmelnitsky YL, Hilhorst R, Veeger C. (1988). *Eur. J. Biochem.* 176: 265-271

## Alkyl mannosides produced by alcoholysis with $\beta$ -mannanases from the fungi *Trichoderma reesei* and *Aspergillus nidulans*

Anna Aronsson<sup>1</sup>, Johan Svantesson Sjöberg<sup>1</sup>, Eva Nordberg Karlsson<sup>2</sup>, Patrick Adlercreutz<sup>2</sup> and Henrik Ståhlbrand<sup>1</sup>

anna.aronsson@biochemistry.lu.se

1. Biochemistry and Structural Biology, Lund University, PO Box 124, S-221 00, Lund, Sweden

2. Biotechnology, Lund University, PO Box 124, S-221 00, Lund, Sweden

Biomass has the potential to substitute fossil resources, the main cause of anthropogenic climate change, in numerous sectors, e.g. energy and chemicals. It is, therefore, important to proceed the development of biotechnology. Alkyl glycosides are a group of biodegradable and non-toxic compounds with surfactant potential. It is possible to enzymatically synthesise alkyl glycosides from carbohydrates and alcohols with glycoside hydrolases [1]. With the aim to produce a set of alkyl mannosides possessing different properties, three fungal  $\beta$ -mannanases were selected based on the difference in hydrolysis products and previously shown alcoholysis capacities; *TrMan5A* and its mutant R171K from *Trichoderma reesei* and *AnMan5C* from *Aspergillus nidulans* [2,3]. Alkyl mannosides produced by the enzymes were detected with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS). With methanol as acceptor and mannotetraose as donor substrate *AnMan5C* produced methyl mannobioside as the unique alcoholysis product and showed the highest efficiency of alcoholysis. With *TrMan5A* R171K it was possible to synthesise methyl mannotrioside to a significant extent. Ongoing work using hexanol as acceptor have resulted in the synthesis of hexyl mannobioside by both *TrMan5A* and *AnMan5C*. Forthcoming work intends to use an organic solvent to increase the yield of hexyl mannosides. A decrease in water activity can favour alcoholysis over hydrolysis as seen in a study of a  $\beta$ -glucanase where acetonitrile was used [4]. Moreover, hexyl mannobiose was detected with reversed phase chromatography. This technique is planned to be further used together with nuclear magnetic resonance (NMR) to quantify this new potential surfactant that is environmentally friendly produced.

### Acknowledgements

Pontus Lundemo is thanked for his supervision in connection to reversed phase chromatography. The study is supported by the BIOSTREAM research project (supported by VINNOVA 2013-0324).

### Literature

1. von Rybinski, W. and K. Hill, *Alkyl Polyglycosides—Properties and Applications of a new Class of Surfactants*. Angewandte Chemie International Edition, 1998. 37(10): p. 1328-1345
2. Rosengren, A., et al., *The role of subsite +2 of the Trichoderma reesei  $\beta$ -mannanase TrMan5A in hydrolysis and transglycosylation*. Biocatalysis and Biotransformation, 2012. 30(3): p. 338-352.
3. Rosengren, A., et al., *An Aspergillus nidulans  $\beta$ -mannanase with high transglycosylation capacity revealed through comparative studies within glycosidase family 5*. Applied Microbiology and Biotechnology, 2014.
4. Akiba, S., K. Yamamoto, and H. Kumagai, *Transglycosylation activity of the endo- $\beta$ -1,4-glucanase from Aspergillus niger IFO31125 and its application*. Journal of Bioscience and Bioengineering, 1999. 87(5): p. 576-580

## Development of microbial production processes for levan polysaccharide

*Ozlem Ates<sup>1</sup> and Ebru Toksoy Oner<sup>2</sup>*

ozlem.ates@nisantasi.edu.tr

1. Department of Medical Services and Techniques, Nisantasi University, Istanbul, Turkey

2. Department of Bioengineering, Marmara University, Istanbul, Turkey

Levan is a  $\beta(2-6)$ -linked fructose homopolysaccharide that is extracellularly produced from sucrose-based substrates by a variety of microorganisms. As a microbial EPS, levan has valuable properties like water solubility, strong adhesivity, film-forming ability, low viscosity, high solubility in oil, compatibility with salts and surfactants, stability to heat, stability to acid and alkali, high holding capacity for water and chemicals, and good biocompatibility(1). Due to its outstanding properties levan that is mainly associated with high-value applications, has a high commercialization potential for various sectors like pharmaceutical, chemical and food industries and there is a growing body of interest in research associated with levan and its applications. Since it is microbially produced using mesophiles at very high production costs, economic hurdles need to be overcome (2). Hence development of novel microbial production processes for levan holds great industrial importance. Recently, halophilic bacterium *Halomonas smyrnensis* AAD6<sup>T</sup> has been reported as a high-level levan producer extremophile for the first time by our research group. Considering the advantages associated with the use of a halophilic producer organism and high industrial potential of levan biopolymer, research activities were focused on developing feasible and low-cost production processes. Using both conventional and systems based approaches production yields in semi-chemical media were substantially improved. Improved production yields with concomitant reduction in production costs were obtained using various biomass resources and sucrose containing sugary wastes as low-cost substrates. Moreover, genome-scale metabolic model of *Chromohalobacter salexigens* was refined for reconstruction of the generic metabolic model of *H. smyrnensis* AAD6<sup>T</sup> to elucidate the relationship between levan biosynthesis and other metabolic processes. Results of the metabolic systems analysis indicated the stimulatory effect of mannitol on levan biosynthesis which was further verified experimentally (1). Furthermore, the systematical analysis was performed to figure out the effects of basal medium components and boric acid was found to be the most effective stimulator of levan production (2).

### Acknowledgements

The financial support of The Scientific and Technological Research Council of Turkey (TUBITAK) through project 114M239 is gratefully acknowledged.

### Literature

1. Ates, O., Arga, K. Y., and Toksoy Oner, E. "The stimulatory effect of Mannitol on levan biosynthesis: lessons from metabolic systems analysis of *Halomonas smyrnensis* AAD6<sup>T</sup>", Biotechnol. Prog., 29, 1386e1397 (2013).
2. Kazak Sarilmiser, H., Ates, O., Ozdemir, G., Arga, K.Y. and Toksoy Oner, E. "Effective stimulating factors for microbial levan production by *Halomonas smyrnensis* AAD6<sup>T</sup>", Journal of Bioscience and Bioengineering, doi: 10.1016/j.jbiosc.2014.09.019 (2014).

## Roles of starch and sucrose in exopolysaccharide formation by *Lactobacillus reuteri*

*Yuxiang Bai*<sup>1</sup>, *Justyna M. Dobruchowska*<sup>1</sup>, *Rachel M. van der Kaaij*<sup>1</sup>, *Albert Woortman*<sup>2</sup>, *Johannis P. Kamerling*<sup>1</sup>, *Lubbert Dijkhuizen*<sup>1</sup>

y.bai@rug.nl

1. Department of Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

2. Department of Macromolecular Chemistry and New Polymeric Materials, Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Oral diseases such as dental caries and periodontitis occur when the equilibrium of the indigenous bacteria is compromised in the oral cavity (1). A strong correlation has been established between the saliva lactobacilli count and salivary constituents with dietary sugars (2). Glucansucrase (GS) enzymes secreted from lactic acid bacteria play an important role by converting sucrose into  $\alpha$ -glucan exopolysaccharides (EPS) that function in dental biofilms (3, 4). Maltose and malto-oligosaccharides, produced from starch by salivary  $\alpha$ -amylase, also act as acceptor substrates during  $\alpha$ -glucan synthesis by GS. The combination of sucrose and starch is potentially more cariogenic than either compound alone. Glycoside hydrolase family 70 includes not only GS enzymes, but also the recently described 4,6- $\alpha$ -glucanotransferase (4,6- $\alpha$ -GTase) enzymes which are inactive with sucrose but cleave ( $\alpha$ 1-4) linkages in starch (hydrolysates) and synthesize  $\alpha$ -glucans with increased percentages of ( $\alpha$ 1-6) linkages (5). Both GS and 4,6- $\alpha$ -GTase enzymes exclusively occur in lactic acid bacteria. Occasionally, they are found in the same strain, e.g. GS GTFA and 4,6- $\alpha$ -GTase GTFB in *Lactobacillus reuteri* 121 (5).

Besides the previously characterized GS GTFA EPS35-5 produced from sucrose, feeding *L. reuteri* 121 with starch hydrolysates as carbon source also resulted in EPS synthesis produced by 4,6- $\alpha$ -GTase GTFB. SEC results showed that the size of both EPS products reached up to 50 MDa. *In vitro* incubations of the *L. reuteri* 121 GTFA plus GTFB enzymes with sucrose plus starch (hydrolysate) yielded initial oligosaccharides and EPS with different structures compared to the products obtained from either substrate alone, evident from 1D/2D <sup>1</sup>H and <sup>13</sup>C NMR, TOFMS, as well as HPAEC analyses. To sum up, EPS formed by *L. reuteri* 121 varies, reflecting the individual GS GTFA (with sucrose) and 4,6- $\alpha$ -GTase GTFB (with starch hydrolysates) activities, but also the cooperation of both enzymes in the presence of both major dietary sugars, sucrose and starch.

### Literature

1. Badet, C., Thebaud, N. B.: *Open J. Microbiol.*, 2, 38-48 (2008).
2. Klein, M. I., Duarte, S., Xiao, J., Mitra, S., Foster, T. H., Koo, H.: *App. Environ. Microbiol.*, 75, 837-41 (2009).
3. Vujičić-Žagar, A., Pijning, T., Kralj, S., Lopez, C. A., Eeuwema, W., Dijkhuizen, L., Dijkstra, B. W.: *P. Natl. Acad. Sci. USA*, 107, 21406-21411 (2010).
4. Rolla, G.: *Scand. J. Dent. Res.*, 97, 115-119 (1989).
5. Kralj, S., Grijpstra, P., van Leeuwen, S. S., Leemhuis, H., Dobruchowska, J. M., van der Kaaij, R. M., Malik, A., Oetari, A., Kamerling, J. P., Dijkhuizen, L.: *App. Environ. Microbiol.*, 77, 8154-8163 (2011).



## Towards the set-up of a recombinant protein production facility for fungal carbohydrate-active enzymes using the yeast *Pichia pastoris*

Mireille Haon<sup>1,2</sup>, Sacha Grisel<sup>1,2</sup>, David Navarro<sup>1,2</sup>, Antoine Gruet<sup>1,2,3</sup>, Jean Guy Berrin<sup>1,2,\*</sup>, Christophe Bignon<sup>4,5,\*</sup>

jean-guy.berrin@univ-amu.fr ; bignon@afmb.univ-mrs.fr

1. INRA, UMR 1163 Biodiversité et Biotechnologie Fongiques, 13288 Marseille, France.
2. Aix-Marseille Université, Polytech Marseille, UMR1163 Biodiversité et Biotechnologie Fongiques, 13288 Marseille, France.
3. The Rockefeller University, Laboratory of Protein and Nucleic Acid Chemistry, 1230 York Avenue, New York, NY 10065 USA
4. Aix-Marseille University, Architecture et Fonction des Macromolécules Biologiques (AFMB) UMR 7257, 13288, Marseille, France
5. CNRS, AFMB UMR 7257, 13288, Marseille, France

Filamentous fungi are the predominant source of lignocellulolytic enzymes used in industry for the transformation of plant biomass into high-value molecules and biofuels. The rapidity with which new fungal genomic and post-genomic data are being produced is vastly outpacing functional studies. This underscores the critical need for producing enzymes lacking confident functional annotation, a prerequisite to their functional and structural study. The yeast *Pichia pastoris* has become increasingly popular as a host for the production of fungal carbohydrate-active enzymes (CAZymes). Therefore, the objective of this study was to try to further streamline recombinant protein expression in *Pichia pastoris*.

Fungal CAZymes glycoside hydrolase (GH) 5, GH11, and GH45 (Couturier et al., 2011 a and b) were used to evaluate the following steps of the protocol set-up by Invitrogen<sup>TM</sup> for expressing recombinant proteins in *P. pastoris*: (i) expression constructs, (ii) selection and (iii) culture of transformants, (iv) protein purification and (v) expression conditions. The main achievements reported in this study are (i) a plating-free selection of transformants using frozen cells, (ii) the miniaturization of liquid cultures to deep well microplate format using both inducible (AOX1) and constitutive (GAP) promoters and (iii) the automated purification of secreted His-tagged recombinant proteins.

We have set-up a shorten protocol for expressing fungal CAZymes in *P. pastoris* that is applicable to other proteins.

### Acknowledgements

This study was funded by the French National Research Agency (ANR FUNLOCK ANR-13-BIME-0002-01).

### Literature

1. Couturier M, Feliu J, Haon M, Navarro D, Lesage-Meessen L, Coutinho PM, Berrin JG: A thermostable GH45 endoglucanase from yeast: impact of its atypical multimodularity on activity. Microb Cell Fact 2011a, 10:103.
2. Couturier M, Haon M, Coutinho PM, Henrissat B, Lesage-Meessen L, Berrin JG. Podospora anserina hemicellulases potentiate the *Trichoderma reesei* secretome for saccharification of lignocellulosic biomass. Appl Environ Microbiol 2011b, 77:237-46.

## Structural analysis of chitin oligosaccharide deacetylases – the “subsite capping model”

*Xevi Biarnés<sup>1</sup>, Hugo Aragunde<sup>1</sup>, David Albesa-Jové<sup>2</sup>, Marcelo Guerin<sup>2</sup>, and Antoni Planas<sup>1</sup>*

antoni.planas@iqs.edu

1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, 08017 Barcelona, Spain.
2. Unidad de Biofísica, Centro Mixto Consejo Superior de Investigaciones Científicas-Universidad del País Vasco/Euskal Herriko Unibertsitatea, 48940 Leioa, Bizkaia, Spain

Chitin deacetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and chito oligosaccharides (COSs). A major challenge is to understand how CDAs specifically define the distribution of GlcNAc and GlcNH<sub>2</sub> moieties in the oligomeric chain. The particular acetylation patterns of natural COSs dictate their role in cell signaling and other biological activities.

We present here the structural bioinformatics analysis of this family of enzymes that lead us to the proposal of a model to rationalize the de-*N*-acetylation specificity exhibited by different CDAs: the “subsite capping model” [1]. According to this model, the acetylation pattern exhibited by different CDAs is governed by critical loops that shape and differentially block accessible subsites in the binding cleft of these enzymes.

### Literature

1. Andrés E., Albesa-Jové D., Biarnés X., Moerschbacher B.M., Guerin M.E., Planas A. Structural basis of chitin oligosaccharide deacetylation. *Angew. Chem. Int. Ed. Engl.* 53, 6882-6887 (2014).

The abstract has been withdrawn

## HEXPIN: Hetero-exopolysaccharide – milk protein interactions

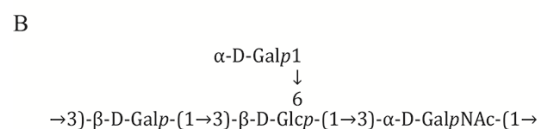
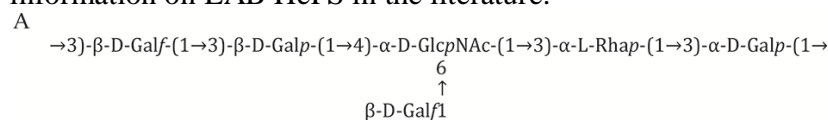
*Johnny Birch<sup>1</sup>, Hörður Kári Harðarson<sup>1</sup>, Maher Abou Hachem<sup>1</sup>, Richard Ipsen<sup>2</sup>, Marie-Rose Van Calsteren<sup>3</sup>, Christel Garrigues<sup>4</sup>, Kristoffer Almdal<sup>5</sup>, Birte Svensson<sup>1</sup>*

Birch@bio.dtu.dk

1. Enzyme and Protein Chemistry, Dept. of Systems Biology, Technical University of Denmark, DK- 2800 Kgs. Lyngby, Denmark.
2. Dept. of Food Science, University of Copenhagen, DK-1958 Frederiksberg C, Denmark.
3. Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec J2S 8E3, Canada.
4. CED-Discovery, Chr Hansen A/S, DK-2970 Hørsholm, Denmark.
5. Dept. of Micro- and Nanotechnology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

Today's food industry often uses restricted chemically modified polysaccharides (PS) in dairy products as thickeners and stabilizers. Hetero-exopolysaccharides (HePS) excreted by various lactic acid bacteria (LAB) strains are *generally recognized as safe* (GRAS) and have a similar positive impact on the textural properties in fermented milk products [1]. The textural effect stems in part from associative complexation between HePS and milk proteins, both caseins and whey proteins which complex with the casein micelle in milk during thermal processing. However, the molecular basis for the HePS-protein interactions and impact on textural properties is poorly understood and there is demand for understanding the HePS-protein interaction in order to be able to naturally increase viscosity in the final consumer product.

We produced and purified a series of HePS of known structures in yields of 41–135 mg l<sup>-1</sup> from different *Streptococcus thermophilus* strains and 8–10 mg l<sup>-1</sup> from *Lactobacillus rhamnosus* GG grown in skimmed milk medium. By using surface plasmon resonance (SPR), binding of the HePS to the whey proteins  $\beta$ -lactoglobulin or  $\alpha$ -lactalbumin was monitored at different pH, ionic strength and temperature. HePS from *Lactobacillus rhamnosus* GG (A) and *Streptococcus thermophilus* Sfi6 (B) demonstrated an optimum in the binding capacity around pH 4.0 and 70 mM NaCl and decreasing binding capacity with increasing temperature in the range 15–35°C. Interaction at pH 4.0 between HePS A and B and native or heat denatured  $\beta$ -lactoglobulin was supported by retardation in affinity gel electrophoresis. As a tool for exploring the potential of the structural variations represented by HePS, we established a database of structural and biophysical properties based on information on LAB HePS in the literature.



## Acknowledgements

HEXPIN activities are supported by the Danish Research Council for Independent Research | Technical and Production Sciences and | Natural Science and a joint PhD stipend from DTU (to JB).

## Literature

- 1 Badel, S., Bernardi, T. and Michaud, P. (2011). *Biotechnol Adv*, Elsevier Inc. **29**, 54–66.

## A single point mutation near the active center is responsible for high efficiency of the *Thermotoga maritima* $\alpha$ -galactosynthase in the synthesis of known amylase substrate

Kirill Bobrov<sup>1</sup>, Anna Borisova<sup>1,2</sup>, Elena Eneyskaya<sup>1</sup>, Dina Ivanen<sup>1</sup>, Daria Chervyakova<sup>1</sup>, Konstantin Shabalin<sup>1,3</sup>, Georgy Rychkov<sup>1,3</sup> and Anna Kulminskaya<sup>1,3</sup>

bobrov@omrb.pnpi.spb.ru

1. National Research Center “Kurchatov Institute”, B.P.Konstantinov Petersburg Nuclear Physics Institute, Gatchina, Russia
2. Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden
3. St.-Petersburg State Polytechnical University, St.-Petersburg, Russia

Alpha-amylase is an enzyme that helps to digest starch and glycogen. It is produced mainly by exocrine pancreas and salivary glands. For many years, the determination of the level of alpha-amylase in serum and plasma has been used in patients to diagnose acute pancreatitis. Early methods were based on the titration of iodine and a change in the optical density of the highest dilution of a complex between iodine and starch, cleavable by alpha-amylase. Now, most of the methods are based on the production of *p*-nitrophenol from a saturated oligosaccharide substrate with blocking groups attached to the ends of sugar molecules. The maltooligosaccharides with chromophoric group are perspective substrates for the  $\alpha$ -amylases. They don't need the additional enzymes for their hydrolysis, so that further polymerization of products and substrate doesn't occur. Here, we present enzymatic approach for the synthesis of known substrate for the  $\alpha$ -amylase based on the use of the modified  $\alpha$ -galactosidase from the hyperthermophilic bacteria *Thermotoga maritima*. This enzyme is well studied and the  $\alpha$ -galactosynthase derived from it was recently reported [1]. Recently, we have reported that GH family 36  $\alpha$ -galactosidase from *Thermotoga maritima* demonstrates ability to form different  $\alpha$ -galactosidic linkages and predicted several single amino acid substitutions leading to changes in the transglycosylation patterns of this enzyme [2,3]. On the basis of simple assumptions made during molecular modeling, residues were identified whose mutation could yield increased (1,2)-regioselectivity of transglycosylation. Two mutations indeed produced mainly  $\alpha$ (1,2)-linked galactosides using *p*-nitrophenyl  $\alpha$ -D-galactopyranoside as a substrate in self-condensation reactions. Here, we present a simple protocol for the synthesis of 2-chloro-nitrophenyl  $\alpha$ -galactomaltoside using  $\alpha$ (1,2)-specific galactosylsynthase produced on the basis of the enzyme and show a role of the P402 in the binding of the acceptor, 2-chloro-4-nitrophenyl-maltoside, in the enzyme active center.

### Literature

1. B. Cobucci-Ponzano, C. Zorzetti, A. Strazzulli, S. Carillo, E. Bedini, M. Corsaro, D. Comfort, R. Kelly, M. Rossi, and M. Moracci. Glycobiology vol. 21 no. 4 pp. 448–456, 2011.
2. K. S. Bobrov, A. S. Borisova, E. V. Eneyskaya, D. R. Ivanen, K. A. Shabalin, A. A. Kulminskaya, G. N. Rychkov. Biochemistry (Mosc). 2013 Oct;78(10):1112-23
3. Borisova AS, Ivanen DR, Bobrov KS, Eneyskaya EV, Rychkov GN, Sandgren M, Kulminskaya AA, Sinnott ML, Shabalin KA. Carbohydr Res. 2015 Jan 12;401:115-21.

## Insights into LPMO diversity from structural and functional characterization of NcLPMO9C, a broad-specificity lytic polysaccharide monooxygenase

*Anna S. Borisova*<sup>1</sup>, *Trine Isaksen*<sup>2</sup>, *Maria Dimarogona*<sup>1</sup>, *Aniko Varnai*<sup>2</sup>, *Morten Sørli*<sup>2</sup>, *Aasmund K. Røhr*<sup>2</sup>, *Christina M. Payne*<sup>1,3</sup>, *Jerry Ståhlberg*<sup>1</sup>, *Mats Sandgren*<sup>1</sup>, *Vincent G. H. Eijsink*<sup>2</sup>

Mats Sandgren <mats.sandgren@slu.se> , Vincent Eijsink <vincent.eijsink@nmbu.no>

1. Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
2. Department of Chemistry, Biotechnology, and Food Science, Norwegian University of Life Sciences, P. O. Box 5003, N-1432 Ås, Norway.
3. Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506, USA.

Recently it was shown that oxidative hydrolysis of cellulose is catalyzed by so called Lytic Polysaccharide MonoOxygenases (LPMOs)[1] which play an essential role in plant biomass degradation along with well-studied cellulases[2-4]. LPMOs are copper-dependent biocatalysts that use molecular oxygen and an electron donor to break glycosidic bonds[5-7]. Electrons may be provided by a reducing agent such as ascorbic acid, gallate or reduced glutathione, or by a co-secreted enzyme such as cellobiose dehydrogenase. We have conducted structural and functional studies of an LPMO from *Neurospora crassa*, NcLPMO9C, that is unique in that is active on soluble cello-oligosaccharides and hemicellulosic substrates, in addition to cellulose[8]. NcLPMO9C is a two-domain protein, containing an N-terminal LPMO-domain and a family 1 carbohydrate-binding module (CBM1) that are connected through a serine- and threonine-rich linker comprising approximately 50 residues. The crystal structure of the catalytic domain shows an extended and highly polar substrate-binding surface that conceivably has the possibility to interact with a variety of sugar substrates. The structure also revealed features that are likely responsible for the strict oxidative regioselectivity of NcLPMO9C (C4 oxidizing). The unique ability of NcLPMO9C to act on soluble substrates was exploited to study enzyme-substrate interactions. ITC studies revealed binding affinities in the low micromolar range for polymeric substrates. Truncation of the CBM1 reduced these affinities and the activity on polymeric tamarind xyloglucan, while activity on phosphoric acid-swollen cellulose did not change. Analyses of the copper site by EPR spectroscopy, copper affinity determinations by ITC, and determination of the redox potential yielded results similar to those obtained previously for cellulose-active LPMOs, indicating that the unique functionality of NcLPMO9C is primarily due to its extended polar substrate-binding surface and, to some extent, the CBM1.

### Literature

1. Horn, S.J., et al., *Biotechnol Biofuels*, 2012. 5(1): p. 45.
2. Forsberg, Z., et al., *Protein Sci*, 2011. 20(9): p. 1479-83.
3. Harris, P.V., et al., *Biochemistry*, 2010. 49(15): p. 3305-16.
4. Vaaje-Kolstad, G., et al., *Science*, 2010. 330(6001): p. 219-22.
5. Aachmann, F.L., et al., *Proc Natl Acad Sci U S A*, 2012. 109(46): p. 18779-84.
6. Beeson, W.T., et al., *J Am Chem Soc*, 2012. 134(2): p. 890-2.
7. Quinlan, R.J., et al., *Proc Natl Acad Sci U S A*, 2011. 108(37): p. 15079-84.
8. Isaksen, T., et al., *J Biol Chem*, 2014. 289(5): p. 2632-42.

## How to quantify enzyme activity and kinetics in "non-bulk" systems? An example through the enzymatic hydrolysis of hemicellulose thin films

Amal Zeidi<sup>1,2,3</sup>, Lucie Dianteill,<sup>1,2,3</sup> Claire Dumon<sup>1,2,3</sup> Cédric Montanier,<sup>1,2,3</sup> Régis Fauré,<sup>1,2,3</sup> Jérôme Morchain,<sup>1,2,3</sup> Nouredine Lebaz,<sup>1,2,3</sup> Childéric Séverac,<sup>4,5</sup> Antoine Bouchoux<sup>1,2,3</sup>

Antoine.Bouchoux@insa-toulouse.fr

1. Univ. de Toulouse, INSA, UPS, INP, LISBP, 135 av. de Rangueil, F-31077 Toulouse, France
2. INRA, UMR792 Ing. des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
3. CNRS, UMR5504, F-31400 Toulouse, France
4. CNRS, ITAV-USR 3505, F-31106 Toulouse, France
5. Univ. de Toulouse, ITAV-USR 3505, F-31106 Toulouse, France

In the carbohydrate-based bioindustry, enzymes are often used in conditions where they have to work on solid surfaces and/or penetrate within structures that are locally highly concentrated. The effect of such physical constraints on the enzyme activity and kinetics is however poorly understood; mostly because following and quantifying the hydrolysis in such conditions is still a challenge.

With this work, our intention is to provide a detailed characterization of an enzyme's activity when its substrate is both concentrated and immobilized at a solid interface. This is essentially done by monitoring the *in-situ* degradation of a thin film of a model hemicellulose using a Quartz Crystal Microbalance with Dissipation (QCM-D).

The thin film is composed of a unique arabinoxylan, extracted from wheat bran, and that is chemically modified for covalently binding onto gold.<sup>1</sup> The film is partly swollen by water, and its water content (hence its local dry concentration) can be tuned by partially removing the L-arabinofuranosyl units that decorate the xylan chain.<sup>2</sup> The film is then put into contact with a solution containing an endo-1,4- $\beta$ -xylanase (*NpXyn11A*<sup>3</sup>), into the QCM-D cell, and the loss of mass in the film is followed with time as degradation occurs. Using mathematical models that are under development in our laboratory, we aim at converting the raw QCM-D data into kinetics curves that give the reaction rate as a function of the polymer concentration in the film. Such a procedure would allow us to accurately compare the behavior of the enzyme in a film with its "bulk" behavior; the latter having been characterized classically with a dilute solution of the same substrate. Our results should reveal precious indications about the effect of substrate immobilization and conformation/concentration on the action of an enzyme.

### Literature

1. Nordgren N., Eklöf J., Zhou Q., Brumer H., Rutland M.W., *Biomacromolecules* 2008, 9, 942–948
2. Köhnke T., Östlund Å., Brelid H., *Biomacromolecules* 2011, 12, 2633–2641
3. Vardakou M., Dumon C., Murray J.W., Christakopoulos P., Weiner D.P., Juge N., Lewis R.J., Gilbert H.J., Flint J.E., *J. Mol. Biol.* 2008, 375, 1293–1305

## The CBMomes of cellulolytic bacteria colonizing different ecological niches present distinct carbohydrate specificities

*Joana L.A. Brás<sup>1</sup>, Diana Ribeiro<sup>2</sup>, Maria J. Romão<sup>2</sup>, Ana L. Carvalho<sup>2</sup>, Wengang Chai<sup>3</sup>, Yan Liu<sup>3</sup>, Ten Feizi<sup>3</sup>, José A.M. Prates<sup>1,4</sup>, Luís M.A. Ferreira<sup>1,4</sup>, Carlos M.G.A. Fontes<sup>1,4</sup>, Angelina S. Palma<sup>2</sup>*

joana.bras@nzytech.com

1. NZYTech - genes & enzymes - Estrada do Paço do Lumiar, Campus do Lumiar, Edif. E, R/C, 1649-038 Lisboa, Portugal
2. UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
3. Glycosciences Laboratory, Department of Medicine, Imperial College London, London W12 0NN, United Kingdom
4. CIISA – Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal

Plant cell walls, predominantly composed of cellulose, are the most abundant source of organic carbon on earth. The energetic constraints posed by anaerobic ecosystems lead to the evolution of a remarkable highly efficient supramolecular enzyme complex, termed cellulosome<sup>1</sup>. Cellulosomes are multi-enzyme complexes of Carbohydrate Active enZymes (CAZymes) and play a pivotal role in the degradation of plant cell wall carbohydrates. *Clostridium thermocellum* and *Ruminococcus flavefaciens* are two highly efficient cellulolytic bacteria that produce very complex but well characterized cellulosomes. The two bacteria colonize different, highly dynamic and populated ecological niches, the soil and the rumen of mammals, respectively. Here we have cloned, expressed and purified all known Carbohydrate Binding Modules (CBMs), the CBMome, of *C. thermocellum* and *R. flavefaciens*. Ligand specificity of the two CBMomes was analysed using a carbohydrate microarray platform<sup>2,3</sup>. derived from target polysaccharide glycomes. This technique enabled the screening of a wide range of protein-carbohydrate interactions in a high-throughput manner using only minute amounts of sample. The data revealed that the two bacteria present CBMomes expressing different carbohydrate-binding capacities. Overall this work suggests that the physico-chemical properties of different ecological niches modulate the evolution of CAZymes presenting distinct ligand specificities.

### Literature

1. Fontes, C. M., Gilbert, H. J. 2010. *Annu. Rev. Biochem.*, 79, 655-681.
2. Palma, A.S., Feizi, T., Childs, R.A., Chai, W., Liu, Y. 2014. *Curr. Opin. Chem. Biol.*, 18C, 87-94.
3. Palma, A. S., Liu Y., Zhang H., Zhang Y., McCleary B. V., Yu G., Huang Q., Guidolin L. S., Ciocchini A. E., Torosantucci A., Wang D., Carvalho A. L., Fontes C. M., Mulloy B., Childs R. A., Feizi T., Chai W. (2015). *Mol Cell Proteomics* 14(4):974-88.



## Determination of mammalian sialic acids in infant formula

*Deanna Hurum<sup>1</sup>, Cees Bruggink<sup>2</sup>, Terri Christison<sup>1</sup>, Jeff Rohrer<sup>1</sup>, and Detlef Jensen<sup>3</sup>*

cees.bruggink@thermofisher.com

1. Thermo Fisher Scientific, Sunnyvale, USA
2. Thermo Fisher Scientific, Breda, Netherlands
3. Thermo Fisher Scientific, Dreieich, Germany

Dietary sialic acids are important for critical immune system and cognitive development in infants. Although these functionalized neuraminic acids are present in all mammalian milk, the proportions and amounts are species specific. Human milk contains sialyl-conjugates with N-acetylneuraminic acid (Neu5Ac) but not N-glycolylneuraminic acid (Neu5Gc). In contrast, bovine milk has primarily Neu5Ac, but also a small proportion of oligosaccharides possessing Neu5Gc. Additionally, bovine milk provides 75% less total sialic acid content than that of human milk. As th, infant formula manufacturers have begun enriching infant formula products with sialic acids. Sialic acids determinations in a complex matrix such as dairy products can be challenging. In addition sialic acids are conjugated and must be released for determinations. Adding another level of complexity, these conjugates are different: oligosaccharides in human milk and glycoproteins for infant formula. Acid hydrolysis is typically used to release the bound sialic acids. However additional carbohydrates are also released causing analytical interferences which can lead to inaccurate reporting of sialic acid concentrations. Sialic acid and other carbohydrates are poor chromophores and therefore require costly, and labor intensive derivitization for spectrophotometric detection. High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a well established direct sensitive and selective method to determine carbohydrates. Here we compare and contrast using ion-exchange and enzyme digestion as sample preparation methods. Sialic acids are determined by HPAE-PAD.

## Cellobiohydrolase and endoglucanase respond differently to surfactants during the hydrolysis of cellulose

Chia-wen C. Hsieh<sup>1</sup>, David Cannella<sup>1</sup>, Henning Jørgensen<sup>2</sup>, Claus Felby<sup>1</sup> and Lisbeth G. Thygesen<sup>1</sup>

dac@ign.ku.dk

1. Faculty of Science, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

2. Center for Bioprocess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søtofts Plads, Building 229, DK-2800 Kgs. Lyngby, Denmark

Non-ionic surfactants such as polyethylene glycol (PEG) can increase the glucose yield from enzymatic saccharification of pretreated lignocellulosic substrates. In a hydrolysis study using Avicel and PASC, we have shown that the boosting effect of PEG differs for the individual cellulases. For both substrates during hydrolysis with a monocomponent exo-cellulase CBH1, the presence of PEG led to an increase in the final glucose concentration, while there was no change in hydrolysis yield with an endoglucanase (EG). Also, PEG had no effect on the activity of  $\beta$ -glucosidases. PEG increased the thermal stability of both the CBH1 and the EG, but only the activity of the CBH1 increased in the presence of PEG. We propose that this has to do with either the processive mechanism of CBH1 affecting the  $k_{cat}$ , or a change in the adsorption/desorption rate ( $k_{off}$ ) of the CBH1.

We have also observed that the properties of the hydrolysis liquid phase change when PEG is present in solution. Using LF-NMR relaxometry we found that PEG increases the relaxation time of the hydrolysis liquid phase, i.e. water is more active. Thus, CBH1 seems to benefit from water being more active, while this is not the case for EG. Based on these results, the properties of the liquid phase have a direct impact on the hydrolysis yield using commercial cellulase mixtures, as CBH1 makes up most of the *T. reesei* secretome from which most cellulolytic enzyme mixtures are produced industrially.

## From waste to health care product: Pectic oligosaccharides produced from citrus peels by treatment of endo-pectate lyase (PL1B) inhibiting colon cancer cells

*Soumyadeep Chakraborty and Arun Goyal*

s.chakraborty@iitg.ernet.in, soumyadeep.chakraborty@gmail.com

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India

An endo pectate lyase (PL1B) from *Clostridium thermocellum* ATCC 27405 cellulosome was cloned and expressed as a 40 kDa protein. It was found to be active on pectic substrates and mainly produced unsaturated di- and tri-galacturonates after enzymatic cleavage of polygalacturonic acid and pectin [1]. The natural pectin was isolated from waste citrus peels of *Citrus limetta* (Sweet lemon) by acid extraction, yielding 12% powdered pectin. The structure of isolated natural pectin was characterized by NMR spectroscopy and was found to contain 77% methylation in the polysaccharide chain. Enzymatic treatment of the isolated natural pectin by PL1B produced unsaturated di-galacturonate as the major product. This was confirmed by Thin Layer Chromatography (TLC) analysis after running the enzymatic reaction samples of different time intervals from 0 to 60 min. The oligosaccharides were then separated by gel filtration using Bio-Gel P2 matrix, where deionized water was used as the mobile phase. The purified oligosaccharides were free from any other contaminants and were freeze dried and used for further studies. The effect of these oligosaccharides at varying concentrations from 0.05 to 0.5 mg/ml was tested on colon cancer cell lines (HT-29) for 3, 6, 12, 24 h. The population of viable cells present after the treatment was measured by MTT assay, where the cell viability is measured by the colorimetric changes. The results showed the 70% inhibition of the growth of HT-29 cells by 0.5 mg/ml oligosaccharides after treatment for 24 h. This result was further verified by observing the changes in the cell morphology upon the treatment of oligosaccharides. Light microscopic images of 0.5 mg/ml oligosaccharides treated HT-29 cells showed disintegrated cell membrane as compared to the untreated cells. Pectin being large polymeric compound is difficult to get absorbed by the alimentary canal hence enzymatic degradation facilitates the production of smaller oligosaccharides which gets easily absorbed by the alimentary canal. Hence, the pectic oligosaccharides can be produced on a large scale by the recombinant endo pectate lyase degradation of citrus peels. The citrus peel which is generally considered as waste can serve as source for a potent healthcare commodity.

### Literature

1. Chakraborty, S., Fernandes, V.O., Dias, F.M.V., Prates, J.A.M., Ferreira, L.M.A., Fontes, C.M.G.A., Goyal, A. and Centeno, M.S.J. (2015) Role of pectinolytic enzymes identified in *Clostridium thermocellum* cellulosome. *Plos One*, 10.1371/journal.pone.0116787.

## Enzymatic synthesis of lipid II and analogues

*Linya Huang, Shi-Hsien Huang, Ya-Chih Chang, Wei-Chieh Cheng, Ting-Jen Rachel Cheng, Chi-Huey Wong*

tingjenc@gate.sinica.edu.tw

Genomics Research Center, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei 115, Taiwan

The emergence of antibiotic resistance has prompted active research in the development of antibiotics with new modes of action. Among all essential bacterial proteins, transglycosylase polymerizes lipid II into peptidoglycan and is one of the most favorable targets because of its vital role in peptidoglycan synthesis. Described in this study is a practical enzymatic method for the synthesis of lipid II, coupled with cofactor regeneration, to give the product in a 50-70% yield. This development depends on two key steps: the overexpression of MraY for the synthesis of lipid I and the use of undecaprenol kinase for the preparation of polyprenol phosphates. This method was further applied to the synthesis of lipid II analogues. It was found that MraY and undecaprenol kinase can accept a wide range of lipids containing various lengths and configurations. The activity of lipid II analogues for bacterial transglycolase was also evaluated.

## Modification of cell wall glucuronoxylans by expressing a GH115 $\alpha$ -glucuronidase in *Arabidopsis thaliana*

Sun-Li Chong<sup>1</sup>, Marta Derba-Maceluch<sup>2</sup>, Sanna Koutaniemi<sup>1</sup>, Maija Tenkanen<sup>1</sup>, and Ewa Mellerowicz<sup>2</sup>

sun-li.chong@helsinki.fi

1. Department of Food and Environmental Sciences, Faculty of Agriculture and Forestry, P.O. Box 27, 00014 University of Helsinki, Finland
2. Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 901-83 Umeå, Sweden

Expressing microbial polysaccharide-modifying enzymes *in planta* is an attractive approach to tailor lignocellulose properties and to study the importance of specific cell wall structure to plant. Glycoside hydrolase family 115  $\alpha$ -glucuronidase (AGU) from white rot fungus, *Schizophyllum commune* cleaves the internal  $\alpha$ -D-(4-*O*-methyl)glucopyranosyluronic acid ((Me)GlcA) from *O*-acetylglucuronoxylans (AcGXs) or xylooligosaccharides<sup>1</sup>. Therefore, the enzyme can be an interesting tool to modify the structures of AcGXs present within the cell walls. In this work, the ScAGU115 was constitutively expressed in model plant, *Arabidopsis thaliana*. The recombinant ScAGU115 was active on the internally substituted aldopentaouronic acids. Surprisingly, the cell wall (Me)GlcA and other non-cellulosic sugars, as well as the lignin content, remained unchanged. In contrast, disrupting the endogenous glucuronyltransferases was shown to be effective reducing the (Me)GlcA substituents in *gux1gux2* mutant plants<sup>2</sup>. The stem section of ScAGU115-expressing plants exhibited a decreased signal from UX1 antibody that recognizes (Me)GlcA substituents, the signal was not affected by the transgene when the sections were deacetylated prior to labeling. In contrast, *gux1gux2* mutant lacked the UX1 signals in both native and deacetylated cell walls. This indicates that the ScAGU115 affects only those (Me)GlcA moieties, which are accessible to UX1 antibody, and constitute a small fraction in AcGXs of *Arabidopsis*. However, majority of the (Me)GlcA substituents were resistant to ScAGU115 modification, likely due to the shielding of neighboring acetyl substitutions. Therefore, a better strategy for tailoring side groups of AcGXs *in planta* can be co-expressing with an acetyl xylan esterase to harness synergism between these side groups removing enzymes.

### Literature

1. Tenkanen and Siika-aho. 2000. *J Biotechnol* **78**(2):149-161.
2. Mortimer et al. 2010. *Proc Natl Acad Sci* **107**(40):17409-17414.

## Biochemical characterization of a new GH-70 enzyme from *Leuconostoc citreum* NRRL B-1299

Marion Claverie<sup>1</sup>, Marlène Vuillemin<sup>1</sup>, Etienne Severac<sup>1</sup>, Pierre Monsan<sup>1,2</sup>, Gianluca Cioci<sup>1</sup>, Claire Moulis<sup>1</sup>, Magali Remaud-Siméon<sup>1</sup>

remaud@insa-toulouse.fr

1. Université de Toulouse, France, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France, CNRS, UMR5504, F-31400 Toulouse, France, INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse.
2. Toulouse White Biotechnology, Parc Technologique du canal, 3 rue des satellites, F-31400 Toulouse, France.

Glucansucrases (GS) from glycoside hydrolase family 70 (GH70) are transglucosylases found in several lactic acid bacteria from the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Weissella*. They catalyze the polymerization of glucosyl residues from sucrose, an economical and abundant agrosresource. Depending on the enzyme specificity, the synthesized  $\alpha$ -glucans vary in terms of size, structure, types of glucosidic bonds and degree of branching. This composition variety confers to  $\alpha$ -glucans diverse properties, making them useful for several applications in food and pharmaceutical industries<sup>1</sup>.

The sequencing of *Leuconostoc citreum* NRRL B-1299 genome allowed the identification of a new GS called DSR-M, which is highly similar (94% sequence identity) to the recently identified GSE16-5 glucansucrase from *L. citreum* LBAE-E16<sup>2</sup>. This 229kDa enzyme has the particularity to synthesize a low molar mass dextran (around 27kDa) composed of exclusively  $\alpha$ -(1→6) linkages.

To allow the structural and biochemical characterization of this enzyme, a truncated version was constructed, expressed and purified. Kinetic study and crystallization trials are in progress. The original results recently obtained will be presented and discussed.

### Literature

1. Leemhuis H, Pijning T, Dobruchowska JM, van Leeuwen SS, Kralj S, Dijkstra BW & Dijkhuizen L (2013) Glucansucrases: Three-dimensional structures, reactions, mechanism,  $\alpha$ -glucan analysis and their implications in biotechnology and food applications. *J. Biotechnol.* **163**, 250–272
2. Amari M, Gabriel V, Robert H, Morel S, Moulis C, Gabriel B, Remaud-Siméon M & Fontagné-Faucher C (2014) Overview of the glucansucrase equipment of *Leuconostoc citreum* LBAE-E16 and LBAE-C11, two strains isolated from sourdough. *FEMS Microbiol. Lett.*, fnu024.

## Discovery of novel carbohydrate active enzymes for plant biomass degradation by metagenomics of hyperthermophilic communities

*Beatrice Cobucci-Ponzano*<sup>1</sup>, *Andrea Strazzulli*<sup>1</sup>, *Rosa Giglio*<sup>1</sup>, *Roberta Iacono*<sup>1</sup>, *Federica Bitetti*<sup>1</sup>, *Corinna Schiano di Cola*<sup>1</sup>, *Federico M. Lauro*<sup>2</sup>, *Yizhuang Zhou*<sup>3</sup>, *Jin Xu*<sup>3</sup>, *Vincent Lombard*<sup>4</sup>, *Bernard Henrissat*<sup>4</sup>, *Vania Cardoso*<sup>5</sup>, *Carlos MGA Fontes*<sup>5</sup> and *Marco Moracci*<sup>1</sup>

beatrice.cobucciponzano@ibbr.cnr.it

1. Institute of Biosciences and BioResources – CNR, Via P. Castellino 111, 80131, Naples, Italy
2. School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW, 2052, Australia
3. BGI Hong Kong, Limited, 16Dai Fu Street, Tai Po Industrial estate, Tai Po, New Territories, Hong Kong
4. Architecture et Fonction des Macromolécules Biologiques, UMR 7257 - CNRS & Aix-Marseille Université, 163 Avenue de Luminy, 13288 Marseille, France
5. NZYTech genes & enzymes, Campus do Lumiar, 1649-038 Lisboa, Portugal

Hyperthermophilic microorganisms, most of them belonging to the domain of Archaea, thrive at temperatures >80°C and attract remarkable interest in basic and applied research for the uncommon intrinsic stability of their enzymes. In the framework of the discovery of new enzymes for biocatalysis and biotransformation for industrial applications, we investigate, through a metagenomic approach, the complex communities of hyperthermophiles populating volcanic sites to understand their adaptation and evolution to extreme conditions and to exploit them as a rich source of novel biocatalysts [1, 2].

A metagenomic analysis of the microbial communities in two neighboring mud/water pools in the solfataric field of Pisciarelli, Agnano (Naples, Italy), showed that the *phylum* Crenarchaeota was prevalent, but differentially abundant at the genus level in the two pools that differ in T and pH (pool1 T=85°C and pH 5.5; pool2 T=94°C and pH 1.5, respectively). To identify enzymes to be exploited in the conversion of lignocellulosic biomasses for second-generation biofuels, we enriched *in-lab* pool1 community to select microorganisms able to grow on different plant biomasses. Total DNA was extracted from the microbial community growing in the enriched samples and, again by metagenomics, we determine the full set of carbohydrate active enzymes (CAZymes) of these microbial communities. The detailed phylogenetic and physiological analysis of the enriched samples and their CAZymes family composition will be discussed.

This work was supported by the project PON01\_01966 “ENERBIOCHEM” of the Programma Operativo Nazionale Ricerca e Competitività 2007-2013 -MIUR-Italy and by the Short-term mobility Programme “STM 2013” of the National Research Council of Italy

### Literature

1. Cobucci-Ponzano B., et al. J Biol Chem, 2010, 20691-20703
2. Cobucci-Ponzano, B., et al. Biochimie, 2010, 92, 1895-907

## Structural and functional investigation of a lytic polysaccharide monooxygenase (LPMO) by NMR spectroscopy

*Gaston Courtade*<sup>1</sup>, *Simone Balzer*<sup>2</sup>, *Zarah Forsberg*<sup>3</sup>, *Gustav Vaaje-Kolstad*<sup>3</sup>, *Vincent G. H. Eijsink*<sup>3</sup>, *Finn L. Aachmann*<sup>1</sup>

finn.l.aachmann@ntnu.no

1. Department of Biotechnology, Norwegian University of Science and Technology, Sem Sælands vei 6/8, 7491 Trondheim, Norway
2. Department of Molecular Biology, SINTEF Materials and Chemistry, Sem Sælands vei 2A, 7465 Trondheim, Norway
3. Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5004, 1432 Ås, Norway

Lytic polysaccharide monooxygenases (LPMOs) are abundantly present in biomass-degrading microbes and are currently classified as auxiliary activity auxiliary activity (AA) families 9 (AA9, formerly GH61), 10 (AA10, formerly CBM33) and 11 (AA11) (see [www.cazy.org](http://www.cazy.org)<sup>4</sup>). LPMOs have been shown to catalyze the cleavage of glycosidic bonds in chitin and cellulose through hydroxylation of either carbon within the scissile bond and are thus likely to play important roles in future biorefining<sup>3, 5-7</sup>. However, the molecular basis of their unprecedented catalytic activity remains largely unknown. We have used NMR techniques to address structural and functional aspects of BILPMO10A, a chitin-active AA10. NMR structural and relaxation studies showed that the core of BILPMO10A is a compact  $\beta$ -sandwich with a flat surface that includes the protein N-terminus. These observations are in agreement with previously investigations on other AA10 LPMOs<sup>1, 2, 8</sup>.

**Acknowledgements** This work was financed by the Norwegian Research Council (NRC) BioTek2021 MARPOL #221576, NTNU-SO. In addition, we would like to extend our thanks to Reinhard Wimmer.

### Literature

1. Aachmann FL, Sørli M, Skjåk-Bræk G et al (2012) *PNAS*. doi:10.1073/pnas.1208822109
2. Hemsworth GR, Davies GJ, Walton PH (2013) *Curr Opin Struct Biol*. doi:10.1016/j.sbi.2013.05.006
3. Kim S, Ståhlberg J, Sandgren M et al (2014) *PNAS*. doi:10.1073/pnas.1316609111
4. Levasseur A, Drula E, Lombard V et al (2013) *Biotechnol Biofuels*. doi:10.1186/1754-6834-6-41
5. Phillips CM, Beeson WT, Cate JH, Marletta MA (2011) *ACS Chem Biol* doi:10.1021/cb200351y
6. Quinlan RJ, Sweeney MD, Leggio LL et al (2011) *PNAS*. doi:10.1073/pnas.1105776108
7. Vaaje-Kolstad G, Westereng B, Horn SJ et al (2010) *Science* doi:10.1126/science.1192231
8. Vaaje-Kolstad G, Horn SJ, van Aalten DMF et al (2005) *JBC*. doi:10.1074/jbc.M504468200



## **A novel carbohydrate esterase isolated from an Arctic environmental metagenome**

*Concetta De Santi*<sup>1</sup>, *Nils-Peder Willassen*<sup>1</sup>, *Arne Oskar Smalås*<sup>1</sup>, *Adele Williamson*<sup>1</sup>

concetta.d.santi@uit.no

1. The Norwegian Structural Biology Centre, Department Of Chemistry, UiT - The Arctic University of Norway, Tromsø.

Carbohydrate-active enzymes (CAZymes) are involved in the metabolism and synthesis of saccharides. Degradative CAZymes form part of the hemicellulolytic and cellulolytic enzyme systems of microbes which break down plant biomass, and have possible applications in biotechnology (1).

Here we describe the biochemical characterisation of a family 15 carbohydrate esterase (CE-15) 'MZ0003' which was cloned from a metagenomic DNA library derived from marine arctic sediment. MZ0003 appears to be of bacterial origin, and has low sequence homology (20%) with previously characterized GH15 enzymes from mesophilic and thermophilic fungi.

In silico and in vitro studies indicate that MZ0003 has structural and biochemical properties that differ from these fungal homologs. In particular, MZ0003 has a broader acetylated substrate range and lower temperature optimum than the previously characterized CE-15 enzymes. A study of the inhibition of activity by site-directed mutagenesis was used to investigate the role of predicted active site residues in catalysis, while a combination of homology-based modelling and preliminary crystallization studies are being undertaken to provide more structural information.

These studies on MZ0003 represent the first instance of biochemical characterization of a bacterial member of the GH15 CAZy family.

### **Literature**

1. van den Brink, J., and de Vries, R. P. (2011) Appl Microbiol Biotechnol 91, 1477-1492.

## **Towards monoglycosylation of organic molecules with glucansucrases: reaction –and enzyme engineering**

*Tim Devlamynck<sup>1</sup>, Evelien te Poele<sup>1</sup>, Xiangfeng Meng<sup>1</sup>, Wim Soetaert<sup>2</sup>, Lubbert Dijkhuizen<sup>1</sup>*

t.devlamynck@rug.nl

1. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, the Netherlands
2. Centre of Expertise for Industrial Biotechnology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University, Belgium

Glucansucrases (GS) are exclusively found in lactic acid bacteria and catalyze the conversion of sucrose into  $\alpha$ -glucan polysaccharides. However, in the presence of suitable acceptor substrates, GS also use sucrose as a donor substrate to glycosylate these acceptors. Glycosylation can significantly improve the physicochemical and biological properties of small molecules like vitamins, antibiotics, flavours and fragrances. Typically, the solubility and stability of the glycosylated compounds are increased, improving also their bioavailability<sup>1</sup>.

Recently, we observed that various GS glycosylate a wide range of phenolic and alcoholic compounds. Moreover, it was shown that GS are capable of adding multiple glucosyl moieties to one acceptor molecule, producing glycosides of different size and structure. On the one hand multiglycosylation leads to an extra increase in solubility and stability of the glycosylated organic molecules. On the other hand, however, multiglycosides lose most of their functional activity. Consequently, monoglycosylation is generally preferred over multiglycosylation.

Two strategies were followed in order to push the reaction towards monoglycosylation: reaction – and enzyme engineering. The former strategy consisted of selecting the optimal reaction conditions. It was found that high acceptor concentrations lead to relatively more monoglycosylation. However, the obtained results were not satisfying yet and therefore the biocatalyst itself was optimized by means of mutagenesis of L981<sup>2</sup> and W1065<sup>2</sup>, residues involved in the positioning of saccharide acceptors in the active site. The resulting mutants lost most of their ability to accept saccharides while retaining a high specificity for phenolic and alcoholic molecules. Hence, the conversion of monoglycosides into multiglycosides was blocked. Furthermore, by eliminating  $\alpha$ -glucan synthesis, the most important side reaction, higher glycosylation yields were obtained. Altogether, this lead to an improvement in monoglycosylation yield of 50% for several phenolic and alcoholic compounds (catechol, resorcinol, hydroquinone, butanol, hexanol and octanol), indicating the general applicability of this strategy.

### **Literature**

1. Desmet *et al.* (2012), Enzymatic glycosylation of small molecules: challenging substrates require tailored catalysts, *Chemistry-A European Journal* 18: 10786-10801.
2. Meng *et al.* (2015, in preparation)

## The feruloyl esterase gene family of *Aspergillus niger*

*Adiphol Dilokpimol*<sup>1</sup>, *Miia R. Mäkelä*<sup>2</sup>, *Olga Belova*<sup>1</sup>, *Sadegh Mansouri*<sup>2</sup>, *Ronald P. de Vries*<sup>1</sup> and *Kristiina Hilden*<sup>2</sup>

Kristiina.S.Hilden@helsinki.fi

1. Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
2. Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland

Ferulic acid (FA) is a major phenolic acid component of plant cell walls. FA and to a lesser extent other hydroxycinnamic acids (e.g., *p*-coumaric acid) are covalently linked mainly to xylan and pectin through ester linkages. They cross-link the cell wall polysaccharides to themselves and to the phenolic polymer lignin. This increases the physical strength and integrity of plant cell walls as well as reduces their biodegradability by microorganisms. Feruloyl esterases (or ferulic acid esterases, FAEs) [E.C. 3.1.1.73] are responsible for the release of FA from plant cell walls. As they are able to liberate phenolic acids from natural plant sources as well as agro-industrial by-products, FAEs are widely used in the food, feed, pulp-paper, and pharmaceutical industries as well as in biofuel production. These broad application fields require various types of FAEs to fit specific pH, temperature, and other conditions.

*Aspergillus niger* is one of the most well-known and important industrial fungi, which produces a wide range of plant cell wall-degrading enzymes. Among others, two FAEs from *A. niger* (AnFaeA and AnFaeB) have been characterized and are among the most thoroughly studied FAEs. Phylogenetic analysis of fungal FAEs performed by Benoit et al. (2008) has led us to numerous novel FAE candidates including several from *A. niger*. In this study, the putative FAEs from *A. niger* were investigated using phylogeny, gene expression and enzyme characterization. Highlights from this study will be presented.

### Acknowledgements

This work was supported by the European Union, Grant agreement no: 613868 (OPTIBIOCAT).

### Literature

Benoit, I., Danchin, E.G., Bleichrodt, R.J., de Vries R.P. (2008) *Biotechnol Lett.* 30:387–396.

## Structural and functional studies of a *Fusarium oxysporum* cutinase with polyethylene terephthalate modification potential

*Maria Dimarogona<sup>1,3</sup>, Efstratios Nikolaivits<sup>1</sup>, Maria Kanelli<sup>1</sup>, Paul Christakopoulos<sup>2</sup>, Mats Sandgren<sup>3</sup> and Evangelos Topakas<sup>1</sup>*

vtopakas@chemeng.ntua.gr

1 Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

2 Biochemical Process Engineering, Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden

3 Department of Chemistry and Biotechnology, Swedish University of Agricultural Science, Uppsala, Sweden

Cutinases (EC 3.1.74, categorized in CE family 5 of the CAZy database), are enzymes that degrade plant polyester waxes, like suberin and cutin, the major constituent of plant cuticle. The cuticle shields leaves, shoots and other exposed parts of plants from microorganisms such as fungi and bacteria. Cutinases are employed by plant pathogens to deconstruct the protective cuticle and then invade the plant by secreting an armory of carbohydrate degrading enzymes (1). Recent findings have revealed that cutinases are capable of hydrolysing or modifying a variety of synthetic esters and polyesters such as polyethylene terephthalate (PET), a property which renders them interesting biotechnological targets. Moreover, degradation products, some of which are found almost exclusively in the suberized plant cell walls, could have considerable value as sources of oleochemicals. Here we report the expression, characterization and crystal structure of FoCut5a, a cutinase from the mesophilic fungus *Fusarium oxysporum*. The recombinant enzyme was heterologously expressed either in the cytoplasmic or periplasmic space of *Escherichia coli*, taking advantage of the pelB signal sequence. The crystal structure of FoCut5A was determined at 1.9 Å resolution and solved by molecular replacement, using *Fusarium solani* cutinase structure (PDB code 1CEX) as starting model (2). The two enzymes have high sequence identity and their overall structures are very similar, however, there are small differences between the structures that can potentially explain the variations in the biochemical properties of the two enzymes. FoCut5A has a maximum activity at 40 °C, when tested on the three *p*-nitrophenyl synthetic esters of aliphatic acids (C<sub>2</sub>, C<sub>4</sub>, C<sub>12</sub>), with the highest catalytic efficiency found against the butyl ester. FoCut5A was also found capable of hydrolyzing PET model substrates, indicating a potential use of this enzyme in surface modification of synthetic polymers.

### Literature

1. Chen et al., Biotechnology Advances, 2013, 31: 1754-1767
2. Longhi et al., Journal of Molecular Biology, 1997, 268(4): 779-799

## The hydrophilic character of cytotoxic payloads affects functional properties of antibody-drug conjugates

*Tero Satomaa<sup>1,2</sup>, Anja Vilkmann<sup>1</sup>, Titta Kotiranta<sup>1</sup>, Filip S. Ekholm<sup>1,3</sup>, Virve Pitkänen<sup>1</sup>, Ritva Niemelä<sup>1</sup>, Annamari Heiskanen<sup>1</sup>, Henna Pynnönen<sup>1</sup>, Jari Helin<sup>1,2</sup> and Juhani Saarinen<sup>1</sup>*

juhani.saarinen@glykos.fi

1. Glykos Finland Ltd, Helsinki, Finland

2. Department of Biochemistry and Biotechnology, University of Helsinki, Finland

3. Laboratory for Instruction in Swedish, Department of Chemistry, University of Helsinki, Finland

Antibody-drug conjugates (ADCs) were prepared using closely related auristatin and dolastatin payloads that have different hydrophilic character. The formed ADCs and the free drugs were characterized in biochemical and cellular assays. Free drugs with increased hydrophilic character were less cytotoxic to cancer cells when applied to the cell culture medium of cancer cells, reflecting their diminished ability to pass through the cell membrane compared to more hydrophobic drugs. However, when conjugated to antibodies (as ADCs), the payloads triggered similar cytotoxicity as their hydrophobic counterparts. Therefore, by increasing the hydrophilicity of the ADC payloads, ADCs with less cytotoxicity to non-target cells can be constructed. Hydrophilic drugs are also known to have less bystander efficacy, *i.e.* ability to attack nearby cells after destroying a target cell. We propose that by selection of a suitable hydrophilic ADC payload, both safety to non-target cells and bystander effect can be optimized.

With these prerequisites, ADCs with high drug-to-antibody ratios (DAR > 4) were generated. With these ADCs, it was demonstrated that the more hydrophilic payloads improved the ADCs stability (less aggregation) and biocompatibility, while the cytotoxic activity remained unaltered. In conclusion, the choice of payload chemistry can affect functional properties in addition to the direct cytotoxicity of ADCs.

## Assisting effect of a carbohydrate binding module on Glycosynthase-catalyzed polymerization

Victoria Codera<sup>1</sup>, Magda Faijes<sup>1</sup>, and Antoni Planas<sup>1</sup>

magda.faijes@iqs.url.edu

1. Laboratory of Biochemistry, Bioengineering Department, Institut Químic de Sarrià, Universitat Ramon Llull, 08017 Barcelona, Spain

The development of polysaccharides have been of great interest for their use in biomaterials and biomedical applications. As alternative to source extraction and chemical synthesis, enzymatic synthesis is a powerful synthetic strategy. Particularly, the glycosynthase technology has provided a broad range of oligosaccharides, glycoconjugates and polysaccharides [1]. Artificial polysaccharides with regular and defined structure and, in some cases crystalline morphology, have been obtained using glycosynthase-catalyzed polymerization of simple activated glycosyl donors [2,3]. The donor is self-condensed by the non-nucleophilic glycosynthase mutant and polymer products can be elongated until they become insoluble.

We envisioned the *in vitro* application of non-catalytic carbohydrate binding modules (CBMs) in the glycosynthase polymerization reaction. In nature, CBMs are found as discrete domains of polysaccharide degrading enzymes where they play critical roles in the recognition of inaccessible polysaccharides and potentiate the activity of their cognate catalytic modules against insoluble substrates [4]. These considerations prompted us to explore the glycosynthase-catalyzed polysaccharide synthesis in the presence of CBMs. CBMs could enhance the solubility of the growing polymeric chain formed during the reaction, increasing the turn-over of the enzyme and obtaining insoluble polymers with probably higher molecular weights and lower polydispersion indexes. We combined a glycosynthase derived from *B. licheniformis* 1,3-1,4- $\beta$ -glucanase [2] and the CBM11 domain of *Clostridium thermocellum* Lic26A-Cel5E enzyme, which binds  $\beta$ -1,3-1,4-glucans [5]. We here report the effects of the CBM11 domain as a free protein or fused to the catalytic glycosynthase domain on the formation of these mixed-linked glucans.

We acknowledge Dr. Harry Gilbert, Newcastle University, for providing the CBM11 gene. VC acknowledges a predoctoral fellowship from IQS. This work was supported by grant GLYCOZYMES (BIO2013-49022) from MICINN, Spain.

### Literature

1. Mackenzie, Wang, Warren, Withers, J. Am. Chem. Soc. 1998, 120, 5583; Malet, Planas, FEBS Lett. 1998, 440, 208; Armstrong, Withers, Biopolymers 2013, 10, 666; Cobucci-Ponzano, Moracci, Nat. Prod. Rep. 2012, 29, 697; Wang, Huang, Curr. Opin. Chem. Biol. 2009, 13, 592.
2. Pérez, Faijes, Planas, Biomacromolecules 2011, 12, 494.
3. Spadiut, Ibatullin, Peart, Gullfot, Martinez-Fleites, Ruda, Xu, Sundqvist, Davies, Brumer, J. Am. Chem. Soc. 2011, 133, 10892; Faijes, Imai, Bulone, Planas, Biochem. J. 2004, 380, 635; Gullfot, Ibatullin, Sundqvist, Davies, Brumer, Biomacromolecules 2009, 10, 1782; Fort, Boyer, Greffe, Davies, Moroz, Christiansen, Schüle, Cottaz, Driguez, J. Am. Chem. Soc. 2000, 122, 5429.
4. Gilbert, Knox, Boraston, Curr. Op. Struct. Biol. 2013, 23,1.
5. Carvalho, Goyal, Prates, Bolam, Gilbert, Pires, Ferreira, Planas, Romão, Fontes, J. Biol. Chem. 2004, 279, 3478.

## Crystallographic studies of a member of the lytic polysaccharide monooxygenase family AA13

*Kristian E.H. Frandsen<sup>1</sup>, Jens-Christian N. Poulsen<sup>1</sup>, Maria A. Stringer<sup>2</sup>, Morten Tovborg<sup>2</sup>, Katja S. Johansen<sup>2</sup>, Leonardo De Maria<sup>2</sup>, Gideon J. Davies<sup>3</sup>, Paul H. Walton<sup>3</sup>, P. Dupree<sup>4</sup>, Bernard Henrissat<sup>5</sup> and Leila Lo Leggio<sup>1</sup>*

Kristian.frandsen@chem.ku.dk

1. Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
2. Novozymes A/S, Smørmosevej 25, 2880 Bagsværd, Denmark
3. University of York, York, UK
4. University of Cambridge, Cambridge, UK
5. Architecture et Fonction des Macromolécules Biologiques, CNRS, Marseille, France

Lytic polysaccharide monooxygenases (LPMOs) are a new class of copper dependent carbohydrate modifying redox enzymes that have gained much interest in the last ten years [1, 2, 3, 4]. Four families of LPMOs are currently classified in CAZY under Auxiliary Activities [5]. AA9 (previously GH61), AA10 (CBM33) and AA11 [6] have all so far been found to act on polysaccharides containing  $\beta$ -1,4-linkages. Recently however, a family of proteins previously shown in the patent literature to boost amylolytic activity [7], has been confirmed to be an LPMO family (AA13) [8, 9] degrading starch and related polysaccharides, which are  $\alpha$ -1,4-linked. Many of the enzymes belonging to AA13 have an associated starch-binding CBM (Carbohydrate Binding Module).

In this contribution we focus on the insight in substrate specificity provided by crystallographic studies on a member of the family naturally devoid of CBM, AoAA13 from *Aspergillus oryzae* [9].

### Acknowledgements

We thank the ERA-IB program for funding to the CESBIC consortium. We also thank MAXLAB and ESRF for synchrotron data collection, the Danish instrument Center DANSCATT and the EU FP7 for funding travel to synchrotrons.

### Literature

1. P. V. Harris et al. *Curr Opin Chem Biol* **19**, 162–170 (2014)
2. G. Hemsworth et al. *Curr Opin Struct Biol* **23**, 660–668 (2013)
3. S. Horn et al *Biotech. Biofuels* **5**, 45 (2012)
4. L. Lo Leggio et al. *Comp. Struct. Biotech. J.* **2**, e201209019 (2012)
5. A. Levasseur et al. *Biotech. Biofuels* **6**, 41 (2013)
6. G. R. Hemsworth et al. *Nat. Chem. Biol.* **10**, 122–126 (2014)
7. P. Harris og M. Wogulis, Patent WO/2010/059413 A2 (2010)
8. V. Vu et al *PNAS*, **111**, 13822–13827, (2014)
9. L. Lo Leggio et al *Nature comm* DOI: NCOMMS6961 (2015)

## Endogenous degradation activity for slimy extracellular polysaccharide produced by *Lactobacillus fermentum* TDS030603

Shinpei Matsumoto, Kenji Fukuda, and Tadasu Urashima

fuku@obihiro.ac.jp

Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine

*L. fermentum* TDS030603 secretes highly viscous extracellular polysaccharides (EPS) into culture media [1]. In our laboratory, an eventual decrease of viscosity of the culture medium has been observed during the stationary and death phases without any fragmentation of the EPS main chain. Recently, Gerwig et al. defined chemical structures of the EPS, and its branched side chains turned out to be heterogeneous [2]. Based on these facts, we hypothesized that side chains of the EPS should be degraded during cultivation that evokes decrease of the culture media viscosity, hence this study was conducted to explore EPS degradation activities of *L. fermentum* TDS030603.

To investigate approximate localization of the EPS degradation activity, crude protein fraction was prepared from the culture supernatant by 80%-saturation ammonium sulphate precipitation, and cell-free extract and cell debris were prepared as follows: cells of *L. fermentum* TDS030603 were harvested after 48 h of static incubation at 30°C, washed with phosphate-buffered saline (PBS) three times, sonicated for 1 min with 30 sec intervals up to 5 min on ice, and centrifuged at 7000 g for 30 min at 4°C. To extract cell-associated substances from *L. fermentum* TDS030603, harvested cells were treated with 2 M guanidine hydrochloride at 37°C for 2 h, or with 0.2 M glycine, 1 M lithium chloride, 40 □g/ml lysozyme, and PBS each at 4°C for 1 h. EPS degradation reaction was carried out at 30°C, for appropriate periods, at pH 4.5, using 0.009% (w/v) of thoroughly purified EPS as substrate. Subsequently, the reaction mixtures were frozen immediately at -80°C and kept until used. After removal of remained EPS in the reaction mixture by ultrafiltration using YM-3 device, 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of mono- and oligo-saccharides were prepared according to the literature [3] and analyzed on high-performance liquid chromatography (HPLC).

Single peak corresponding to standard galactose was observed on HPLC chromatogram only when the cell debris was mixed with the EPS substrate. This peak appeared when the reaction was performed at pH 4.5, but not at pH 7.0. All the five extracts from the cell-surface gave several peaks of PMP-derivatives including the one corresponding to the standard galactose. Among the five, highest peaks were observed toward the extract with 0.2 M glycine, and areas of some of the peaks increased as the reaction time prolonged. The EPS degradation activity of the glycine extracts was abolished by heat treatment at 100°C for 10 min. These results indicated presence of EPS-degrading substance(s) on cell-surface of *L. fermentum* TDS030603.

### Literature

1. Fukuda et al. *Carbohydr. Polym.* **79**, 1040-1045 (2010).
2. Gerwig et al. *Carbohydr. Res.* **378**, 84-90 (2013).
3. Honda et al. *Anal. Biochem.* **180**, 351-357 (1989).



## Activity studies on lytic polysaccharide monooxygenases

Aline L. Gaenssle<sup>1</sup>, David Cannella<sup>2</sup>, Claus Felby<sup>2</sup> and Morten J. Bjerrum<sup>1</sup>

mobj@chem.ku.dk / fcr497@alumni.ku.dk

1. Department of Chemistry, University of Copenhagen, 2100 Copenhagen, Denmark

2. Department of Geosciences and Natural Resource Management, University of Copenhagen, 2100 Copenhagen, Denmark

Lytic polysaccharide monooxygenases (LPMO, former GH61) are enzymes disrupting crystalline cellulose by cleaving glycosidic bonds<sup>1</sup>. However, kinetic studies are difficult due to the lack of fast screening methods. The presented activity assay<sup>2</sup> applies the ability of LPMO to produce hydrogen peroxide in absence of substrate. The produced hydrogen peroxide can be followed spectroscopically using horseradish peroxidase and Amplex red. Presence of substrate decreases production of hydrogen peroxide, limiting substrate studies, but allowing a large array of screenings targeting activity enhancers, inhibitors and optimal conditions. Additionally, a substrate assay<sup>3</sup> based on oxidation of phosphoric acid swollen cellulose by LPMO, followed by high performance anion exchange chromatography was used. Combining both assays, LPMO was studied regarding potential substitutes for molecular oxygen and reducing agents. Furthermore, molecular dynamics simulations were performed to study the interaction of the enzyme with its cellulose substrate showing multiple interaction sites (Fig. 1).



**Fig. 1:** Molecular dynamics simulation with LPMO (PDB ID 2YET) and cellulose, overlay of all frames. LPMO is shown as cartoon with residues located in the active site or assumed to interact with the substrate drawn as sticks. The cellulose strands are indicated by strings with hexagons.

### Literature

1. S. Kim, J. Ståhlberg, M. Sandgren, R.S. Paton, G.T. Beckham, *Proc. Nat. Acad. Sci.* **2014**, 1, 149-154
2. R. Kittl, D. Kracher, D. Burgstaller, D. Haltrich, R. Ludwig, *Biotechnol. Biofuels* **2012**, 5, 79
3. M. Bey, S. Zhou, L. Poidevin, B. Henrissat, P.M. Coutinho, J.-G. Berrin, J.-C. Sigoillota, *Appl. Environ. Microbiol.* **2013**, 72, 2, 488-496

## Characterization of a broad substrate specificity AA9 lytic polysaccharide monooxygenases from *Podospora anserina*

*Soňa Garajová*<sup>1,2</sup>, *Chloe Bennati-Granier*<sup>1,2</sup>, *Maria Rosa Beccia*<sup>3</sup>, *Charlotte Champion*<sup>1,2</sup>, *Sacha Grisel*<sup>1,2</sup>, *Mireille Haon*<sup>1,2</sup>, *Simeng Zhou*<sup>1,2</sup>, *Bruno Guigliarelli*<sup>3</sup>, *Isabelle Gimbert*<sup>1,2</sup>, *Eric Record*<sup>1,2</sup> and *Jean-Guy Berrin*<sup>1,2</sup>

jean-guy.berrin@univ-amu.fr

1. INRA, UMR1163 BBF, F-13288 Marseille, France
2. Aix Marseille Université, Polytech Marseille, F-13288 Marseille, France
3. CNRS, BIP, UMR7281, F-13402 Marseille, France

Plant cell walls, made up of cellulose and hemicelluloses, represent a high potential renewable resource for the production of 2<sup>nd</sup> generation biofuels and platform molecules for the chemical industry. In the last few years, significant progress in enzymatic polysaccharide degradation was achieved with the identification of a new class of fungal secreted enzymes, the lytic polysaccharide monooxygenases (LPMOs) that facilitate the degradation of cellulosic biomass. AA9 LPMOs (formerly classified into the GH61 family) are copper-dependent enzymes found exclusively in fungi. They act on the relatively crystalline surface regions of their substrates (mainly cellulose) by oxidizing at different positions of the glycosidic bond in cellulose. Here, we characterized in depth a family AA9 from *Podospora anserina* (*Pa*LPMO9H) with biochemical and biophysical approaches. This copper monooxygenase, which bear a family 1 Carbohydrate Binding Module (targetting cellulose) at its C-terminus was expressed heterologously using the methylotrophic yeast *Pichia pastoris* and produced in bioreactor. Using chromatographic and fluorescent methods, we showed that *Pa*LPMO9H is versatile in terms of substrate specificity as it displayed activity on cellulose and  $\beta$ -(1,4)-linked hemicellulose polysaccharides (xyloglucan and mixed-linkage  $\beta$ -glucan). Ability to oxidatively cleave cello-oligosaccharides was also demonstrated. Sequence similarities and differences with the *Neurospora crassa* NcLPMO9C (Isaksen et al., 2013; Agger et al., 2014) will be presented. The broad specificity of *Pa*LPMO9H makes it attractive to tackle the recalcitrance of lignocellulosic biomass.

### Acknowledgements

This study was carried out in the frame of Futurol and Funcopper projects with financial support from OSEO and the AMIDEX foundation.

### Literature

1. Agger JW, Isaksen T, Várnai A, Vidal-Melgosa S, Willats WGT, Ludwig R, Horn SJ, Eijsink VGH, Westereng B: Discovery of LPMO activity on hemicelluloses shows the importance of oxidative processes in plant cell wall degradation. Proc Natl Acad Sci U S A 2014, 111:6287–92.
2. Isaksen T, Westereng B, Aachmann FL, Agger JW, Kracher D, Kittl R, Ludwig R, Haltrich D, Eijsink VGH, Horn SJ: A C4-oxidizing lytic polysaccharide monooxygenase cleaving both cellulose and cello-oligosaccharides. J Biol Chem 2013, 289:2632–42.

## Molecular cloning, expression and characterization of novel endo- $\beta$ -1, 4-mannanase of a family 10 glycoside hydrolase from *Pedobacter saltans* DSM12145

Kedar Sharma, Anil Kumar Verma and Arun Goyal

arungoyl@iitg.ernet.in

Indian Institute of Technology Guwahati, Assam, India.

Microbial mannanases have become biotechnologically important since they target the hydrolysis of complex polysaccharides of plant tissues into simple molecules like manno-oligosaccharides and mannose. The role of mannanases in the paper and pulp industry is well established and more recently they have been reported being applied in the food and feed industry.  $\beta$ -Mannanase is an excellent biocatalyst for the production of gluco-manno-oligosaccharides (GMOS). Several studies have confirmed that GMOSs with the degree of polymerization (DP) of 2 to 6 have the health benefits on humans. GMOS could increase the growth of intestinal microorganisms, decrease the populace of pathogenic bacteria and improve the integrity of intestinal mucosa [1]. This study reports an endo- $\beta$ -1,4-Mannanase (*PsMan*) from *Pedobacter Saltans* DSM 12145 belonging to family 10 glycoside hydrolase. The nucleotide sequence encoding *PsMan* was accessed from the CAZy (Carbohydrate Active Enzymes, [www.cazy.org](http://www.cazy.org)) database [2]. *PsMan* was amplified by PCR, the amplicon was cloned into pET-28a(+) vector and expressed in *E. coli* BL21 (DE3) cells. The recombinant endo-1,4- $\beta$ -mannanase was soluble protein and showed a homogeneous protein of molecular size, approximately, 43 kDa band after purification by immobilized  $\text{Ni}^{2+}$  ion affinity chromatography. The specific activity of the purified *PsMan* was 15.08 U  $\text{mg}^{-1}$  using Konjac-glucomannan as substrate. *PsMan* displayed optimal temperature, 37°C and optimum pH, 6.5. The  $K_m$  and  $V_{max}$  values for Konjac-glucomannan, are 0.255  $\text{mg mL}^{-1}$  and 15.67  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ , respectively. The presence of 1 mM of  $\text{Cu}^{2+}$  ions increased the activity of *PsMan* by 2 fold. Analysis of hydrolyzed products showed that *PsMan* could hydrolyze different types of mannan based polysaccharides, releasing manno-oligosaccharides. The main products released were mannobiose, mannotriose and other higher oligosaccharides which confirmed that *PsMan* is an endo-acting enzyme.

### Literature

1. Zhang, Y.Z., Zhang, M., Chen, X.L., et al. Purification and functional characterization of endo-beta-mannanase MAN5 and its application in oligosaccharide production from konjac flour, Applied Microbiology and Biotechnology, 83, 865-873, 2009.
2. Lombard, V., Golaconda, R. H., Drula, E, Coutinho, P. M and Henrissat, B. (2014) The Carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research, 42, D490-D495.

## Insights into the mechanism of glucuronoxylan hydrolysis revealed by the 3-dimensional crystal structures of glucuronoxylan-xylanohydrolase (CtXyn30A) from *Clostridium thermocellum*

Anil Kumar Verma<sup>1\*</sup>, Arun Goyal<sup>1</sup>, Filipe Freire<sup>2</sup>, Carlos M.G.A. Fontes<sup>2</sup> and Shabir Najmudin<sup>2</sup>

arungoyl@iitg.ernet.in

1. Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati, 781039, Assam, India.
2. CIISA-Faculdade de Medicina Veterinária, Avenida da Universidade Técnica, 1300-477, Lisbon, Portugal.

The family 30 glycoside hydrolase (GH30) from *Clostridium thermocellum* is a modular carbohydrate active enzyme. The full length enzyme CtXynGH30 contains an N-terminal catalytic module, CtXyn30A followed by a family 6 carbohydrate binding module, CtCBM6 and a dockerin type I at the C-terminal [1]. CtXyn30A was cloned, expressed and purified as a soluble ~45 kDa protein. CtXyn30A preferably hydrolysed xylan based substrates like beechwood-, birchwood- and glucurono-xylan and displayed optimum activity at pH 6.0 and at 70°C. Zymogram analysis of CtXyn30A with beechwood xylan confirmed its xylanase activity. CtXyn30A gave maximum activity of 30.9 U mg<sup>-1</sup> with beechwood xylan. Kinetic studies of CtXyn30A with beechwood xylan gave a  $K_m$  of 0.2184 mg mL<sup>-1</sup> and  $K_{cat}$  of 1.205×10<sup>5</sup> min<sup>-1</sup>. The TLC analysis of hydrolysed products of beechwood xylan by treatment with CtXyn30A produced a series of higher xylo-oligosaccharides showing that it is an endo-xylanase. Whereas, on prolonged treatment of xylohexaose by CtXyn30A did not lead to formation of any lower xylo-oligosaccharides or monomer xylose. This suggested that CtXyn30A does not act on linear xylo-oligosaccharides and has an absolute requirement of substitution of glucuronic acid or 4-O-methyl glucuronic acid on xylans for enzymatic cleavage. Crystallization of CtXyn30A was achieved by sitting-drop vapour diffusion method and X-ray structure was solved by molecular replacement using the crystal structure of XynC from *Bacillus subtilis* 168 (PDB code 3gtn) as search model [2]. The three dimensional structure of CtXyn30A display a (β/α)<sub>8</sub> TIM barrel core with a side associated β-sheet domain. Structural data collected from crystals grown in different conditions showed that CtXyn30A can bind a variety of ligands such as the proteolysed His-tag peptide, HEPES, glycerol, tartaric or malonic acid in the catalytic site. The analysis of the residues interacting with the various ligands bound in the CtXyn30A structures suggested that Trp81, Tyr139, Trp143, Gln173, Tyr200, Tyr227, Ser231, Trp264, Tyr265, Arg268 and Tyr270 are important for ligand binding. A comparison with homologous structures solved in complex with different ligands confirmed that the equivalent residues are involved defining a consensus catalytic site. Thus, CtXyn30A catalytic site displays specificity pockets either side of active site, enabling GH30-8 members to bind to a number of different ligands that may mimic the different decorations of the xylan backbone.

### Literature

1. Anil K. Verma and Arun Goyal (2014) *In silico* structural characterization and molecular docking studies of first glucuronoxylan-xylanohydrolase (Xyn30A) from family 30 glycosyl hydrolase (GH30) from *Clostridium thermocellum*. Molecular Biology, 48, 278-286.
2. Anil K. Verma, Arun Goyal, Freire F., Bule P., Venditto I., Bras J. L. A., Santos H., Cardoso V., Bonifacio C., Thompson A., Romao M. J., Prates J. A. M., Ferreira L. M. A., Fontes C. M. G. A., and Najmudin S. (2013) Overexpression, crystallization and preliminary X-ray crystallographic analysis of glucuronoxylan-xylanohydrolase (Xyn30A) from *Clostridium thermocellum*, Acta Crystallogr. F69, 1440-1442.

## Enhanced saccharification and effective pretreatment of corn cob by utilizing recombinant cellulase and hemicellulase from *Clostridium thermocellum* for bioethanol production

Ashutosh Gupta, Debasish Das and Arun Goyal

arungoyl@iitg.ernet.in

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India

Exploitation of readily available agro waste such as corn cob (*Zea mays* var. *saccharata*) is one of the most promising renewable energy sources for production of bioethanol. Cost-effective saccharification and fermentation of lignocellulosic biomass encompasses the efficiencies of i) pretreatment, ii) carbohydrate degrading enzymes and iii) fermentative microbes. The present study involves the comparative analysis of two individual pretreatments viz. wet oxidation and ammonia fiber expansion (AFEX) and a mixed pretreatment strategy involving both, for efficient hydrolysis by recombinant cellulase and hemicellulase from *Clostridium thermocellum*. The analysis of structural carbohydrates content of untreated corn cob revealed cellulose, 52.2 (% w/w); hemicellulose, 28.0 (% w/w) and lignin 14.3 (% w/w), whereas after the mixed pretreatment (wet oxidation and AFEX) the content was 49.3, 23.0 and 6.2 (% w/w), respectively, displaying significant removal of lignin content. Fourier transform infrared (FT-IR) spectroscopic and field emission scanning electron microscopy (FESEM) analyses confirmed the structural deterioration with increased porosity and lignin breakdown of mixed pretreated corn cob. *E. coli* cells containing recombinant family 5 glycoside hydrolase (GH5) a cellulase [1] or GH43 a hemicellulase from *Clostridium thermocellum* [2] were used in saccharification process for the release of hexose and pentose sugars from pretreated lignocellulosic biomass. 1% (w/v) Simultaneous saccharification and fermentation (SSF) trials with individual pretreatment strategy of wet oxidation and AFEX comprising enzymatic consortium of GH5 and GH43 along with fermentative microbes, *Candida shehatae* and *Saccharomyces cerevisiae* gave 1.4 g/L and 1.2 g/L ethanol titres, respectively. However, improved ethanol titre of 1.7 g/L and yield of 0.235 (g of ethanol/g of substrate) was obtained with mixed pretreated 1% (w/v) biomass. Increasing the substrate concentration to 5% (w/v), SSF shake flask contributed a 5.5-fold higher ethanol titre (9.30 g/L) and yield (0.257 g of ethanol/g of pretreated substrate).

### Literature

1. Sangeeta Bharali, Ravi K. Purama, Avishek Majumder, Carlos M.G.A. Fontes and \*Arun Goyal (2005) Molecular cloning and biochemical properties of family 5 glycoside hydrolase of bi-functional cellulase from *Clostridium thermocellum*. Indian Journal of Microbiology 45(4), 317-321.
2. Shadab Ahmed, Ana Sofia Luís, Joana L.A. Brás, Arabinda Ghosh, Saurabh Gautam, Munishwar N. Gupta, Carlos M.G.A. Fontes and \*Arun Goyal (2013) A novel arabinofuranosidase of family 43 glycoside hydrolase (Ct43Araf) from *Clostridium thermocellum* releasing both  $\alpha$ -L arabinofuranose and arabinopyranose from xylan side chains. Plos One, 8(9), e73575.

## Structural and functional studies of a copper-dependent lytic polysaccharide monooxygenase from *Bacillus Amyloliquefaciens*

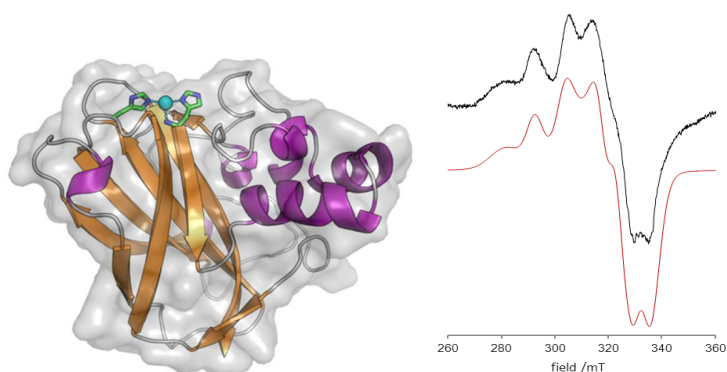
*Rebecca Gregory*<sup>1</sup>, *Gideon Davies*<sup>1</sup> and *Paul Walton*<sup>2</sup>

rcg502@york.ac.uk

1. York Structural Biology Laboratory, Department of Chemistry, University of York, UK

2. Department of Chemistry, University of York, UK

The enzymatic degradation of polysaccharides is a major goal of the biotechnology industry, most notably for both first (starch) and second (cellulose/chitin) generation biofuel processes. In recent years, the production of second generation biofuels has become increasingly more important to prevent unnecessary food usage. In addition, more efficient techniques of degradation of these second generation materials have become a requirement for scientific research. One such focus for increased efficiency is the class of copper-containing enzymes discovered in 2010<sup>1</sup>, known as Lytic Polysaccharide Monooxygenases (LPMOs). These enzymes were originally thought to possess no catalytic activity, so were incorrectly classified at the time of discovery. They were re-classified as “Auxiliary Activity” enzymes in 2013<sup>2</sup> (AA9 and AA10). The aim of my work is to study the bacterial LPMOs from the AA10 family, to determine their substrate specificity and ultimately oxidative mechanism. The enzyme I am currently working on is from the bacterium known as *Bacillus amyloliquefaciens*, the 3-D structure of which had been solved previously.<sup>3</sup> Here I showcase the enzymatic degradation of chitin by the *BaAA10*, as well as structural and mechanistic studies using various techniques, including EPR and X-ray crystallography.



**Figure 1.** 3-D structure and EPR spectrum (black – raw data; red – simulated) of *BaAA10*.<sup>3</sup>

### Acknowledgements

This work is funded by the BBSRC White Rose Doctoral Training Partnership.

### Literature

1. Vaaje-Kolstad, G.; Westereng, B.; Horn, S. J.; Liu, Z.; Zhai, H.; Sorlie, M.; Eijsink, V. G. *Science* **2010**, 330, 219.
2. Levasseur, A.; Drula, E.; Lombard, V.; Coutinho, P. M.; Henrissat, B. *Biotechnol Biofuels* **2013**, 6, 41.3. Hemsworth, G. R.; Taylor, E. J.; Kim, R. Q.; Gregory, R. C.; Lewis, S. J.; Turkenburg, J. P.; Parkin, A.; Davies, G. J.; Walton, P. H. *Journal of the American Chemical Society* **2013**, 135, 6069.

## Metagenome mining of novel enzymes for the bioethanol industry

*Noam Grimberg<sup>1,2</sup> and Yuval Shoham<sup>2</sup>*

noamgri@tx.technion.ac.il

1. The Nancy & Stephen Grand Technion Energy Program, Technion – Israel Institute of Technology.
2. Faculty of Biotechnology and Food Engineering, Technion – Israel Institute of Technology.

Lignocellulosic biomass is considered an attractive and immediate source for liquid biofuel. The hydrolysis of lignocellulose requires the synergistic actions of glycoside hydrolases (GHs), thus, novel enzymes with enhanced activity and production are necessary to improve the economics of the process. In the framework of this research, metagenomics and biochemical approaches were combined to identify and characterize novel enzymatic systems geared for lignocellulose degradation.

Our view on the GHs world is restricted to the current known sequences available in the databases. Thus, when analyzing metagenomes with conventional methods novel GHs genes with low sequence similarities are usually not identified.

Our sequence-based screening was designed to elicit novel GH genes from metagenomic data. We designed our search algorithm under three principles: a) a GHs designated algorithm, rather than a full data analysis; b) Genomic Neighborhood approach assimilation for sieving excess data ; and c) Conserved Neighborhood approach for putative novel GH genes evaluation .

Our search algorithm managed to reduce the analyzed data, and allowed us to focus on several clusters, which potentially harbor novel GHs. Following the scan of 75 thermal-springs metagenomes, 97 DNA scaffolds with 778 hemicellulolytic genes have been identified. Two clusters with putative cellulose degradation pathways were chosen for further evaluation and three putative novel GHs had taken for biochemical analysis. To-date, we identified a gene product with CMCase activity in each cluster, belonging to families GH5 and GH44. These results supports our working hypothesis that these clusters are involved in cellulose hydrolysis. Several of the remaining genes in the clusters are potentially new GH families and are in the process of biochemical characterization.

This work was supported by the Grand Technion Energy Program (GTEP), and comprises part of The Leona M. and Harry B. Helmsley Charitable Trust reports on Alternative Energy series of the Technion, Israel Institute of Technology, and the Weizmann Institute of Science.

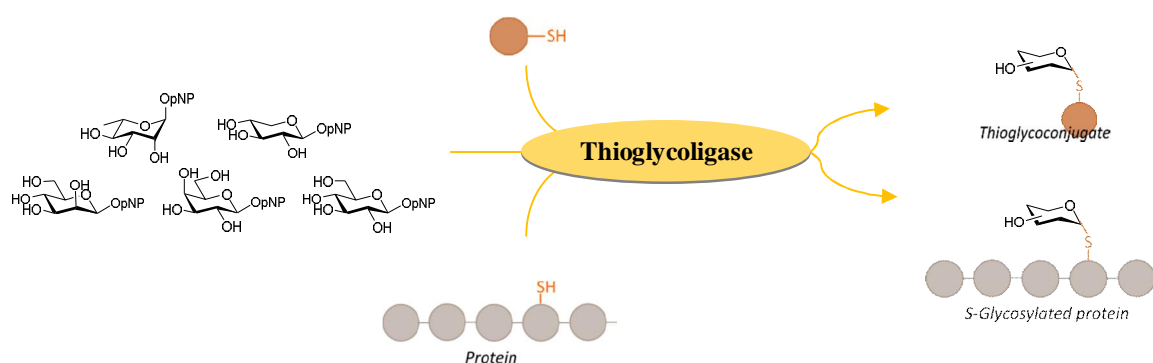
## Thioglycoligases : innovative biocatalytic tools for S-glycosylated proteins synthesis

*Laure Guillotin<sup>1</sup>, Pierre Lafite<sup>1</sup> and Richard Daniellou<sup>1</sup>*

laure.guillotin@univ-orleans.fr

1. Univ. Orléans, CNRS, ICOA, UMR 7311, rue de Chartres F-45067 Orléans, France

Glycosylation is one of the most complex co- and post-translational modifications of proteins and is involved in numerous biological processes as protein folding, metabolic stability, intracellular trafficking... As a consequence, errors in glycosylation have dramatic impacts on those processes affording apparition of several diseases (diabete, ancer, auto-immune disease...).<sup>1</sup> During the last past decades, advances made on carbohydrate chemistry allowed synthesis of glycoproteins and new glycoconjugates in order to understand their role and generate new therapeutics. Amongst them, thioglycoconjugates, in which a sulfur atom has replaced the glycosidic oxygen atom, became of great importance through their ability to resist to chemical and enzymatic cleavage.<sup>2</sup> The ambition of our project is therefore to develop a novel method based on chemoenzymatic synthesis to access S-glycosylated proteins.



Recent advances on protein bioengineering highlighted a new class of biocatalysts, thioglycoligases<sup>3</sup>, able to generate thioglycosidic linkage. In order to take up this challenge a bank of thermophilic mutated GHs from *D. thermophilum* was developed. All enzymes were assayed for thioglycosylation using a range of various thiols as glycoside acceptors. At the end, best thioglycoligase candidates will be used to generate S-linked glycosylated amino acid paving the way for S-glycoproteins synthesis.

**Acknowledgements** This research is supported by Region Centre and Glycodiag funding.

### Literature

1. A. Varki. *Glycobiology*, 1993, **3**, 97-130
2. P. Lafite, R. Daniellou. *Nat. Prod. Rep.*, 2012, **29**, 729-738.
3. L. Guillotin, P. Lafite, R. Daniellou. *Carbohydr. Chem*, 2014, **40**, 178-194.
4. L. Guillotin, P. Lafite, R. Daniellou. *Biochemistry*, 2014, **53**, 1447-1455.



## Rational design of a novel cyclodextrin glucanotransferase from *Carboxydocella* to improve alkyl glycoside synthesis

*Kazi Zubaida Gulshan Ara*<sup>1</sup>, *Jonas Jönsson*<sup>1</sup>, *Pontus Lundemo*<sup>1</sup>, *Javier A. Linares-Pastén*<sup>1</sup>, *Patrick Adlercreutz*<sup>1</sup> and *Eva Nordberg-Karlsson*<sup>1</sup>

eva.nordberg\_karlsson@biotek.lu.se

1. Dept of Chemistry, Biotechnology, Lund University, Sweden

Alkyl glycosides have gained huge attention from different industries due to their biodegradability, high surface activity and low toxicity. In many applications defined alkyl glycosides with an elongated carbohydrate moiety is required. The elongation process of an alkyl glycoside can be accomplished by using different cyclodextrins as donors in coupling reactions catalyzed by CGTase [1]. CGTase or cyclodextrin glucanotransferase (EC 2.4.1.19) belongs to GH family 13 and is also capable of catalyzing cyclization, hydrolysis and disproportionation by using substrates like starch. Recently we have characterized a novel CGTase from *Carboxydocella*, which was used to elongate dodecyl- $\beta$ -maltoside [2]. The high disproportionation reactivity limits the usability of this enzyme for producing well-defined alkyl glycosides (Table 1). In order to improve the synthesis of defined alkyl glycosides we have designed several mutations by a site-directed mutagenesis approach. The objective of this mutagenesis is to increase the coupling product formation while decreasing the disproportionation activity of the enzyme. Currently, we are analyzing the effect of these mutations and comparing them with the wild-type.

**Table 1:** Coupling and disproportionation activities of CGTase from *Carboxydocella*. Dodecyl- $\beta$ -maltoside was used as acceptor for coupling and disproportionation.

Enzyme	Donor	Coupling ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	Disproportionation ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )
CGTase ( <i>Carboxydocella</i> )	$\alpha$ -CD	$40.5 \pm 0.5$	$66.0 \pm 10.7$
	$\beta$ -CD	$25.2 \pm 1.9$	$19.6 \pm 4.6$
	$\gamma$ -CD	$65.5 \pm 0.7$	$85.1 \pm 11.2$

### Aacknowledgements

This work was supported by the EU FP7 program AMYLOMICS.

### Literature

1. Svensson D, Ulvenlund S, Adlercreutz P. 2009b. Enzymatic route to alkyl glycosides having oligomeric head groups. *Green Chemistry*, 11:1222-1226.
2. Ara, K.Z.G., et al., A CGTase with high coupling activity using  $\gamma$ -cyclodextrin isolated from a novel strain clustering under the genus *Carboxydocella*. *Glycobiology*, 2014

## **Development and application of a synthetic cellulosome-based screening platform for enhanced enzyme discovery**

*Johnnie Hahm<sup>1</sup>, Elizabeth Znameroski<sup>1</sup>, Fang Liu<sup>1</sup>, Tia Heu<sup>1</sup>, Ian Haydon<sup>1</sup>, Sumati Hasani<sup>1</sup>, Michael Lamsa<sup>1</sup>, Aubrey Jones<sup>1</sup>, William Widner<sup>1</sup>, Ronald Mullikin<sup>1</sup>, Paul Harris<sup>1</sup>, Sarah Teter<sup>1</sup>, Janine Lin<sup>1</sup>*

joh@novozymes.com

1. Novozymes, Inc.

At the forefront of enzyme discovery, Novozymes continues to search for better ways to screen the growing diversity of glycosyl hydrolases in order to improve enzyme cocktails for biomass conversion. To this end, we have successfully integrated a synthetic biology approach into our next generation of enzyme discovery platforms. Specifically, we have leveraged the available natural diversity of cellulosomal components to develop an artificial mini-cellulosome as the basis for a versatile cell-free method to screen combinations of multiple enzyme activities. In the process, we have engineered a highly thermostable scaffoldin comprised of distinct cohesin domains that stably bind cognate dockerins with high specificity for extended periods under lignocellulose hydrolysis conditions. These protein-protein interactions serve as respective binding modules for covalently-linked libraries of different enzyme classes. Our synthetic cellulosome approach has been adapted to an automated HTS system through which enzymes of synergistic quality are more efficiently identified compared to past conventional approaches. Finally, we have successfully employed this novel screening platform to discover promising new enzyme candidates tailored for improved hydrolytic performance on (AFEX-PCS) an industrially-relevant biomass substrate.

## Identification of the catalytic residues of glycosidases from *Paenibacillus thiaminolyticus* as a key into engineering new glycosynthases

*Katarína Hlat-Glembová<sup>1</sup>, Vojtěch Spiwok<sup>1</sup>, Eva Benešová<sup>1</sup>, Blanka Králová<sup>1</sup>*

katarina.hg@gmail.com

1. University of Chemistry and Technology, Prague

Glycosyl hydrolases  $\beta$ -D-galactosidase and  $\alpha$ -L-fucosidase cloned from *Paenibacillus thiaminolyticus*, are interesting not only because of their hydrolase activity but mainly, for their transglycosylation activity, which is important for synthesis of interesting glycosylated molecules. In this project both enzymes were studied using the combination of theoretical and experimental methods. In the theoretical part, the structures of enzymes were predicted and further studies of these structures proposed the possible catalytic residues in both enzymes. The aim of the experimental part is to confirm the predictions of catalytic amino acid residues assumed in the theoretical part. Here we report the final results in which mutated enzymes  $\beta$ -D-galactosidase\_mut233,  $\alpha$ -L-fucosidase\_mut186 and  $\alpha$ -L-fucosidase\_mut239 were produced and purified. Furthermore  $\beta$ -D-galactosidase\_mut157 was purified in earlier experiments. All mutants were tested on several substrates. The results of these activity tests are helpful for better understanding the catalytic machinery of studied enzymes and lead us to possibility of transforming them into glycosynthases i.e. modified glycosyl hydrolases that lack their natural hydrolytic activity, because of mutation of nucleophilic amino acid residue, but maintain their transglycosylation activity. On this poster we also show the first progress in designing of these glycosynthases.

**Acknowledgements** The project was supported by COST actions MultiGlycoNano (CM1102, LD13024). Participation at the conference is supported by specific university research (MSMT No 20/2015, No MSMT 21/2015).

### Literature

1. Benešová E., Lipovová P., Dvořáková H., Králová B.:  $\beta$ -D-Galactosidase from *Paenibacillus thiaminolyticus* catalyzing transglycosylation reactions. *Glycobiology* 20, 442-451 (2010).
2. Lammerts van Bueren A., Ardevol A., Fayers-Kerr J., Luo B., Zhang Y., Sollogoub M., Blériot Y., Rovira C., Davies G. J.: Analysis of the reaction coordinate of  $\alpha$ -L-fucosidases: A combined structural and quantum mechanical approach. *J. Am. Chem. Soc.* 132, 1804-1806 (2010).

## Identification and characterization of a novel unclassified de-N-acetylase from *Sulfolobus solfataricus*

Roberta Iacono<sup>1</sup>, Beatrice Cobucci-Ponzano<sup>1</sup>, Andrea Strazzulli<sup>1</sup> and Marco Moracci<sup>1</sup>

roberta.iacono@ibbr.cnr.it

1. Institute of Biosciences and BioResources – CNR, Via P. Castellino 111, 80131, Naples, Italy

In the last few years, our research topic has been focused extensively on the carbohydrate active enzymes from hyperthermophilic Bacteria and Archaea (thermozymes) in order to discover new enzymatic activities, understand the molecular basis of thermal adaptation and, eventually, to design new stabilized biocatalysts for industrial applications [1,2]. Therefore, we embarked on the identification of novel enzymatic activities from the thermoacidophilic Archaeon *Sulfolobus solfataricus*.

Here we report on the identification and the characterization of a hypothetical protein codified by the ORF sso2901. According to BLAST alignments, sso2901 encodes for a putative N-acetylglucosaminyl-phosphatidylinositol-(GlcNAc-PI)-de-N-acetylase (EC 3.5.1.89), which belongs to PIG-L family (Pfam 02585). This family includes the de-N-acetylases that catalyses the second step of GPI anchor biosynthesis [3]. Although the activities on GlcNAc-PI are included in the CAZy database in CE14, sso2901 is not classified in CE families. The biochemical characterization on the recombinant SSO2901 (rSSO2901) on GlcNAc revealed that the maximum activity is observed at pH 8.5 and 70°C, with  $k_{CAT}$   $1.94 \pm 0.19$  s<sup>-1</sup>,  $K_M$   $22.01 \pm 4.91$  mM and  $k_{CAT}/K_M$   $88 \times 10^{-3}$  s<sup>-1</sup> mM<sup>-1</sup>. In addition, rSSO2901 has shown activity on pNp- $\alpha$ -GlcNAc and pNp- $\beta$ -GlcNAc, but not on chitobiose, GalNAc, ManNAc, GlcNAc-1P, GlcNAc-6S, UDP-GlcNAc and hyaluronic acid. These results indicate that SSO2901 represents the first de-N-acetylase identified from *S. solfataricus* belonging to PIG-L family. In addition, close to sso2901, we have identified the ORF sso2900, codifying for a hypothetical GT4. Surprisingly, we have observed that the recombinant SSO2900 is able to hydrolyze UDP-GlcNAc. So, we speculate that both enzymes might be involved in the same metabolic pathway and their biochemical characterizations might shed light on their function *in vivo*.

This work was supported by the project PON01\_01966 “ENERBIOCHEM” of the Programma Operativo Nazionale Ricerca e Competitività 2007-2013 -MIUR-Italy and by the Short-term mobility Programme “STM 2013” of the National Research Council of Italy

### Literature

1. Cobucci-Ponzano B. et al., Protein Eng Des Sel. 2011 Jan;24(1-2):21-6.
2. Ferrara MC. et al. Biochim Biophys Acta. 2014 Jan;1840(1):367-77.
3. Watanabe R. et al.; Biochem J 1999;339:185-192.

## Development of novel enzymatic tools for the production of xylose-based products within a lignocellulosic biorefinery concept

*Eleni Ioannou<sup>1,2,3,4</sup>, Claire Dumon<sup>1,2,3</sup>, David Bryant<sup>4</sup>, Narcis Fernandez-Fuentes<sup>4</sup> and Michael O'Donohue<sup>1,2,3</sup>*

michael.odonohue@insa-toulouse.fr

1. Université de Toulouse, INSA, UPS, INP; LISBP, 135 avenue de Rangueil, F- 31077, Toulouse, France

2. INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F- 31400, Toulouse, France

3. CNRS, UMR5504, F- 31400, Toulouse, France

4. IBERS, Gogerddan Campus, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3EE, United Kingdom

Advanced biorefining is a key element of the targeted transition towards a sustainable bioeconomy, which will use plant biomass as a primary carbon source instead of petroleum resources. Utilization of plant biomass is followed by the breakdown of its complex structure into fermentable sugars via a treatment step that uses enzymatic cocktails. Biomass degrading enzymes, such as Glycoside Hydrolases (GHs) [1], often work in synergy with other enzymes and there are numerous examples of natural bi- or multifunctional enzyme assemblies [2]. Microorganisms can be considered as the source of biomass degrading enzymes and the functional screening of their metagenomic libraries, can mediate the discovery of new enzymatic activities [3].

In this project, a novel GH was discovered after the functional screening of a metagenomic library [3] and was annotated as a xylanase. Its amino acid sequence was predicted to also encode a Carbohydrate Binding Module (CBM) [1] and it was then used to construct a 3D structural model. The molecular modeling contributed in understanding the architecture of the domains, revealing that it is a multimodular enzyme consisted of a catalytic GH domain and two CBMs. This novel multimodular enzyme will serve as a blueprint for further enzyme engineering; where the CBM domains could be replaced by another GH, in order to design tailored multifunctional enzymes with multiple catalytic activities for use in biorefineries.

### Literature

1. Lombard, V. et al. (2014). "The Carbohydrate-active enzymes database (CAZy) in 2013." *Nucleic Acids Res.* **42**: D490–D495
2. Himmel, M. E. et al. (2010). "Microbial enzyme systems for biomass conversion: emerging paradigms" *Biofuels* **1** (2): 323-341
3. Bastien, G., G. Arnal, et al. (2013). "Mining for hemicellulases in the fungus-growing termite *Pseudacanthotermes militaris* using functional metagenomics." *Biotechnology for biofuels* **6**(1): 78

## Biochemical characterization of a novel aldose-ketose isomerase, mannose isomerase from *Marinomonas mediterranea*

*Nongluck Jaito<sup>1</sup>, Wataru Saburi<sup>1</sup>, Yuka Tanaka<sup>1</sup>, and Haruhide Mori<sup>1</sup>*

nongluck@chem.agr.hokudai.ac.jp

1. Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

*N*-acetylglucosamine 2-epimerase (AGE) superfamily enzymes including AGE, aldose-ketose isomerase, and cellobiose 2-epimerase share a catalytic domain formed by an ( $\alpha/\alpha$ )<sub>6</sub>-barrel and an active-site structure composed by two catalytic His residues acting as general acid and base catalysts. A possible member of AGE superfamily, Marme\_2490 from *Marinomonas mediterranea*, is an uncharacterized protein with low amino acid sequence similarity to any characterized enzyme, and its biological functions are unclear. In this study, we investigated biochemical properties of recombinant Marme\_2490 produced in *Escherichia coli*.

The recombinant Marme\_2490 was successfully produced as soluble protein in *E. coli*, and purified to homogeneity by Ni-chelating column chromatography. From 1 L of the culture broth, 18.8 mg of purified recombinant protein was obtained. To examine the activity for Marme\_2490, the protein was incubated with 21 kinds of sugars and the reaction mixture was analyzed on thin layer chromatography. D-Mannose and D-fructose were converted to D-fructose and D-mannose, respectively, indicating that Marme\_2490 has D-mannose isomerase activity. Thus, Marme\_2490 was designated as *Mm*MI. The optimum pH and temperature of *Mm*MI, investigated using D-mannose as a substrate, were pH 7.3 and 30° C, respectively. The enzyme was stable in a range of pH 5.5-9.4 and below 35°C after the pH (4° C for 24 h) and heat (pH 7 for 30 min) treatments, respectively. The  $k_{cat}$ ,  $K_m$ , and  $k_{cat}/K_m$  of *Mm*MI for the epimerization of D-mannose were  $463 \pm 3 \text{ s}^{-1}$ ,  $16.7 \pm 1.8 \text{ mM}$ , and  $27.7 \text{ s}^{-1}\text{mM}^{-1}$ , respectively. *Mm*MI also had a weak isomerization activity to disaccharides,  $\beta$ 1-4 mannobiose, 4-*O*- $\beta$ -D-glucosyl-D-mannose, and 4-*O*- $\beta$ -D-galactosyl-D-mannose (epilactose). Isomerization activity to oligosaccharides has been first found in D-mannose isomerases. In contrast to *E. coli* and *Salmonella enterica* aldose-ketose isomerases (YihS proteins), which catalyze interconversion of D-mannose, D-glucose, and D-fructose [1], *Mm*MI did not act on D-glucose (reaction velocity was estimated to be  $\leq 0.22 \text{ }\mu\text{mol/min/mg protein}$ ,  $\leq 0.052\%$  of that to D-mannose) and produce D-glucose from D-fructose and D-mannose. *N*-acetyl-D-glucosamine and cellobiose did not serve as substrates of *Mm*MI. Consequently, *Mm*MI has been classified to be a member of aldose-ketose isomerase, and it is a novel type of mannose isomerase.

### Acknowledgements

Part of this work was supported by Noda Institute for Scientific Research GRANT (2014 Young Investigator Research Grant).

### Literature

1. Itoh, T., Mikami, B., Hashimoto, W., and Murata, K. *J. Mol. Biol.*, **377**, 1443-1459 (2008).

## Neopullulanase subfamily and related specificities of the family GH13 - *in silico* study focused on domain evolution

*Stefan Janecek and Andrea Kuchtova*

Stefan.Janecek@savba.sk

Laboratory of Protein Evolution, Institute of Molecular Biology, Slovak Academy of Sciences, SK-84551 Bratislava, Slovakia

The  $\alpha$ -amylase family GH13 [1] containing ~30 different enzyme specificities and more than 21,000 sequences, represents one of the largest CAZy glycoside hydrolase (GH) families [2]. Earlier, based on a characteristic sequence motif in their 5<sup>th</sup> conserved sequence region, the two closely related subfamilies, the so-called oligo-1,6-glucosidase and neopullulanase subfamilies were described [3]. Currently the two subfamilies cover several CAZy-defined GH13 subfamilies [4] because the  $\alpha$ -amylase family GH13 has been officially divided into 40 subfamilies [5]. The present *in silico* analysis was undertaken in an effort to shed some light on the evolution of modular arrangement within the neopullulanase subfamily (the subfamily GH13\_20). The main goal was to reveal the evolutionary relationships between the catalytic domain representing the enzyme specificity and the non-catalytic starch-binding domains (SBDs). The subfamily GH13\_20 contains, in addition to neopullulanase, also cyclomaltodextrinase and maltogenic amylase [2-4]. They usually possess the N-terminal SBD classified as the carbohydrate-binding module (CBM) family CBM34 [2,6]. The studied set, extracted mostly from the CAZy database subfamily GH13\_20 and family CBM34, consisted of 70 family GH13 members with the emphasis given on biochemically characterized enzymes. The set was further completed by some closely related amylolytic enzymes either from the subfamily GH13\_39 having also CBM48 or from GH13\_20 with CBM20, or even lacking any CBM.

### Acknowledgements

This work was supported by the Slovak Research Agency VEGA grant No. 2/0150/14.

### Literature

1. Janecek S., Svensson B. & MacGregor E.A.: *Cell. Mol. Life Sci.* 2014, 71: 1149-1170.
2. Lombard V., Golaconda Ramulu H., Drula E., Coutinho P.M. & Henrissat B.: *Nucleic Acids Res.*, 2014, 42: D490-D495.
3. Oslancova A. & Janecek S.: *Cell. Mol. Life Sci.* 2002, 59: 1945-1959.
4. Majzlova K., Pukajova Z. & Janecek S.: *Carbohydr. Res.* 2013, 367: 48-57.
5. Stam M.R., Danchin E.G., Rancurel C., Coutinho P.M. & Henrissat B.: *Protein Eng. Des. Sel.* 2006, 19: 555-562.
6. Janecek S., Svensson B. & MacGregor E.A.: *Enzyme Microb. Technol.* 2011, 49: 429-440.

## Characterization of a GH30 glucuronoxylan specific xylanase from *Streptomyces turgidiscabies* C56

Tomoko Maehara<sup>1</sup>, Zui Fujimoto<sup>2</sup>, Kei Kamino<sup>3</sup>, Yoshiaki Kitamura<sup>4</sup>, and Satoshi Kaneko<sup>5</sup>

sakaneko@agr.u-ryukyu.ac.jp

1. Food Biotechnology Division, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
2. Biomolecular Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba 305-8602, Japan
3. National Institute of Technology and Evaluation, 2-5-8 Kazusa, Kisarazu 292-0818, Japan
4. Applied Bacteriology Division, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
5. Department of Subtropical Biochemistry and Biotechnology, Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan

The gene belonging to glycoside hydrolase family 30 (StXyn30A) was cloned from *Streptomyces turgidiscabies* C56 and the recombinant protein which is a putative xylanase was purified and characterized. This enzyme showed hydrolytic activity on xylans containing glucuronic acid side chains, but not on linear xylooligosaccharides, suggesting that this enzyme positively recognize the glucuronic acid side chain to hydrolyze the main chain of xylan. By thin-layer chromatography and mass spectrometry analysis of GH30 xylanase hydrolysate treated  $\beta$ -xylosidase, it was confirmed that the hydrolysis product from glucuronoxylan has glucuronic acid at the second xylopyranosyl residue from the reducing end.

To understand the mechanism how StXyn30A recognizes glucuronic acid substitution, we focused R296 residue of GH30 from *S. turgidiscabies* C56 which is conserved in bacterial GH30s but not in fungal GH30s. Because molecular modeling analysis and previous studies (ref) suggested the R296 residue in StXyn30A interact with carboxyl group of glucuronic acid side chain of xylan, we constructed R296 mutant enzymes to analyze the activities for glucuronoxylans and hydrolysis products. Significant reduction of the hydrolytic activities for glucuronoxylans were observed in all of R296 mutants.  $\beta$ -xylosidase treatment of hydrolysis products by R296 mutants suggested R296 mutants lose the ability to recognize the glucuronic acid side chains.



## Chitinases in a lignin-producing cell culture of Norway spruce

Kaija Porkka<sup>1</sup>, Silvia Vidal-Melgosa<sup>2</sup>, Julia Schückerl<sup>2</sup>, Sanna Koutaniemi<sup>3</sup>, William G. T. Willats<sup>2</sup> and Anna Kärkönen<sup>1</sup>

kaija.porkka@helsinki.fi

1. University of Helsinki, Department of Agricultural Sciences, Finland

2. University of Copenhagen, Department of Plant and Environmental Sciences, Denmark

3. University of Helsinki, Department of Food and Environmental Sciences, Finland

A suspension culture of Norway spruce (*Picea abies* (L.) Karst.) secretes extracellular lignin into the culture medium (1). Interestingly, a high chitinolytic activity was detected in the medium. Polyacrylamide gel electrophoresis in a chitin-containing gel indicated chitinolytic activity in enzymes of 16–24 kDa, and isoelectric focusing showed the presence of 8–10 acidic and basic chitinolytic enzymes. Some activity was also observed in the higher molecular weight region. Since chitin is not present in plants, we hypothesize that the observed chitinases in the aseptic Norway spruce cell culture may act upon not just chitin but also against endogenous plant substrates.

Chitinases of the culture medium were purified with affinity, size exclusion and ion exchange chromatography. They were characterized by western blotting with antibodies against chitinases. At least group I, II and IV chitinases of 18–35 kDa were observed. The enzyme activity of two cationic chitinases was tested against several cell wall-related polymers using chromogenic polysaccharide hydrogel (CPH) substrates and carbohydrate microarray-based technology. The chromogenic substrates did not reveal any obvious substrate alternatives for the chitinases, but microarray analyses indicated a low mannan response. Currently some of the chitinases expressed in spruce wood and in the cell culture are being cloned and will be produced heterologously. Produced proteins will be further characterized and utilized in substrate analyses in order to eliminate possible contaminant enzyme activities carried over in the purification process of chitinolytic enzymes.

### Acknowledgements

We thank the Academy of Finland and the Niemi Foundation for funding.

### Literature

1. Kärkönen et al. 2002. *Physiologia Plantarum* 114: 343–353.

## Enzyme properties affecting enzyme adsorption onto lignin in high solid environments

Miriam Kellock,<sup>1,2</sup>, Jenni Rahikainen<sup>1</sup> and Kristiina Kruus<sup>1</sup>

ext-miriam.kellock@vtt.fi

1. VTT Technical Research Centre of Finland, Espoo, Finland

2. Department of Food and Environmental Sciences, University of Helsinki, Finland

Lignocellulosic biomass is a renewable raw material that can be refined to various products including paper, packaging, composites, fuels and chemicals. In the industry, enzymatic hydrolysis of lignocellulosic biomass into fermentable sugars is preferentially carried out in high solid consistency, i.e. over 15 % (w/w) solids concentration<sup>1</sup>. Currently there is a limited amount of data available on enzyme performance in high solids concentrations. In high consistency environments enzyme inhibitions are typically stronger, for instance unproductive binding onto lignin. This study aims to elucidate the effect of enzyme properties on unproductive binding in low and high consistency enzymatic hydrolysis. Enzyme binding onto lignin rich residues isolated from steam explosion pretreated spruce and wheat straw was studied with purified *Trichoderma reesei* monocomponent cellulases and a xylanase as well as *Aspergillus niger*  $\beta$ -glucosidase -enzymes. Enzyme binding was studied using conventional adsorption assays as well as quartz crystal microgravimetry technique (QCM). Enzymes containing large hydrophobic patches have been hypothesized to bind more readily onto lignin model films<sup>2</sup>. Nevertheless, thermostable enzymes are expected to be less prone to the presence of lignin<sup>3</sup>. This paper discusses the effects of enzyme properties, such as surface characteristics and thermostability, on enzyme adsorption and inactivation onto lignin surfaces.

### Acknowledgements

Fortum foundation is greatly acknowledges for funding this work. Arja Paananen is thanked for her assistance and expertise with atomic force microscopy imaging. Technicians at VTT are thanked for their excellent assistance throughout the work.

### Literature

1. Modenbach, A. A., & Nokes, S. E. (2013). Enzymatic hydrolysis of biomass at high-solids loadings - A review. *Biomass and Bioenergy*, 56, 526-544.
2. Sammond, D. W., Yarbrough, J. M., Mansfield, E., Bomble, Y. J., Hobdey, S. E., Decker, S. R., Taylor, L. E., Resch, M. G., Bozell, J. J., Himmel, M. E., Vinzant, T. B. & Crowley, M. F. (2014). Predicting enzyme adsorption to lignin films by calculating enzyme surface hydrophobicity. *The Journal of Biological Chemistry*, 289(30), 20960–9.
3. Rahikainen, J. L., Moilanen, U., Nurmi-Rantala, S., Lappas, A., Koivula, A., Viikari, L., & Kruus, K. (2013). Effect of temperature on lignin-derived inhibition studied with three structurally different cellobiohydrolases. *Bioresource Technology*, 146, 118–25.

## Solution structures of glycosaminoglycans and their complexes with complement Factor H: implications for disease

Sanaullah Khan<sup>1,2</sup>, Jayesh Gor<sup>1</sup>, Barbara Mulloy<sup>3</sup> and Stephen J. Perkins<sup>1</sup>

sank@nanotech.dtu.dk

1. Institute of Structural and Molecular Biology, Division of Biosciences, Darwin Building, University College London, Gower Street, London WC1E 6BT, UK
2. Department of Micro- and Nanotechnology Ørsted's Plads, building 345E DK-2800 Kgs. Lyngby, Denmark.
3. National Institute of Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, U. K.

Factor H (FH) is a major regulator of complement and is genetically associated with the development of age-related macular degeneration (AMD) in the elderly. Heparin and heparan sulphate (HS) bind to sites found on at least the SCR-6/8 and SCR-19/20 regions of FH. Despite its importance, no molecular structures of free HS and large heparin fragments have been reported. By combining analytical ultracentrifugation (AUC), small angle x-ray scattering (SAXS), and constrained scattering modeling, we have analyzed the solution structures for a range of purified heparin and HS fragments. Solution structures of heparin and HS complexes with FH are likewise not known, and were determined. To date, our solution structures for the heparin complex with FH SCR-6/8 shows that FH SCR-6/8 forms large aggregates in the presence of heparin. Intact FH forms 5-15% of dimeric and higher oligomer structures in the absence of heparin. When heparin is titrated with FH, our AUC and X-ray data shows that heparin promotes further strong FH aggregation (up to 63%) as well as conformational changes in FH. These conformational changes and aggregation results provide the first molecular picture of how FH interacts with host cell surfaces and have a direct relevance for the sub-retinal pigmented endothelial deposits that form in Bruch's membrane during the development of AMD. SPR of immobilized heparin with full length FH gave K<sub>d</sub> values of 1–3 μM, and weaker K<sub>d</sub> values of 4–20 μM for the FH SCR-6/8 and FH SCR-16/20 fragments, confirming co-operativity between the two binding sites.

### Acknowledgments

We thank University College London, the Medical Research Council, the Biotechnology and Biological Sciences Research Council, Biolin Scientific, the Fight for Sight Charity, the Henry Smith Charity.

### Literature

1. Khan, S., Fung, K. W., Rodriguez, E., Patel, R., Gor, J., Mulloy, B. and Perkins, S. J. (2013). The solution structure of heparan sulphate differs from that of heparin: implications for function. *J. Biol. Chem.* **288**: 27737–51.
2. Khan, S., Nan, R., Gor, J., Mulloy, B. and Perkins, S. J. (2012). Bivalent and co-operative binding of complement factor H to heparan sulphate and heparin. *Biochem. J.* **444**: 417–28.

## A novel sialic acid-specific lectin from the mushroom *Hericium erinaceum*

Seonghun Kim<sup>1,2</sup>

seonghun@kribb.re.kr

1. Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsingil, Jeongeup 580-185, Korea
2. Biosystems and Bioengineering Program, University of Science and Technology (UST), Daejeon 305-350, Korea

Sialoglycoconjugates have a variety of biological functions and involve the mediation and/or modulation of numerous biological phenomena [1]. Mushroom lectins harboring carbohydrate binding specificity are a powerful tool to detect sialoglycoconjugate with  $\alpha(2,3)$ -,  $\alpha(2,6)$ -, and  $\alpha(2,8)$ -linkages [2]. However limited numbers of sialic acid-specific binding lectins are available to detect linkage of glycoconjugates [3]. In this study, a sialic acid-specific binding lectin was identified and characterized from the fruiting body extract of a mushroom *Hericium erinaceum*. The sialic acid binding lectin was purified by a combination of ion-exchange column, an immobilized fetuin column and gel filtration chromatography. The purified lectin was designed as HEL (*H. erinaceum* lectin). Tricine-PAGE, MALDI-TOF mass spectrometry, and N-terminal amino acid sequencing indicated that the native HEL has an identical form with a molecular weight of approximate 15 kDa. Isoelectric focusing of the lectin showed bands near pI 5.4. Hemagglutination assay displayed the agglutination activity of HEL was more effective against porcine erythrocyte rather than other animal red blood cells. When HEL was analyzed with glycan microarray, it was observed that the lectin binds to Neu5Ac, Neu5Gc and other sialic acid derivatives. Furthermore, HEL bound to  $\alpha(2,3)$ -sialylated fetuin and not to asialofetuin. Thus, HEL will be a promising tool for detection of linkage-specific sialic acid in glycoconjugates.

### Acknowledgements

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2013R1A1A1061657) and by Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009783) funded by Rural Development Administration, Korea.

### Literature

1. Kim, S., Oh, D.B., Kang, H.A. and Kwon, O. (2011) Features and applications of bacterial sialidases. Appl. Microbiol. Biotechnol. 91: 1-15.
2. Varrot, A., Basheer, S.M., and Imberty, A. (2013) Fungal lectins: structure, function and potential applications. Curr. Opin. Struct. Biol. 23: 678-685.
3. Lehmann, F., Tiralongo, E., Tiralongo, J. (2006) Sialic acid-specific lectins: occurrence, specificity and function. Cell. Mol. Life Sci. 63: 1331-1354

## Enzymatic production of a natural solubilizer rubusoside using a thermostable lactase from *Thermus thermophilus*

Doman Kim<sup>1,2</sup>, Thi Thanh Hanh Nguyen<sup>2</sup>, Jaeyoung Cho<sup>2</sup>, Ye-seul Suh<sup>2</sup>, Eunbae An<sup>1</sup>, Jiyoun Kim<sup>1</sup>, and Shin-Hye Yu<sup>1</sup>

kimdm@snu.ac.kr

1. Graduate School of International Agricultural Technology, Seoul National University, Korea
2. Institutes of Green Bio Science & Technology, Institute of Food Industrialization, Seoul National University, Korea

The major challenge with the design of oral dosage is its poor solubility and low permeability. Among the bioactive compounds obtained from plant sources, it has been discovered that some steviol glycosides such as stevioside (Ste), rebaudioside A, and rubusoside possess solubilizing properties. Rubusoside (RU) is a natural sweetener and a solubilizing agent with antiangiogenic and ant allergic properties. However, currently, its production is quite expensive, and therefore to find enzymes for more efficient conversion of Ste to Ru, thirty commercial enzymes were tested, having the mixed activities of pectinase, cellulases, hemicellulases,  $\alpha$ -galactosidase,  $\beta$ -galactosidase and/or  $\beta$ -glucanase, along with a purified recombinant lactase. Among these, the recombinant lactase from *Thermus thermophilus* showed the highest Ru productivity. Therefore, it was chosen for immobilization on sodium alginate beads, and used to prepare Ru. Recombinant  $\beta$ -galactosidase of *Thermus thermophilus* (6 KU) was mixed with 20 mL of 3% (w/v) sodium alginate solution to give a final 300 U.mL<sup>-1</sup> of sodium alginate bead. The free enzyme was kept at 70°C and the thermo stability was compared with the immobilized enzyme. Immobilized lactase was prepared and used for the production of rubusoside; twelve reaction cycles were repeated with 95.4% of Ste hydrolysis and 49 g.L<sup>-1</sup> of Ru was produced. The optimum rubusoside synthesis yield was 86% at 200 g.L<sup>-1</sup>, 1200 U lactase. The reaction mixture containing 20 mL of 1% (w/v) Ste solution in Tris-HCl buffers (40 mM, pH 7.0) and 10 mL alginate beads (300 U.mL<sup>-1</sup> beads) were incubated at 70°C in a water bath. After the enzymatic reaction had proceeded for 24 h. One hundred mg RU and 10 mg insoluble compounds were weighted and mixed at the ratio of 10:1 (w/w). One mL of absolute ethanol was added in a tightly sealed tube and mixed for 15 min. The mixture solution was centrifuged at 12,000 rpm for 10 min and the supernatant was transferred to another Eppendorf tube. The ethanol was evaporated. The purified 10% rubusoside solution showed increased water solubility of liquiritin from 0.98 mg.mL<sup>-1</sup> to 4.70  $\pm$  0.12 mg.mL<sup>-1</sup> and 0 mg.mL<sup>-1</sup> to 3.42  $\pm$  0.11 mg.mL<sup>-1</sup> in the case of teniposide. (Poster Presentation Abstract)

### Aacknowledgement

This work was partially supported by the National Research Foundation of Korea (NRF) grant, funded by the Korea government (MEST) (No. NRF-2012R1A2A2A01045995).

### Literature

Production of rubusoside from stevioside by using a thermostable lactase from *Thermus thermophiles* and solubility enhancement of liquiritin and teniposide. Enzyme Microb Technol 2014 Oct 11;64-65:38-43. 2014.

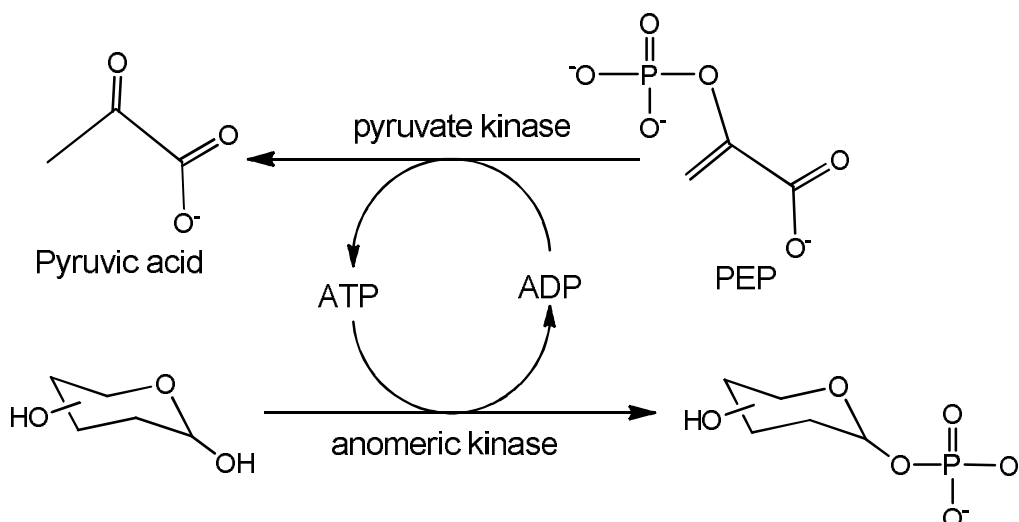
## Practical preparation of sugar 1-phosphates

*Motomitsu Kitaoka, Yuan Liu, and Mamoru Nishimoto*

mkitaoka@affrc.go.jp

National Food Research Institute, National Agriculture and Food Research Organization

Sugar 1-phosphates are biochemically important compounds for precursors of NDP-sugars as well as substrates for phosphorylases. We here show the preparations of these compounds. Some of sugar 1-phosphates were produced by the phosphorolysis of oligosaccharides using phosphorylases and others were by phosphorylation of monosaccharides using anomeric kinases in the presence of an ATP-regeneration system. The sugar 1-phosphates produced were isolated by employing electrodialysis steps using two different membrane cassettes and finally crystalized as certain salts. We have prepared the following sugar 1-phosphates in more than gram-scale:  $\beta$ -D-glucose 1-phosphate (by maltose phosphorylase),  $\alpha$ -D-mannose 1-phosphate, *N*-acetyl- $\alpha$ -D-glucosamine 1-phosphate, *N*-acetyl- $\alpha$ -D-galactosamine 1-phosphate (by *N*-acetylhexosamine 1-kinase),  $\alpha$ -D-galactose 1-phosphate,  $\alpha$ -D-galacturonic acid 1-phosphate,  $\beta$ -L-arabinose 1-phosphate (by galactokinase),  $\beta$ -L-fucose 1-phosphate,  $\alpha$ -D-arabinose 1-phosphate (by fucokinase).



**Fig 1.** Anionic phosphorylation by anionic kinase with an ATP-regeneration system

### Acknowledgements

This work was supported in part by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

### Literature

1. Liu *et al.* (2015) *Carbohydr. Res.*, **401**, 1-4

## Structural and functional insights into the CBM50s of two plant GH18 chitinases

*Yoshihito Kitaoku*<sup>1</sup>, *Toki Taira*<sup>2</sup>, *Tomoyuki Numata*<sup>3</sup>, *Tamo Fukamizo*<sup>1</sup>, *Takayuki Ohnuma*<sup>1</sup>

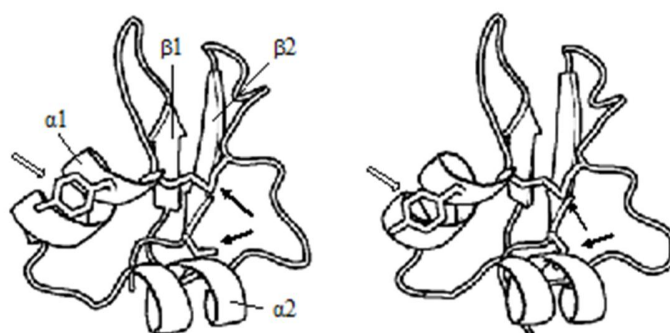
1333690010c@nara.kindai.ac.jp

1. Department of advanced bioscience, Kinki university, 3327-204 Naka-machi, Nara-shi, Nara, Japan

2. Department of Bioscience and Biotechnology, University of Ryukyus, 1 Senbaru, Nishihara, Okinawa, Japan

3. Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba 305-8566, Japan

Family 50 carbohydrate-binding modules (CBM50s) are also known as LysM domains. They are protein modules that are widely distributed in eucaryotes and procaryotes. Although CBM50s are found in various proteins, main chain structure, *i.e.*  $\beta\alpha\alpha\beta$  fold, and ligand binding sites are well conserved. In the present study, we solved the crystal structure of two CBM50s of plant GH18 chitinases, *Pteris ryukyuensis* chitinase-A (PrChi-A) and *Equisetum arvense* chitinase-A (EaChi-A) at 1.8 and 2.5 Å resolution, respectively. In these two structures, two disulfide bonds were well conserved, suggesting that these bonds are important in the conformational stability of the modules (Fig 1). Interestingly, these bonds are not conserved in other CBM50s. The chitin binding sites of the CBM50s from PrChi-A and EaChi-A were estimated based on the NMR titration experiments. Based on the results obtained, we identified the binding sites located in a shallow groove formed by the loop between strand 1 and helix 1, the N-terminal part of helix 1, the C-terminal part of the helix 2, and the loop between helix 2 and strand 2. It should be noted that the dramatic line broadening of the NMR signal of the solvent exposed tyrosine residues (Y72 of PrChi-A and Y15 of EaChi-A) were observed upon ligand binding. To verify the involvement of these residues in chitin binding, binding affinity of the wild-type and Tyr mutants (Y72A, Y72F, Y72W) to chitohexaose was measured using isothermal titration calorimetry (ITC). Mutation of these residues to alanine resulted in  $K_a$  values which are about one order of magnitude lower than that of the wild-type, indicating the crucial role of aromatic residues in chitin binding.



**Fig 1.** Crystal structures of two CBM50s from plant chitinases. Crystal structures of PrChi-A LysM2 (left, PDB; 4PXV) and EaChi-A LysM (right) are illustrated in ribbon models. White arrow: surface exposed tyrosine residue, black arrow: intra-domain disulfide bonds.

## New glucuronoyl esterases for wood processing

*Sylvia Klaubauf<sup>1</sup>, Silvia Hüttner<sup>2</sup>, Hampus Sunner<sup>1</sup> and Lisbeth Olsson<sup>1,2</sup>*

klaubauf@chalmers.se

1. Wallenberg Wood Science Centre, Department of Biology and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden
2. Department of Biology and Biological Engineering, Division of Industrial Biotechnology, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

The development of new wood-based materials is of great interest to the forest industry. Wood tissue is composed of a complex biopolymer mixture containing cellulose, hemicellulose and lignin. Covalent bonds between lignin and different polysaccharides form closely associated structures known as lignin-carbohydrate complexes (LCCs). As a result, the successful extraction and separation of wood polymers poses a major challenge for materials biorefinery concepts. Enzymes that target lignin-carbohydrate (LC) bonds are especially useful for biorefinery applications as they can facilitate the isolation of individual wood components in combination with mild chemical treatments.

The main LCCs present in wood are believed to be esters, benzyl ethers and phenyl glycosides [1,2]. Glucuronoyl esterases (GEs) have been proposed to degrade ester bonds between glucuronic acids in xylans and lignin alcohols. GEs belong to the carbohydrate esterase (CE) 15 family and are present in the genomes of a wide range of fungi and bacteria.

The aims of our study were to characterize new GE enzymes, to investigate their capacity in disconnecting hemicellulose from lignin and to apply them in the extraction process. Selected candidate genes encoding novel GEs from a diverse range of filamentous fungi were produced in the eukaryotic enzyme production host *Pichia pastoris*. Purified enzymes were tested on model substrates as well as LCC fractions and their applicability in wood processing is investigated.

### Acknowledgements

Research on glucuronoyl esterases at Chalmers is funded by the Knut and Alice Wallenberg Foundation. Selection of candidate genes was performed in the context of OPTIBIOCAT (FP7 KBBE. 2013.3.3-04), which is gratefully acknowledged.

### Literature

1. Balakshin MY, et al. (2007) MWL fraction with a high concentration of lignin-carbohydrate linkages: Isolation and 2D NMR spectroscopic analysis. *Holzforschung* 61:1–7.
2. Watanabe T (1995) Important properties of lignin-carbohydrates complexes (LCCs) in environmentally safe paper making. *Trends in Glycoscience and Glycotechnology* 7:57–68.



## Comparison of transglycosylation abilities of two $\alpha$ -L-fucosidase isozymes from *Paenibacillus thiaminolyticus*

*Terézia Koval'ová<sup>1</sup>, Patricie Buchtová<sup>1</sup>, Eva Benešová<sup>1</sup>, Tomáš Koval<sup>2</sup>, Petra Lipovová<sup>1</sup>*

terezia.kovalova@gmail.com

1. Department of Biochemistry and Microbiology, University of Chemistry and Technology, Technická 5, 166 28 Prague 6, Czech Republic
2. Institute of Macromolecular Chemistry, Heyrovského nam.2, 162 06 Praha 6-Břevnov, Czech Republic

$\alpha$ -L-Fucosylated oligosaccharides take part in a number of biological processes such as fertilization, cell adhesion, cell proliferation and processes of inflammation. They are also responsible for existence of blood group antigens. Changes in fucosylation of oligosaccharides were observed in association with several diseases, like cancer, rheumatoid arthritis and cystic fibrosis. Fucosylated oligosaccharides are used in pharmaceutical, cosmetics and food industry however their preparation via organic synthesis is a difficult and complicated process, which could be simplified by using enzymatic synthesis instead. Our previous research indicate, that  $\alpha$ -L-fucosidase from *Paenibacillus thiaminolyticus* has great potential for artificial fucosylation [1], [2], [3]. There exist two isozymes of this protein. Both recombinant isozymes were produced, isolated and characterized. Preliminary test of substrate specificity was conducted using selected substrates. Ability to catalyze transglycosylation reactions of studied enzymes was tested and compared. Gained results indicate that isozyme 1 shows higher potential to catalyze transglycosylation reactions. Crystallographic experiments were also carried out on both isozymes.

### Acknowledgements

The project was supported by COST actions MultiGlycoNano (CM1102, LD13024). Participation at the conference is supported by specific university research (MSMT No 20/2015, No MSMT 21/2015).

### Literature

1. Becker, D. J., Lowe, J. B. (2003). Fucose: biosynthesis and biological function in mammals. *Glycobiology*, **13**, 41R-53R.
2. Ma, B., Simala-Grant, J. L., Taylor, D. E. (2006): Fucosylation in prokaryotes and eukaryotes. *Glycobiology*, **16**, 158R-184R.
3. Benešová E. (2010): *Enzymes with fucosidase activity produced by the bacterium Paenibacillus thiaminolyticus*. Doctoral thesis, ICT Prague, Czech Republic.

## Variations in the substrate specificity of cellobiose dehydrogenase

*Daniel Kracher, Marita Preims, Alfons Felice, Dietmar Haltrich and Roland Ludwig*

Daniel.Kracher@BOKU.ac.at

Laboratory of Food Biotechnology, University of Natural Resources and Life Sciences, Vienna

Cellobiose dehydrogenase (CDH) is an extracellular redox enzyme secreted by wood-rotting fungi during lignocellulose degradation. Recently it was shown that CDH can act as a reductase for lytic polysaccharide monooxygenases (LPMOs), which boosts the depolymerization of crystalline cellulose, hemicellulose or starch. CDH's unusual architecture comprises a catalytic flavodehydrogenase domain connected to a mobile cytochrome domain. This bipartite structure not only is unique for an extracellular protein but is also essential for the interaction with LPMOs and a key feature for its technical utilization in biosensors or the production of aldonic acids.

Data mining in available fungal genomes provided more than 300 putative *cdh* genes, which form three phylogenetic branches: Class I CDHs from basidiomycetes and class II or class III CDHs from ascomycetes. Recombinant production and characterization of more than 15 CDHs from different branches shows a correlation to the phylogenetic classification. Differences are not only of structural, but mostly of catalytic nature. Class I CDHs generally show a preference for the natural substrate cellobiose, while substrate spectra of class II CDHs extend to carbohydrates found in hemicellulose such as mannopentaose, xylo-oligosaccharides or maltose and malto-oligosaccharides. In addition, some class II CDHs show neutral or alkaline pH optima not observed for the classically known (acidic) class I CDHs. These abilities clearly distinguish class II CDHs from the well-studied class of basidiomycetous CDHs and are an important step towards the development of CDH-based biocatalytic processes and biosensors. The enigmatic class III CDHs, which have not been characterized so far, show a dramatically altered active site architecture. First results of their expression and characterization will be presented. Taken together, these varying substrate specificities may help to understand the interaction with LPMOs and are of interest for the production of various aldonic acids.

### Literature

- 1 C.M. Phillips, W.T. Beeson *et al.* ACS Chem Biol. 6 (2011) 1399–1406.
- 2 Harreither W, Sygmond C, *et al.* Appl Environ Microbiol. (2011) 1804-15
- 3 C. Sygmond, D. Kracher *et al.* Appl. Environ. Microbiol. 78 (2012) 6161–71.
- 4 R. Ludwig, W. Harreither *et al.* ChemPhysChem. 11 (2010) 2674–97.
- 5 J. Tanne, D. Kracher *et al.* Biosensors. 4 (2014) 370–86.

## The first transglycosidase derived from a GH20 $\beta$ -N-acetylhexosaminidase

Kristýna Slámová<sup>1</sup>, Jana Krejzová<sup>1</sup>, Natalia Kulik<sup>2</sup> and Vladimír Křen<sup>1</sup>

kren@biomed.cas.cz

1. Institute of Microbiology AS CR, Vídeňská 1083, CZ 14220 Prague, Czech Republic
2. Institute of Nanobiology and Structural Biology of GCRC AS CR, Zámek 136, CZ 37333 Nové Hradky, Czech Republic

The  $\beta$ -N-acetylhexosaminidase (EC 3.2.1.52, CAZy GH20) from the filamentous fungus *Talaromyces flavus* has been shown to possess unique substrate flexibility and remarkable synthetic ability. However, the yields of the transglycosylation reactions are significantly lowered by the undesired hydrolysis of the glycosidic bonds of the substrate and product. To overcome this problem, **mutant variants of *T. flavus*  $\beta$ -N-acetylhexosaminidase** were designed based on a computational model of the active site of this enzyme, aiming at diminishing its hydrolytic activity and retaining transglycosylation activity at once, e. g. at the preparation of a transglycosidase [1].

Three mutants of  $\beta$ -N-acetylhexosaminidase were produced in the *Pichia pastoris* expression system in high yields, purified from the culture media and characterized. All of the **mutant variants featured strongly (ca 200-fold) reduced hydrolytic activity** while maintaining high transglycosylation activity reaching extraordinary conversion rates. Reaction with *p*NP-GlcNAc as a substrate yielded mixtures of ***p*NP-chitooligosaccharides containing up to 8 N-acetylglucosamine units**. Also natural substrates - chitooligomers (DP 2 - 3) - were rearranged by these transglycosidases to **rare oligomers with higher degrees of polymerization (DP 4 – 8)**. GlcNAc-linker-*t*Boc was extended to *t*Boc-chitooligosaccharides enabling thus simple creation of multivalent molecules. This enzyme has a potential to attach  $\beta$ -GlcNAc units to a variety of other acceptors analogously as the wild enzyme but in a considerably higher yield. In summary, we have designed and prepared three variants of a novel transglycosidase derived from  $\beta$ -N-acetylhexosaminidase from *T. flavus*, able to catalyze the synthesis of valuable N-acetylchitooligomers without unfavourable hydrolysis.

This is **the first report on a transglycosidase** stemming from an *exo*-glycosidase employing the **substrate-assisted catalytic mechanism** via the oxazoline reaction intermediate.

**Acknowledgements:** The financial support by the project from Czech Academy of Sciences M200201204 and by the Czech Science Foundation Grant 15-02578J is gratefully acknowledged.

### Literature

1. K. Slámová, J. Krejzová, P. Marhol, L. Kalachova, N. Kulik, H. Pelantová, J. Cvačka, V. Křen: Synthesis of derivatized chitooligomers using transglycosidases engineered from the fungal GH20  $\beta$ -N-acetylhexosaminidase. *Adv. Synth. Catal.* in press (2015) DOI 10.1002/adsc.201500075

## Carbohydrate composition in spruce bark

Katariina Kemppainen, Matti Siika-aho and Kristiina Kruus

Kristiina.kruus@vtt.fi

VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, FI-02044 VTT, Finland

Norway spruce (*Picea abies*) bark is an abundant lignocellulosic feedstock having potential as a source of biofuels and biochemicals based on phenolics and carbohydrates. This poster summarizes the overall carbohydrate profile of spruce bark, discusses the solubility and extractability of different carbohydrate fractions, and compares the results to spruce wood carbohydrates based on published<sup>1</sup> and unpublished data.

Spruce bark dry matter was found to contain circa 20% cellulose, 21-24% non-cellulosic neutral carbohydrates and 7-9% acidic carbohydrates. Cellulose content of bark was significantly lower than cellulose content of spruce wood (35%). Non-cellulosic neutral carbohydrates were mainly composed of non-cellulosic glucose (8-12% of bark dry matter), which can be present in bark as a part of non-cellulosic polysaccharides, as free glucose or sucrose, or as glycosides, e.g. as a part of stilbene glycosides. According to glycome profiling analysis carried out with an extensive set of glycan-directed monoclonal antibodies, spruce bark contained xyloglucan, which is one source for non-cellulosic glucose. Starch and  $\beta$ -glucan contents were found to be below 1%. Hot water extracts prepared from bark collected from logs felled in the winter contained free glucose that composed up to 2% of bark dry matter, whereas corresponding samples from bark collected from logs felled in the summer contained very low amounts of free glucose. Apparently the season affected the amount and type of non-cellulosic glucose and glucan in bark. Stilbene glycosides such as isorhapontin and astringin are abundant in spruce bark<sup>2</sup> and there is some indication that glucose may be bound to condensed tannin units as well<sup>3</sup>. Content of mannose-containing polysaccharides in bark was found to be much lower (2-3%) compared to wood (12-13%), but pectin content in bark was significantly high, up to 7% of bark dry matter based on the content of polygalacturonic acid. Glycome profiling showed also the presence of arabinogalactans in bark.

**Acknowledgements** The research leading to these results has received funding from the WoodWisdom-Net Research Program which is a transnational R&D program jointly funded by national funding organizations within the framework of the ERA-NET WoodWisdom-Net 2.

### Literature

1. Kemppainen, K., Siika-aho, M., Pattathil, S., Giovando, S., Kruus, K. 2014. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. *Ind. Crops Prod.* 52, 158–168
2. Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., Willför, S., 2012. Extraction and chemical characterization of Norway spruce inner and outer bark. *Nordic Pulp Paper Res. J.* 27, 6–17.
3. Zhang, L., Gellerstedt, G., 2008. 2D heteronuclear (1H–13C) single quantum correlation (HSQC) NMR analysis of Norway spruce bark components. In: Hu, T.Q. (Ed.), *Characterization of Lignocellulosic Materials. Part I: Novel or Improved Methods for the Characterization of Wood, Pulp Fibers, and Paper.* Blackwell Publishing Ltd., pp. 3–16.

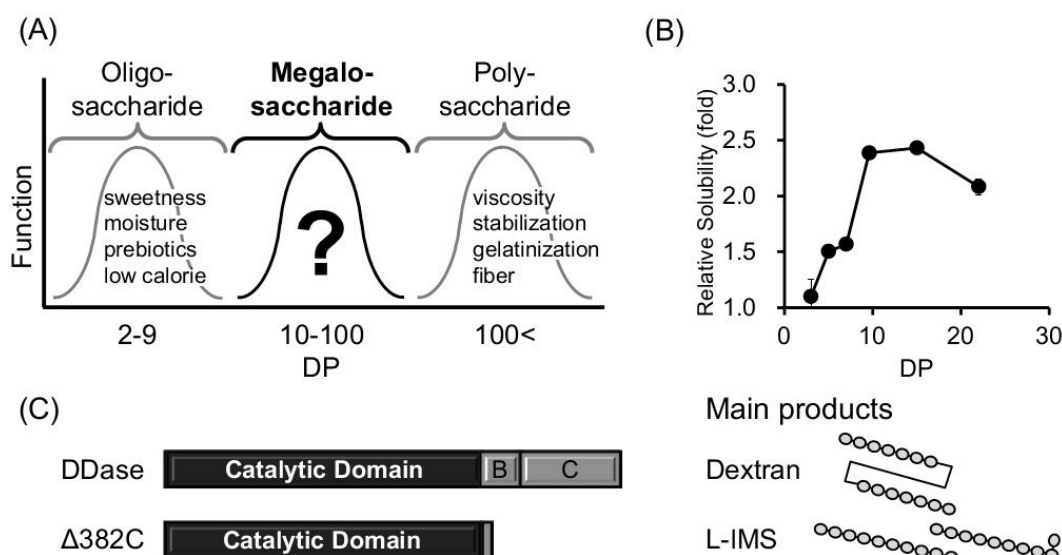
## Enzymatic synthesis of functional linear isomaltomegalosaccharide by *Gluconobacter oxydans* dextran dextrinase

*Yuya Kumagai, Weeranuch Lang, Juri Sadahiro, Masayuki Okuyama, Haruhide Mori, and Atsuo Kimura*

kimura@abs.agr.hokudai.ac.jp

Research Faculty of Agriculture, Hokkaido University

By definition, the megalosaccharide has DP between 10 and 100 (Fig. 1A). Linear isomaltomegalosaccharide (L-IMS), which is linear-type  $\alpha$ -(1 $\rightarrow$ 6)-glucosyl megalosaccharide, possesses beneficial functions of bioavailability of flavonoid glycoside (Fig. 1B) [1-3]. Dextran dextrinase from *Gluconobacter oxydans* (DDase; EC 2.4.1.2), a secreted protein of *G. oxydans* [4], produced dextran from maltooligosaccharides by its transglycosylation activity, whereas C-terminal deletion mutant ( $\Delta$ 382C) could efficiently produce L-IMS (Fig. 1C). The yield of L-IMS against substrate by DDase and  $\Delta$ 382C was 21% and 41% (w/w), respectively. C-terminal truncation study revealed that the deletion of B region increased the yield of L-IMS. Mutational experiment found that the substitution of Asn916 in B region with Ala increased L-IMS productivity. Our study revealed that B region of DDase controls saccharides length of product.



**Fig 1.** Definition of megalosaccharide, and function and production of L-IMS. (A), Size-dependent functions of saccharides; (B), Effect of DP on quercetine-3-*O*- $\beta$ -D-glucoside (Q3G) solubility. Relative solubility of 1.0 is the Q3G-solubility in water; (C), Schematic presentation of the enzymes and their main end products.

### Acknowledgements

This study was partially supported by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry in Japan.

### Literature

1. Shinoki A, *et al.*, *Food Chemistry* **136**, 293-296 (2013)
2. Lang W, *et al.*, *Bioresource Technology* **150**, 298-306 (2013)
3. Lang W, *et al.*, *Bioresource Technology* **169**, 518-524 (2014)
4. Sadahiro J, *et al.*, *Biochemical and Biophysical Research Communication* **456**, 500-505 (2015)

## Genomics, systematics and proteomics of the wood-decomposing white rot Basidiomycota Polypore species *Phlebia radiata*

*Jaana Kuuskeri*<sup>1</sup>, *Olli-Pekka Smolander*<sup>2</sup>, *Heikki Salavirta*<sup>1</sup>, *Pia Laine*<sup>2</sup>, *Ilona Oksanen*<sup>1</sup>, *Miia R. Mäkelä*<sup>1</sup>, *Kristiina Hildén*<sup>1</sup>, *Petri Auvinen*<sup>2</sup>, *Markku Varjosalo*<sup>3</sup>, *Lars Paulin*<sup>2</sup> and *Taina Lundell*<sup>1</sup>

jaana.kuuskeri@helsinki.fi

1. Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, Fungal Biotechnology Laboratory,
2. Institute of Biotechnology, DNA Sequencing and Genomics Laboratory,
3. Institute of Biotechnology, Proteomics Unit, Viikki Campus, University of Helsinki, Helsinki, Finland

The systematically incoherent genus *Phlebia* consists of white rot species which produce a variety of lignocellulose-degrading CAZymes (carbohydrate-acting enzymes) and oxidoreductases. Due to the capability for efficient conversion of wood, plant biomass and harmful organic compounds, the type species of this genus, *P. radiata*, was selected for genome and transcriptome sequencing. The nuclear and mitochondrial genomes of Finnish wild-type heterokaryotic isolate *P. radiata* 79 (FBCC43) were assembled by using PacBio RSII and Roche 454 FLX Titanium sequencing platforms. *P. radiata* mitochondrial genome is the second largest (over 156 kbp) sequenced and gene annotated for fungi [1]. Nuclear genome sequencing produced 4.34 Gbp of data with mean read length of 8095 bp. HGAP3 pipeline assembly produced 127 contigs with 37 longest containing more than 90% of the assembly (contig N50 1.984 Mbp), summing up to 40.92 Mbp haploid genome size.

In addition to genome sequencing, our aim was to provide an assessment of protein coding genes and proteins that function in core fungal metabolism, degradation of plant cell wall lignocelluloses (PCWL) and oxidation of lignin-like and polluting compounds. The fungus was cultivated on spruce wood, and extraction of RNA and proteome were performed weekly for parallel cultures. Extracellular CAZy enzyme activities were followed in liquid and wood cultivations. Secreted wood-decay enzymes were constantly produced when the fungus was growing on spruce wood. Lignin peroxidases (LiP) were dominant oxidoreductases in the proteome. Besides LiPs, other lignin-modifying class II peroxidases (manganese peroxidases), many cellulolytic CAZymes, and auxiliary hydrogen peroxide producing enzymes were the most identified proteins. The results show that *P. radiata* has a functional and versatile wood and plant biomass degrading enzyme machinery.

**Acknowledgements** The study was supported by the Academy of Finland Ox-Red research project, grant 113833, and University of Helsinki Doctoral Programme in Microbiology and Biotechnology.

### Literature

- 1 Salavirta H, Oksanen I, Kuuskeri J, Mäkelä M, Laine P, Paulin L, Lundell T: Mitochondrial genome of *Phlebia radiata* is the second largest (156 kbp) among fungi and features signs of genome flexibility and recent recombination events. PLoS One 2014, 9:e97141.

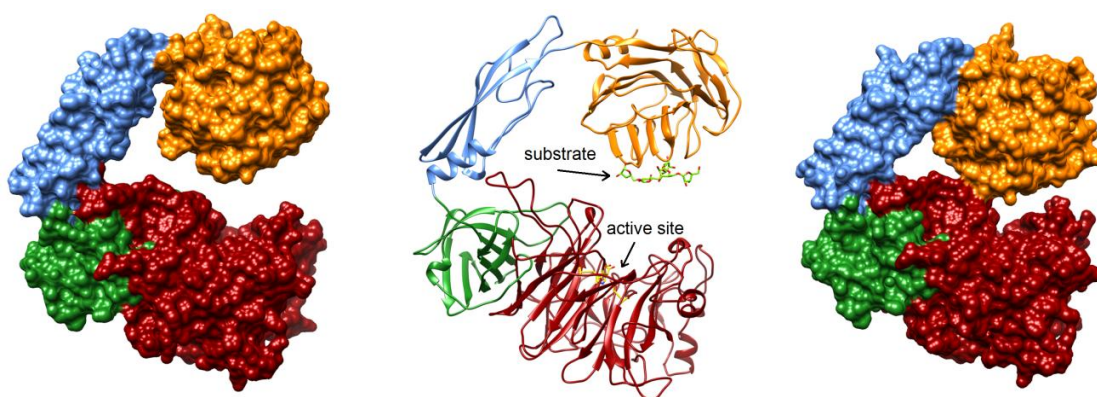
## A unique multi-domain extracellular GH43 arabinanase determined in different conformational states

*Shifra Lansky<sup>1</sup>, Rachel Salama<sup>2</sup>, Omer Shwartzhtien<sup>1</sup>, Yuval Shoham<sup>2</sup> and Gil Shoham<sup>1</sup>*

<sup>1</sup> Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

<sup>2</sup> Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel

$\alpha$ -L-arabinanases are key enzymes in the breakdown of arabinan, one of the main polysaccharides in the plant cell-wall, and hence present a wide range of important potential biotechnological applications. *GsAbn43A* is an extracellular  $\alpha$ -L-arabinanase from the thermophilic Gram-positive bacterium *Geobacillus stearothermophilus*-T6, shown to degrade efficiently linear and naturally-branched arabinan. The enzyme belongs to family GH43, contains 848 amino-acid residues, and possesses relatively low sequence identity to related arabinanases. The 3D structure of *GsAbn43A* has recently been determined by X-ray crystallography, revealing a unique and novel multi-domain architecture, the largest reported so far in the GH43 family. The enzyme is built of four different domains, arranged in a pincer-like structure. The catalytic domain (shown in red in the figures below) corresponds to the five-bladed  $\beta$ -propeller fold observed in GH43 enzymes. The second domain (green) has also been seen before in some homologous two-domain arabinanases, but the third (blue) and fourth (orange) domains are unique to *GsAbn43A*. Interestingly, two different conformational states have been determined for the enzyme, a "closed" state (right figure) and an "open" state (left), differentiated by a  $\sim 12$  Å movement in location of the fourth domain. Substrate-binding structural experiments demonstrate, surprisingly, that an arabinopentaose substrate binds to a dedicated site in the fourth domain, rather than to the expected catalytic domain and active site (central figure). These findings, together with complementary ITC and kinetics experiments, suggest a novel catalytic mechanism for the arabinan degradation action of *GsAbn43A*.



## Structural analysis of Abp, a GH27 $\beta$ -L-arabinopyranosidase from *Geobacillus stearothermophilus*

*Shifra Lansky*<sup>1</sup>, *Rachel Salama*<sup>2</sup>, *Hodaya V. Solomon*<sup>1</sup>, *Yuval Shoham*<sup>2</sup> and *Gil Shoham*<sup>1</sup>

<sup>1</sup>Institute of Chemistry, the Hebrew University of Jerusalem, Jerusalem 91904, Israel

<sup>2</sup>Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel

L-arabinose sugar residues are relatively abundant in plants, found mainly in arabinan polysaccharides and other arabinose-containing polysaccharides, such as arabinoxylans and pectic arabinogalactans. The majority of the arabinose units in plants are present in the furanose form and only a small fraction are present in the arabinopyranose form. We have recently characterized the L-arabinan utilization system in *Geobacillus stearothermophilus* T-6, a Gram-positive thermophilic soil bacterium, where one of the key enzymes was found to be an intracellular  $\beta$ -L-arabinopyranosidase (Abp). The detailed three-dimensional structure of Abp-WT has been determined by X-ray crystallography to 2.28Å resolution, as well as the structure of one of its catalytic mutants (Abp-D197A) in complex with a  $\beta$ -L-arabinose molecule (2.19Å resolution). These structures demonstrate that the 3D structure of the Abp monomer generally correlates with the general fold observed for GH27 proteins, consisting of two main domains, an N-terminal TIM-barrel domain and a C-terminal all- $\beta$  domain. The two catalytic residues are located in the TIM-barrel domain, such that their carboxylic functional groups are about 5.9 Å from each other, consistent with a retaining mechanism. An isoleucine residue (Ile67), located at a key position in the active site, is shown to play a critical role in the substrate specificity of Abp, providing structural basis for the high preference of the enzyme toward arabinopyranoside over galactopyranoside substrates. The crystal structure demonstrates that Abp is a tetramer composed of two "open pincer" dimers, clamped around each other to form a central cavity (Figure). The four active sites of Abp are situated at the inner surface of this cavity, all opened into the central space of the cavity. The biological relevance of this tetrameric structure is supported by independent results obtained from gel-filtration, DLS and SAXS experiments. These data and their comparison to the structural data of related GH27 enzymes are used for a more general discussion concerning structure-selectivity aspects in this GH family.





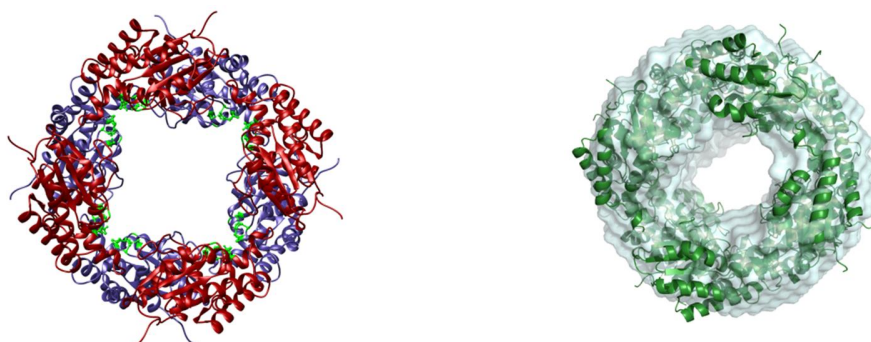
## A unique octameric structure of an acetyl-xylan esterase

*Shifra Lansky<sup>1</sup>, Onit Alalouf<sup>2</sup>, Hodaya V. Solomon<sup>1</sup>, Yuval Shoham<sup>2</sup> and Gil Shoham<sup>1</sup>*

<sup>1</sup>Institute of Chemistry, the Hebrew University of Jerusalem, Jerusalem 91904, Israel

<sup>2</sup>Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel

*Geobacillus stearothermophilus* T-6 is a thermophilic soil bacterium that possesses an extensive and highly regulated hemicellulolytic system, allowing the bacterium to efficiently degrade high molecular weight polysaccharides such as xylan, arabinan and galactan. As part of the xylan degradation system, the bacterium uses a number of side-chain cleaving enzymes, one of which is Axe2, a 219 amino-acids intracellular serine acetylxylan-esterase that removes acetyl side-groups from xylo-oligosaccharides. Axe2 belongs to the lipase GDSL family and represents a new family of carbohydrate esterases. The detailed three-dimensional structure of Axe2 has recently been determined by X-ray crystallography demonstrating it is arranged as a "doughnut-shaped" homo-octamer (left figure). Gel-filtration, TEM and SAXS (right figure) experiments all confirm the octameric torus structure of Axe2 is indeed the biologically relevant structure in solution. The Axe2 octamer is built of two staggered tetrameric rings, where all eight active sites face the relatively wide internal cavity, and is held together by three specific types of monomer–monomer interfaces, each of which involves a number of intermolecular interactions. A series of rational noncatalytic, site-specific mutations were made in order to further examine the functional significance of this oligomerization, some of which lead to a different dimeric form of Axe2 showing a significant reduction in catalytic activity. The structure of one of these mutants, Axe2-Y184F-W190P, has recently been solved by X-ray crystallography, and offers a possible explanation for the functional origin of this unique oligomeric structure.



## Characterization of a Chitin Utilization Locus from *Flavobacterium johnsoniae*

Johan Larsbrink<sup>1</sup>, Sampada S. Kharade<sup>2</sup>, Kurt J. Kwiatkowski<sup>3</sup>, Alasdair MacKenzie<sup>1</sup>, Yongtao Zhu<sup>2</sup>, Nicole Koropatkin<sup>3</sup>, Mark J. McBride<sup>2</sup>, Vincent G. H. Eijsink<sup>1</sup>, Phil B. Pope<sup>1</sup>

johan.larsbrink@nmbu.no

1. Dept. of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, 1432 Ås, Norway
2. Dept. of Biological Sciences, University of Wisconsin—Milwaukee, WI 53201, USA
3. Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109, USA

The bacteria belonging to the Bacteroidetes phylum are well known for their polysaccharide degradation capabilities, with the most well studied species reside in the gastrointestinal tract of animals. The enzymes responsible for the degradation of complex glycans are often parts of distinct gene clusters named Polysaccharide Utilization Loci (PULs). PULs contain outer membrane sugar binding proteins and transporters, as well as sugar sensors and regulatory elements and a varying number of degradative enzymes. Thus far, a multitude of PULs have been identified in Bacteroidetes species from varying habitats, mainly targeting non-crystalline polysaccharides (e.g. hemicelluloses, pectins and host glycans) as well as different types of starch.

A PUL responsible for the degradation of chitin has been identified in the aerobic soil bacterium *Flavobacterium johnsoniae* [1]. Chitin is a crystalline polysaccharide found in shells of arthropods and cell walls of fungi, and is the second most abundant carbohydrate on earth after cellulose. In the current study, the chitin utilization locus (ChiUL), consisting of two SusC/D pairs, a sensor-regulator pair, an inner membrane transporter, and four enzymes, is characterized in detail. The main chitinase of the ChiUL, ChiA, is secreted into the environment via the newly discovered type IX secretion system, therefore not conforming to the typical PUL arrangement, where polysaccharide-active enzymes are attached to the outer membrane. The ChiUL contains three chitinase (GH18) domains, two in ChiA and one in ChiB. These three GH18 enzymes are not highly homologous and display different activities on their substrates, which include both crystalline  $\alpha$ - and  $\beta$ -chitin and chitooligosaccharides. ChiB is predicted to be located in the periplasm together with a GH20 enzyme, facilitating the conversion of imported oligosaccharides into GlcNAc.

In addition to the enzymology, gene knock-out studies have shown how both the SusC/D pairs, the sensor-regulator pair, and the inner membrane transporter are indispensable for growth on chitin. However, ChiA is the only singular enzyme gene essential for growth. Further, isothermal calorimetry has been performed to evaluate the binding affinities of the SusD-like outer membrane proteins for chitooligosaccharides, revealing distinct preferences for sugar length.

The study of the ChiUL from *F. johnsoniae* enables us to understand how highly recalcitrant crystalline polysaccharides can be utilized by the sophisticated carbohydrate degradation systems of the Bacteroidetes.

### Literature

1. Kharade et. al. (2014). J Bacteriol 196(5):961-70

## Recombinant production of an exopolysaccharide of interest for health industry

L. Lebellenger<sup>1</sup>, J. Ratiskol<sup>1</sup>, C. Sinquin<sup>1</sup>, A. Zykwinska<sup>1</sup>, S. Collic-Jouault<sup>1</sup>, M. Dols-Lafargue<sup>2</sup>, C. Delbarre-Ladrat<sup>1</sup>.

Lou.lebellenger@ifremer.fr

1. Microbial Ecosystems and Marine Molecules for Biotechnology, Ifremer, Nantes Cedex 3, France.
2. Université de Bordeaux, ISVV 2110 chemin de Leusotte, 33882 Villenave d'Ornon.

Polysaccharides have many applications in the health and cosmetical industries. These molecules are extracted from plants, algae and animals. Microorganisms are also able to produce innovative exopolysaccharides (EPS). This way of production exhibits a great interest for the industrial demand. On one hand, the microbial diversity allows opportunities to discover high-value new molecules; on the other hand, it provides a production independent of climatic or seasonal changes, easy to optimize and control as well as an easier and safer extraction process. The marine bacteria *Vibrio diabolicus* [1] produces an EPS composed of uronic acid, *N*-acetyl-glucosamine and *N*-acetyl-galactosamine, the EPS HE800 [2]. Its chemical structure is similar to the hyaluronic acid one, an EPS widely used in human health. The EPS HE800 has already shown efficiency on bone regeneration [3]. To improve the EPS HE800 applications and production, an accurate knowledge of its biosynthesis is required. The cluster responsible for the production of the EPS HE800 has been identified. Cloning and expression in *Escherichia coli*, a common heterologous host, is studied in order to improve the control of the production and later plan the production of targeted functionalised molecules. Data on the EPS HE800 recombinant production will be presented. These would include EPS production tests, molecular mechanisms of the polysaccharide biosynthesis, transcriptional analysis of the main genes responsible for the production of this EPS. This study provides a better understanding of the biosynthesis of a new molecule of interest in health industry, in order to improve and control its production.

### Literature

1. Raguenes G, Christen R, Guezennec J, Pignet P and Barbier G, *Vibrio diabolicus* sp. nov., a new polysaccharide-secreting organism isolated from a deep-sea hydrothermal vent polychaete annelid, *Alvinella pompejana*. International Journal of Systematic Bacteriology, 1997. **47**(4): 989-995.
2. Rougeaux H, Kervarec N, Pichon R and Guezennec J, *Structure of the exopolysaccharide of Vibrio diabolicus* isolated from a deep-sea hydrothermal vent. Carbohydrate Research, 1999. **322**(1-2): 40-45.
3. Zanchetta P, Lagarde N and Guezennec J, *A new bone-healing material: A hyaluronic acid-like bacterial exopolysaccharide*. Calcified Tissue International, 2003. **72**(1): 74-79.

## Exploring complex glycan utilization machinery of *Roseburia* spp. implicated in inflammatory and metabolic disorders

*Maria Louise Leth<sup>1</sup>, Morten Ejby Hansen<sup>1</sup> and Maher Abou Hachem<sup>1</sup>*

malole@bio.dtu.dk

1. Dept. of Systems Biology, Technical University of Denmark

The human gastrointestinal tract is colonized by a dense, dynamic and complex microbiota. Humans are unable to degrade most dietary glycans, which instead are metabolised by different gut bacteria in the lower part of the gastrointestinal tract. The metabolism of dietary complex polysaccharides by distinct gut microbiota taxa has a pronounced effect on human health and physiology, which is at least partly associated with the specific short chain fatty acid (SCFA) fermentation products of glycans that vary between different taxa.

To date, a relatively narrow taxonomic range of bacteria involving mainly probiotic strains from *Lactobacillus* and *Bifidobacterium* genera have been explored for their health promoting properties. The therapeutic potential of other gut commensals remains ill-explored. Recent evidence correlates decreased abundance of gut commensal bacteria from the *Roseburia* genus to inflammatory and metabolic disorders such as Crohn's disease and type 2 diabetes (1-2). Furthermore, *Roseburia* spp. are reported to be primary degraders of complex polysaccharides including starch and inulin (3), which are fermented to butyrate. Butyrate production plays a role in the maintenance of colonic homostasis, and increased concentrations of this glycan fermentation product in the colon are associated with anti-inflammatory and anti-tumorigenic effects.

A more detailed investigation of the glycan metabolic repertoire of *Roseburia* spp. is necessary to reveal features underpinning its specialization and ultimately to explore its potential in therapeutic interventions targeting inflammatory and metabolic disorders. This project addresses the gap in our knowledge on the above aspects by characterization of selected glycan active proteins from the taxon. The latest results will be presented and discussed.

### Acknowledgements

This study is supported by a Graduate School DTU Scholarship, Lyngby, Denmark

### Literature

1. Willing et al. 2010, A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes, *Gastroenterology*, 139 (1844–1854)
2. Qin et al. 2012, A metagenome-wide association study of gut microbiota in type 2 diabetes, *Nature*, 490 (55–60).
3. Scott et al. 2011, Substrate-driven gene expression in *Roseburia* inulinivorans: Importance of inducible enzymes in the utilization of inulin and starch, *PNAS*. 108 (4672–4679)

## Structural and functional insights on the glycoside hydrolases involved in the metabolism of xylooligo- and arabinooligosaccharides in lactic acid bacteria

*Javier A. Linares-Pastén<sup>1</sup>, Peter Falck<sup>1</sup>, Reza Faryar<sup>1</sup>, Patrick Adlercreutz<sup>1</sup>  
Derek T. Logan<sup>2</sup>, Eva Nordberg Karlsson<sup>1</sup>*

eva.nordberg\_karlsson@biotek.lu.se

1. Biotechnology and <sup>2</sup>Biochemistry and Structural Biology, Department of Chemistry Lund University, P.O. Box 124, Lund, Sweden

Arabino- and xylo-oligosaccharides (AOS and XOS) have gained increased interest as prebiotics during the last years. AOS and XOS can be produced from major fractions of biomass including agricultural by-products and other low cost raw materials. *Weissella* and *Lactobacillus* can be considered representative genus of lactic acid bacteria. Strains of *Weissella cibaria/confusa* have recently been proposed as potential probiotics based on growth and adsorption to human cells {Lee, 2012 #24}, while *Lactobacillus* strains have been broadly used as models for studies of probiotics. The metabolism of the AOS/XOS is still poorly understood in lactic acid bacteria. Hydrolysis of AOS/XOS is one of the first steps in the metabolism. The main hydrolases involved in converting AOS and XOS to monosugars are arabinosidases and xylosidases. In lactic acid bacteria, these enzymes are found in families GH8, GH43 and GH51 ([www.cazy.org](http://www.cazy.org)), where GH43 seems most broadly present in lactic acid bacteria.

We have done a comparative study of the structure/function of arabinofuranosidases and xylosidases from GH43 in novel strains from *Weissella* and in *L. brevis* DSM 1269. Previously, both activities have shown preference for short natural substrates (di- and tri-saccharides) in enzymes cloned from *L. brevis* DSMZ 20054 {Michlmayr, 2013 #74}, and similar preferences were shown for enzymes cloned from *L. brevis* DSMZ 1269 and from *Weissella* strains. Kinetics studies also suggest that *Weissella* enzymes are more active than the corresponding candidates from *L. brevis*. All studied enzymes are intracellular, lacking signal peptides. Structurally, arabinofuranosidases and xylosidases share a five-bladed  $\beta$ -propeller fold in the catalytic domain, while the studied xylosidases have an additional domain with  $\beta$ -sandwich fold in the C-terminal end. The role of this additional domain is still unknown, however, its mutational deletion have shown it to be fundamental for the xylosidase activity. Crystallographic studies and size exclusion chromatography analysis are consistent with dimerization in native arabinofuranosidases for *Weissella* and *L. brevis* DSM 1269. However, although the xylosidases are present mostly as tetrametric structures, a single mutation in a superficial loop of the catalytic domain could affect the stability of the oligomers. These structural and functional implications will be discussed.

### Literature

1. Lee, K., Park, J., Jeong, H., Heo, H., Han, N. & Kim, J. 2012. Probiotic properties of *Weissella* strains isolated from human faeces. *Anaerobe*, 18, 96-102.
2. Michlmayr, H., Hell, J., Lorenz, C., Böhmendorfer, S., Rosenau, T. & Kneifel, W. 2013. Arabinoxylan oligosaccharide hydrolysis by family 43 and 51 glycosidases from *Lactobacillus brevis* DSM 20054. *Applied and environmental microbiology*, 79, 6747-6754.

## **$\beta$ -D-galactosidase/fucosidase from *Paenibacillus thiaminolyticus* and its transglycosylation properties and immobilization**

*Petra Lipovová<sup>1</sup>, Miroslav Smola<sup>1</sup>, Veronika Kováčová<sup>1</sup>, Eva Benešová<sup>1</sup>, Šárka Musilová<sup>2</sup> and Vojtěch Spiwok<sup>1</sup>*

petra.lipovova@vscht.cz

1. University of Chemistry and Technology, Prague, Technická 3, 166 28 Prague, Czech Republic

2. Czech University of Life Science Prague, Kamýcká 129, 165 21 Prague 6 – Suchbát, Czech Republic

$\beta$ -D-Galactosidases are enzymes which have been for many years applied in biotechnological processes for different purposes. Their ability to catalyze transgalactosylation reactions enables the production of galactooligosaccharides, beneficially affecting intestinal microbiota or galactosylated molecules with the potential for pharmaceutical industry. Some of these widespread enzymes are known to often display not only  $\beta$ -D-galactosidase activity, but also the  $\beta$ -D-fucosidase,  $\beta$ -D-glucosidase, or  $\alpha$ -L-arabinosidase activity.  $\beta$ -D-Galactosidase with  $\beta$ -D-fucosidase activity from the bacterial strain *Paenibacillus thiaminolyticus* was produced in the form of histidine-tagged protein, and purified using affinity chromatography. Kinetic parameters of  $\beta$ -D-galactosidase were determined with *p*-nitrophenyl  $\beta$ -D-galactopyranoside, *p*-nitrophenyl  $\beta$ -D-fucopyranoside and also with *p*-nitrophenyl  $\beta$ -D-glucopyranoside and lactose. The ability to catalyze transfucosylation reactions with varied acceptors was tested. The enzyme catalysed also the transfer of fucosyl moiety to many different acceptor molecules, and some products were confirmed by mass spectrometry and NMR. Also the prebiotic effect of fucosylated saccharides was tested. Further we were concerned to immobilization of this enzyme by entrapping on the surface of the synthetic lipid droplets, and as a lipid coated enzyme. Immobilized  $\beta$ -D-galactosidase was used to catalyze transgalactosylation reactions in the two-phase organic-aqueous systems. This system is suitable for glycosylation of substances poorly soluble in water.

### **Acknowledgements**

This work was supported by ESF COST Chemistry Action CM1102 "MultiGlycoNano" (MŠMT LD13024).

### **Literature**

1. Panesar PS, Panesar R, Singh RS, Kennedy JF, Kumar H. 2006. Microbial production, immobilization and applications of  $\beta$ -galactosidase. *J Chem. Technol. Biotechnol.* 81:530–543
2. Mori T, Fujita S, Okahata Y. 1997. Transglycosylation in a two-phase aqueous-organic system with catalysis by a lipid-coated  $\beta$ -D-galactosidase. *Carbohydr. Res.* 298: 65-73
3. Benesova E, Lipovova P, Dvorakova H, Kralova B. 2010. Beta-D-Galactosidase from *Paenibacillus thiaminolyticus* catalyzing transfucosylation reactions. *Glycobiol.* 20: 442-451.

## Cultivation strategies for Chitinase Expression in *Chitinibacter tainanensis*

Chao-Hsien Yeh<sup>1</sup>, Jin-Ting Chen<sup>1</sup>, Jeen-Kuan Chen<sup>2</sup> and Chao-Lin Liu<sup>1</sup>

f2402002@ms16.hinet.net

1. Department of Chemical Engineering, Ming Chi University of Technology

2. Environment and Biotechnology Department, Refining and Manufacturing Research Institute, CPC Corporation.

Chitin, a kind of polysaccharide, exists abundantly on the earth. Chitin are the major components of most fungal cell walls, insect exoskeletons and the shells of crustaceans. They are also the main components of the cell wall of prokaryotes. Chitin and its derivatives, such as oligochitin, chitosan and N-acetyl glucosamine (NAG), are utilized versatile and ubiquitously. They are applied on industrial, environmental, agricultural and biomedical fields.

Recently, *Chitinibacter tainanensis* with NAG as final unique product of chitin degrading was isolated from the soil in Southern Taiwan. Chitin is degraded with the fermentate of *C. tainanensis*. The yield was 0.75 g/g - 0.98 g/g depending on the substrates. In the work, various conditions of medium were applied during inoculation and fermentation. The morphologies of bacteria and enzyme activity were examined for optimization..



**Fig 1.** The morphology of *Chitinibacter tainanensis*.

### Literature

1. Chen, J. K., Shen, C. R., and Liu, C. L. (2014) *Applied biochemistry and biotechnology* 172, 3827-3834
2. Chen, J. K., Shen, C. R., Yeh, C. H., Fang, B. S., Huang, T. L., and Liu, C. L. (2011) *International Journal of Molecular Sciences* 12, 1187-1195

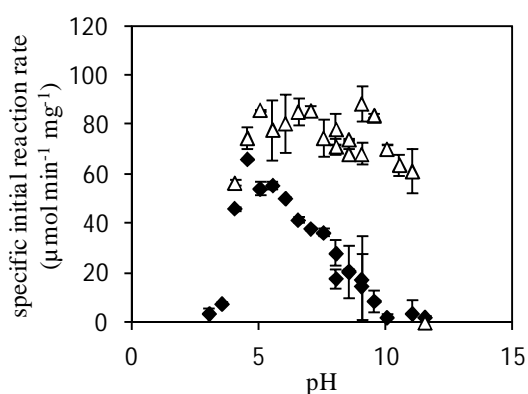
## From glycoside hydrolase to transglycosidase through protein and reaction engineering

Pontus Lundemo<sup>1</sup>, Eva Nordberg Karlsson<sup>1</sup> and Patrick Adlercreutz<sup>1</sup>

pontus.lundemo@biotek.lu.se

1. Lund University, Dept of Chemistry, Biotechnology, P.O. Box 124, SE-221 00 Lund, Sweden

If devoid of their dominant hydrolytic reactivity, glycoside hydrolases would be excellent tools for glycosylation. They are characterized by robustness, absolute stereoselectivity and accept a wide variety of acceptors. Starting from a particularly stable  $\beta$ -glucosidase originating from the thermophile *Thermotoga neapolitana* and belonging to the well-studied glycoside hydrolase family 1, we have attempted to understand the factors governing the predisposition for hydrolysis or transglycosylation. With proper immobilization, low hydration decreased the unwanted hydrolytic reactivity of the glycosidase [1]. However, already at moderate hydration, the activity of the enzyme was reduced significantly. A more successful approach was protein engineering, where a single amino acid replacement improved the yield of our models target compound, hexyl- $\beta$ -D-glucoside, from 17% to 58% [2]. Together with the observation that hydrolysis is more pH dependent than transglycosylation, we can limit the hydrolysis of the enzyme to an almost undetectable level, by running the reactions at high pH (Fig 1).



**Fig 1.** pH dependence of hydrolysis (♦) and transglycosylation (Δ) of *TnBg11A* mutant N220F. Error bars are 1σ.

### Acknowledgements

This work was supported by the Swedish research Council Formas

### Literature

1. Lundemo P, Karlsson EN, Adlercreutz P. 2014. Preparation of two glycoside hydrolases for use in micro-aqueous media. *J Mol Catal B-Enzym*, 108:1-6.
2. Lundemo P, Adlercreutz P, Karlsson EN. 2013. Improved Transferase/Hydrolase Ratio through Rational Design of a Family 1  $\beta$ -Glucosidase from *Thermotoga neapolitana*. *Appl Environ Microb*, 79:3400-3405.



## The impact of polysaccharide chemistry on the regioselectivity of AnAXE from *Aspergillus nidulans*

*Galina Mai-Gisondi<sup>1</sup>, Maija Tenkanen<sup>2</sup> and Emma Master<sup>1,3</sup>*

galina.mai@aalto.fi

1. Department of Chemical Technology and Biotechnology, Aalto University; 00076 Aalto, Kemistintie 1, Espoo, Finland.
2. Department of Food and Environmental Sciences, University of Helsinki, Finland.
3. Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada.

Acetyl xylan esterases (AXEs) targeting polymeric or oligomeric substrates have been classified into 8 CE families, namely CE1-7, and CE12. AXEs from different CE families can differ in terms of their regioselectivity towards acetylated xylan<sup>1-4</sup>. Briefly, characterizations to date indicate that esterases from families CE1, CE5 and CE6 can deacetylate mono- or di-acetylated methyl  $\beta$ -xylopyranoside<sup>2</sup>, whereas family CE4 esterase target 2- and 3-monoacetylated xylopyranosyl residues<sup>5</sup>. Some AXEs are also active on cellulose acetate, a synthetic cellulose derivative, and show a distinct regioselective mode of action dependent on CE family<sup>6,7</sup>. For example, CE1 esterases cleave substituents from the C2- and C3-positions of cellulose acetate. By comparison, the action of CE4 and CE5 esterases on cellulose acetate is restricted towards C3 and C2 substituents, respectively<sup>6</sup>. In addition to acetylated xylan and cellulose acetate, AXEs were previously shown to deacetylate native preparations of galactoglucomannan<sup>8</sup>. However, the regioselectivity of AXEs towards this substrate is unclear. In the current study, we investigate the impact of polysaccharide backbone and branching chemistry on the regioselectivity of a CE1 esterase from *Aspergillus nidulans*, namely AnAXE. In particular, acetylated forms of glucuronoxylan, galactoglucomannan, and cellulose were used in the analysis. Accessory enzymes, including a family GH115  $\alpha$ -glucuronidase and family GH27  $\alpha$ -galactosidase, were also used to distinguish impacts of backbone and branching substituents. Moreover, potential synergism between these debranching enzymes was evaluated.

### Acknowledgements

This work was supported by the Finnish Funding Agency for Technology and Innovation (Tekes) through a FiDiPro fellowship (EM).

### Literature

1. Pawar, P. M.-A.: *Front. Plant Sci.* **4**, 118 (2013).
2. Biely, P.: *Biochim. Biophys. Acta - Gen. Subj.* **1622**, 82–88 (2003).
3. Biely, P.: *Biochim. Biophys. Acta - Gen. Subj.* **1830**, 5075–5086 (2013).
4. Biely, P.: *J. Appl. Glycosci.* **61**, 35–44 (2014).
5. Uhliaríková, I.: *Biochim. Biophys. Acta - Gen. Subj.* **1830**, 3365–3372 (2013).
6. Altaner, C.: *J. Biotechnol.* **105**, 95–104 (2003).
7. Altaner, C.: *Chem. Technol.* **10**, 85–95 (2003).
8. Tenkanen, M.: *Appl. Enzym. to Linogcellulosics* (eds. Mansfield, S. D. & Saddler, J. N.) 211–29 (American Chemical Society, 2003).

## Plant biomass degrading potential of a new *Penicillium* species, *Penicillium subrubescens*

*Sadegh Mansouri*<sup>1</sup>, *Miia R. Mäkelä*<sup>1</sup>, *Ad Wiebenga*<sup>2</sup>, *Ronald P. de Vries*<sup>2</sup>, *Kristiina Hildén*<sup>1</sup>

Sadegh.mansouri@helsinki.fi

1. Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, Viikki Biocenter 1, University of Helsinki, Finland
2. Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

A recently identified *Penicillium* species, *P. subrubescens* (Mansouri et al. 2013), was evaluated for its ability to hydrolyse plant biomass. Growth on various plant biomass related substrates demonstrated the capacity of this species to degrade all the main polysaccharides present in plant biomass as well as metabolise all their monomeric components. The ability to degrade broad range of carbohydrates suggests a high potential in plant biomass saccharification.

To evaluate this in more detail, *P. subrubescens* was grown on wheat bran and sugar beet pulp and a set of extracellular enzyme activities were analyzed from culture liquids. Also the ability to saccharify wheat bran and sugar beet pulp was determined. Compared to *P. chrysogenum*, *P. subrubescens* produced higher levels of  $\beta$ -glucosidase, endoglucanase, endoxylanase and cellobiohydrolase.

Enzyme mixtures produced on wheat bran by *P. subrubescens* were more efficient in saccharification of wheat bran compared to enzymes produced on sugar beet pulp cultures. The opposite result was observed for saccharification of sugar beet pulp. This demonstrates that *P. subrubescens* produces enzyme mixtures that are closely tailored to the available substrate, suggesting the presence of a fine-tuned regulatory system that controls the production of these enzymes.

### Literature

Mansouri S, Houbraken J, Samson RA, Frisvad JC, Christensen M, Tuthill DE, Koutaniemi S, Hatakka A, Lankinen P (2013) *Penicillium subrubescens*, a new species efficiently producing inulinase. *Antonie Van Leeuwenhoek* 103:1343-1357

## Genome and in-lab analysis of cold-tolerant xylanolytic *Paenibacillus* spp isolated from low level radioactive waste repository

Kaisa Marjamaa<sup>1</sup>, Minna Vikman<sup>1</sup>, Erna Storgårds<sup>1</sup>, Heikki Salavirta<sup>1</sup> and Merja Itävaara<sup>1</sup>

kaisa.marjamaa@vtt.fi

1. VTT Technical Research Centre of Finland Ltd

*Paenibacillus* spp are Gram-positive facultatively anaerobic bacteria related to *Bacilli*. They have been isolated from various environments including rhizosphere, hot springs, Antarctic and intestine of insects. For biotechnology, they have been studied for e.g. use as biofertilizers, production of antibiotics and plant biomass degrading enzymes. Here, several bacterial strains belonging to the genus *Paenibacillus* were isolated from low level radioactive repository experiment (Gas generation experiment (GGE), 1) operated by Teollisuuden Voima Oyj in Olkiluoto, Finland. The strains exhibit xylanolytic growth at 5-40°C and pH 5-9 and produce soluble and cell bound enzymes that hydrolyse polymeric xylan and *p*-nitrophenyl β-D-xylopyranoside. Taxonomically these paenibacilli are related to *P. borealis*, *P. odorifer*, *P. glucanolyticus* and *P. thaichungensis*. Genomic DNA of four strains related to *P. borealis*, *P. odorifer* and *P. thaichungensis* was sequenced with Illumina HiSeq followed by de novo assembly and in the case of strains related to both *P. borealis* and *P. odorifer*, mapping to available reference genomes. Genomic sequences are being explored for enzymes related to conversion of xylan and other carbohydrates.

### Acknowledgements

Liisa Heikinheimo, Jenni Sauramo and Tuire Haavisto from Teollisuuden Voima Oyj (Finland) are thanked for provision of samples from GGE. Birgit Hillebrandt-Chellaoui and Helena Hakuli are thanked for technical assistance.

### Literature

1. Small J, Nykyri M, Helin M, Hovi U, Sarlin T, Itävaara M (2008) Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. *Applied Geochemistry* 23, 1383–1418

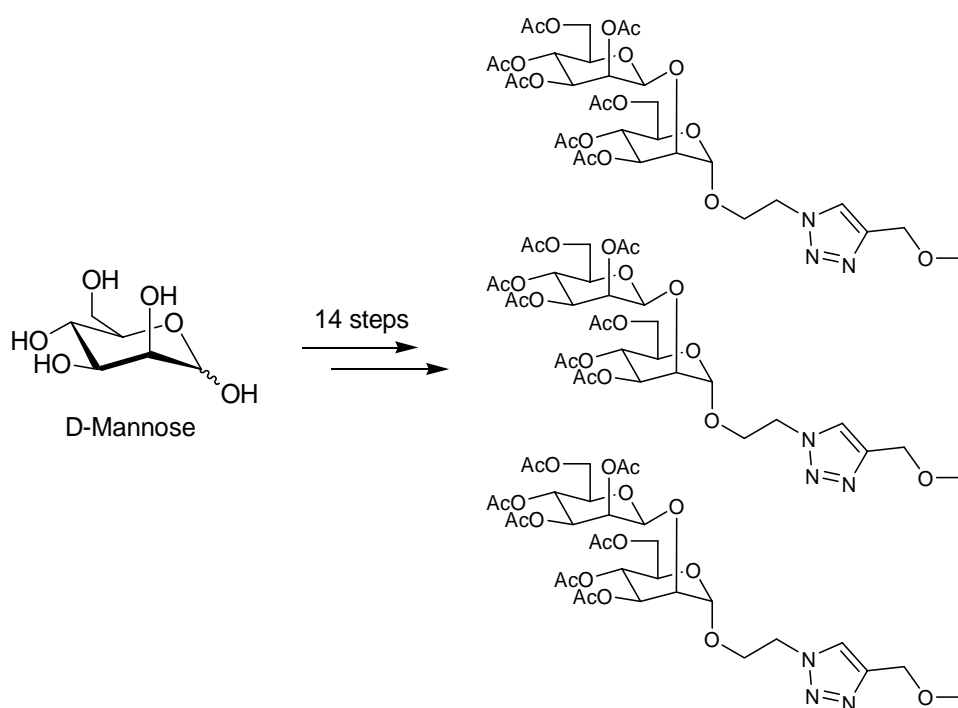
## Up-scaling of the synthetic procedure for preparation of oligosaccharide adjuvant for allergen immunotherapy

*Denys Mavrynsky,<sup>1</sup> Reko Leino<sup>1</sup>*

reko.leino@abo.fi

1. Laboratory of Organic Chemistry, Åbo Akademi University, FI-20500 Åbo, Finland

Our group recently reported the synthesis and characterization of a new oligomannoside derived glycocluster (Fig. 1).<sup>1</sup> This compound has demonstrated considerable suppressive effects on the Th2-type allergic inflammatory response both in vitro and in vivo. In order to evaluate the possibility of bringing this product to clinical trials, a reliable procedure for up-scaling of its synthesis needs to be developed. In the present work, optimization of gram scale synthetic procedures was performed, including substitution of chromatographic purifications with crystallizations and extractions.



**Fig 1.** The target molecule

### Literature

1. Mukherjee, C.; Mäkinen, K. ; Savolainen, J. ; Leino R. *Chem. Eur. J.* **2013**, *19*, 7961-7974

## Beechwood xylan for the measurement of *endo*-1,4- $\beta$ -D-xylanase

*Páraic McGeough<sup>1</sup>, Ida Lazewska and Barry McCleary<sup>1</sup>*

barry@megazyme.com

1. Megazyme International Ireland, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.

*endo*-1,4- $\beta$ -Xylanase ( $\beta$ -xylanase) is most commonly assayed using birchwood xylan with measurement of reducing sugar using the DNSA assay procedure. Recently, the supply of birchwood xylan ceased. Unfortunately, the only other commercially available xylan, beechwood xylan, is not sufficiently pure for  $\beta$ -xylanase assays due to unacceptable high reducing sugar level leading to high background colour in the assay. In the measurement of *endo*-1,4- $\beta$ -glucanase (cellulase) activity with the DNSA reducing sugar procedure, cellobiose is used as the standard instead of glucose. The reason for this is that under the highly alkaline conditions of this assay, some cello-oligosaccharides are chemically hydrolysed during incubation with DNSA, giving inflated hydrolysis values. Using cellobiose as the standard in place of glucose compensates for this. In  $\beta$ -xylanase assays, xylose has been routinely used as the standard because suitably pure xylobiose has not been readily available.

In the current study, we have critically compared birchwood xylan, beechwood xylan and wheat flour arabinoxylan for the measurement of  $\beta$ -xylanase using both the DNSA and the Nelson/Somogyi reducing sugar procedures, and have compared these assays to those using dyed arabinoxylan substrates. Standard curves for xylose and xylobiose in the presence of either the two xylans or arabinoxylan were not linear with the DNSA procedure, but were with the Nelson/Somogyi procedure. Also, with the DNSA procedure the colour response from xylobiose was approximately 140 % of that of an equimolar concentration of xylose. This, of course, has implications on the determined  $\beta$ -xylanase activity. Also, there is a difference in the sensitivity of the three substrates in the assay of  $\beta$ -xylanase. Each of these challenges in the measurement of  $\beta$ -xylanase will be discussed.

## Novel substrates for the measurement of pullulanase

David Mangan<sup>1</sup>, Vincent McKie<sup>1</sup> and Barry McCleary<sup>1</sup>

david@megazyme.com

1. Megazyme International Ireland, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.

Specific and highly sensitive chromogenic and fluorogenic substrate mixtures have been prepared for the measurement of limit-dextrinase and pullulanase activity and assays employing these substrates have been developed. These substrate mixtures contain thermostable  $\alpha$ - and  $\beta$ -glucosidases and either 4,6-*O*-benzylidene-2-chloro-4-nitrophenyl-  $\beta$ -maltotriosyl (1-6)  $\alpha$ -maltotriose (BzCNPG3G3) [I] as a chromogenic substrate, or 4,6-*O*-benzylidene-methylumbelliferyl- $\beta$ -maltotriosyl (1-6)  $\alpha$ -maltotriose (BzMUG3G3) [II] as a fluorogenic substrate. Hydrolysis of substrates [I] and [II] by *exo*-acting enzymes such as amyloglucosidase,  $\beta$ -amylase and  $\alpha$ -glucosidase is prevented by the presence of the 4,6-*O*-benzylidene group on the non-reducing end D-glucosyl residue. The substrates also are not hydrolysed by any  $\alpha$ -amylases studied (e.g. *A. niger*, porcine pancreas or bacterial) and are resistant to hydrolysis by *Pseudomonas* sp. isoamylase. On hydrolysis by pullulanase, the 2-chloro-4-nitrophenyl- $\beta$ -linked maltotrioside is immediately hydrolysed to D-glucose and 2-chloro-4-nitrophenol (or methylumbelliferone). The reaction is terminated by the addition of a weak alkaline solution leading to phenolate ions in solution whose concentration can be determined using either spectrophotometry or fluorimetric analysis. The assay procedure is simple to use, specific, accurate, robust and readily adapted to automation. It should find widespread application in the screening of microbial preparations for this enzyme and also in the routine analysis of industrial pullulanase preparations and of plant limit-dextrinase.

## Residue L940 has a crucial role in the specificity of the glucansucrase GTF180 of *Lactobacillus reuteri* 180

Xiangfeng Meng<sup>1</sup>, Justyna M. Dobruchowska<sup>1</sup>, Tjaard Pijning<sup>2</sup>, Cesar A. López<sup>2</sup>, Johannis P. Kamerling<sup>1</sup> and Lubbert Dijkhuizen<sup>1</sup>

x.meng@rug.nl

1. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
2. Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

Highly conserved GH70 family glucansucrases differ in catalyzing the synthesis of  $\alpha$ -glucans with different structures from sucrose. The structural determinants of glucansucrase specificity have remained unclear. Residue L940 in domain B of GTF180, the glucansucrase of the probiotic bacterium *Lactobacillus reuteri* 180, was shown to vary in different glucansucrases and is close to acceptor binding site +1 in the crystal structure<sup>1</sup>. Here, we show that mutations in L940 of wild-type GTF180- $\Delta$ N all caused an increased percentage of ( $\alpha$ 1 $\rightarrow$ 6) linkages and a decreased percentage of ( $\alpha$ 1 $\rightarrow$ 3) linkages in the products<sup>2</sup>.  $\alpha$ -Glucans with potential different physico-chemical properties [containing 67% to 100% of ( $\alpha$ 1 $\rightarrow$ 6) linkages] are produced by GTF180 and its L940 mutants. Mutant L940W was unable to form ( $\alpha$ 1 $\rightarrow$ 3) linkages and synthesized a smaller and linear glucan polysaccharide with only ( $\alpha$ 1 $\rightarrow$ 6) linkages. Docking studies revealed that the introduction of the large aromatic amino acid residue tryptophan at position 940 partially blocked the binding groove, preventing the isomalto-oligosaccharide acceptor to bind in a favorable orientation for the formation of ( $\alpha$ 1 $\rightarrow$ 3) linkages. Our data showed that the reaction specificity of mutant glucansucrase GTF180 was shifted either to increased polysaccharide synthesis (L940A, L940S, L940E and L940F) or increased oligosaccharide synthesis (L940W). The L940W mutant is capable of producing a large amount of isomalto-oligosaccharides using released glucose from sucrose as acceptors. Thus, residue L940 in domain B is crucial for linkage and reaction specificity of GTF180. This study provides clear and novel insights into the structure-function relationships of glucansucrase enzymes.

### Literature

1. Vujičić-Žagar A, Pijning T, Kralj S, López CA, Eeuwema W, Dijkhuizen L, Dijkstra BW (2010) Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proc Natl Acad Sci USA* 107(50):21406-21411
2. Meng X, Dobruchowska JM, Pijning T, López CA, Kamerling JP, Dijkhuizen L (2014) Residue Leu940 has a crucial role in the linkage and reaction specificity of the glucansucrase GTF180 of the probiotic bacterium *Lactobacillus reuteri* 180. *J Biol Chem* 289(47):32773-32782

## Truncation of domain V of the multidomain glucansucrase GTF180 of *Lactobacillus reuteri* 180 heavily impairs its polysaccharide-synthesizing ability

Xiangfeng Meng<sup>1</sup>, Justyna M. Dobruchowska<sup>1</sup>, Tjaard Pijning<sup>2</sup>, Gerrit J. Gerwig<sup>2</sup>, Johannes P. Kamerling<sup>1</sup> and Lubbert Dijkhuizen<sup>1</sup>

x.meng@rug.nl

1. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
2. Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

Glucansucrases are exclusively found in lactic acid bacteria and synthesize a variety of  $\alpha$ -glucans from sucrose. They are large multi-domain enzymes belonging to the CAZy family 70 of glycoside hydrolase enzymes (GH70). The crystal structure of the N-terminally truncated GTF180 of *Lactobacillus reuteri* 180 (GTF180- $\Delta$ N) reveals that the polypeptide chain follows a U shape course to form five domains, including domains A, B and C, which resemble those of family GH13 enzymes, and two extra and novel domains (domains IV and V), which are attached to the catalytic core<sup>1</sup>. To elucidate the functional roles of domain V, we have deleted the domain V fragments from both the N- and C-terminal ends (GTF180- $\Delta$ N $\Delta$ V). Truncation of domain V of GTF180- $\Delta$ N yielded a catalytically fully active enzyme but with heavily impaired polysaccharide synthesis ability. Instead, GTF180- $\Delta$ N $\Delta$ V produced a large amount of oligosaccharides. Domain V is not involved in determining the linkage specificity and the size of polysaccharide produced as the GTF180- $\Delta$ N and GTF180- $\Delta$ N $\Delta$ V polysaccharides were identical in size and structure. The data indicates that GTF180- $\Delta$ N $\Delta$ V acts non-processively, frequently initiating synthesis of a new oligosaccharide from sucrose, instead of continuing the synthesis of a full size polysaccharide. Mutations L940E and L940F in GTF180- $\Delta$ N $\Delta$ V, which are involved in the acceptor substrate binding<sup>2</sup>, restored polysaccharide synthesis almost to the level of GTF180- $\Delta$ N. These results demonstrated that interactions of growing glucan chains with both domain V and acceptor substrate binding sites are important for polysaccharide synthesis<sup>3</sup>.

### Literature

1. Vujičić-Žagar A, Pijning T, Kralj S, López CA, Eeuwema W, Dijkhuizen L, Dijkstra BW (2010) Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proc Natl Acad Sci USA* 107(50):21406-21411
2. Meng X, Dobruchowska JM, Pijning T, López CA, Kamerling JP, Dijkhuizen L (2014) Residue Leu940 has a crucial role in the linkage and reaction specificity of the glucansucrase GTF180 of the probiotic bacterium *Lactobacillus reuteri* 180. *J Biol Chem* 289(47):32773-32782
3. Meng X, Dobruchowska JM, Pijning T, Gerwig GJ, Kamerling JP, Dijkhuizen L (2015) Truncation of domain V of the multidomain glucansucrase GTF180 of *Lactobacillus reuteri* 180 heavily impairs its polysaccharide-synthesizing ability. *Appl. Microbiol Biot*, DOI 10.1007/s00253-014-6361-8, in Press.



## Identification and engineering of new family AA5 galactose oxidases

*Filip Mollerup*<sup>1</sup>, *Kirsti Parikka*<sup>2</sup>, *Maija Tenkanen*<sup>2</sup> and *Emma Master*<sup>3</sup>

filip.mollerup@aalto.fi

1. Aalto University, Department of Biotechnology and Chemical Technology, Kemistintie 1, Espoo, Finland, FI-0076
2. Department of Food and Environmental Sciences, Agnes Sjöbergin Katu 2, Helsinki, Finland, FI-00014
3. University of Toronto, Department of Chemical Engineering and Applied Chemistry, 200 College Street, Toronto, Ontario, Canada, M5S 3E5

Enzymatic oxidation can facilitate regiospecific modification of plant poly- and oligosaccharides for formation of aero- and hydrogels, as well as site-specific addition of new functional groups under mild aqueous conditions<sup>1,2,3,4</sup>. Galactose oxidase (GaO) from *Fusarium spp.* is classified by the CAZy database as a family 5 auxiliary activity and has been used to oxidize several galactose-containing polysaccharides, thereby expanding their performance in bio-based materials. GaO oxidizes the C6-hydroxyl of terminal galactose substituents and is the only oxidase so far reported to oxidize the primary hydroxyl of terminal sugar-residues in polysaccharides.

Herein this poster, we describe C-terminal fusion of a family 3 carbohydrate binding module to the m-RQW variant of GaO (aka M<sub>3</sub>)<sup>5</sup>, generating M<sub>3</sub>-CBM3. While M<sub>3</sub> gained activity on glucose, the activity of M<sub>3</sub> on galactose decreased approximately 1000-fold<sup>5</sup>. Accordingly, in addition to constructing M<sub>3</sub>-CBM3, three amino acid substitutions were herein introduced in an effort to retain widened substrate preference while maximizing catalytic efficiency. The activities of wild-type and engineered GaO were tested using 23 substrates, including galactose and glucose containing oligosaccharides and malto- and cellooligosaccharides. This analysis revealed that in addition to accepting a broader range of sugars for oxidation, M<sub>3</sub> is less sensitive to glycoside linkage type as compared to wild-type GaO. Although preliminary tests suggest that CBM3 fusion does not promote M<sub>3</sub> activity on cellulose or galactose containing polysaccharides bound to filter paper, M<sub>3</sub>-CBM3 was 2.5 and 4 times more active on galactose and glucose than previously reported values for M<sub>3</sub> alone<sup>6</sup>. M<sub>3</sub>-CBM3 was also able to functionally bind crystalline cellulose, promoting immobilization to cellulosic surfaces.

### Acknowledgements

Funding provided by the Finnish Cultural Foundation and Academy of Finland (Contract No. 1252183).

### Literature

1. Leppänen AS, Xu C, Parikka K, Eklund P, Sjöholm R, Brumer H, Tenkanen M, Willför S. 2014. Carbohydr Polym. 100:46-54.
2. Mikkonen, K. S., Parikka, K., Suuronen, J.-P., Ghafar, A., Serimaa, R., & Tenkanen, M. 2014. RSC Advances, 4(23), 11884. doi:10.1039/c3ra47440b
3. Parikka K, Leppänen AS, Xu C, Pitkänen L, Eronen P, Osterberg M, Brumer H, Willför S, Tenkanen M. 2012. Biomacromolecules. 3(8):2418-28.
4. Xu C, Spadiut O, Araújo AC, Nakhai A, Brumer H. 2012. ChemSusChem. 5(4):661-5.
5. Sun L, Bulter T, Alcalde M, Petrounia IP, Arnold FH. 2002. ChemBiochem. 3(8):781-3
6. Lippow SM, Moon TS, Basu S, Yoon SH, Li X, Chapman BA, Robison K, Lipovšek D, Prather KL. 2010. Chem Biol. 17(12):1306-15.

## Investigating synergism within multimodular glycoside hydrolases during wheat straw cell wall deconstruction

Thierry Vernet<sup>1</sup>, Anne-Marie DiGuilmi<sup>1</sup>, Michael O'Donohue<sup>2,3,4</sup>, Cédric Montanier<sup>2,3,4</sup>

cedric.montanier@insa-toulouse.fr

1. Institut de Biologie Structurale Jean-Pierre Ebel, UMR 5075 (CEA, CNRS, UJF); 41 rue Jules Horowitz, F-38027 Grenoble, France
2. Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France
3. INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
4. CNRS, UMR5504, F-31400 Toulouse, France

In Nature, the plant-based organic carbon contained within plant cell walls is mainly recycled by the action of cellulolytic microorganisms, such as bacteria and fungi, which produce complex arrays of cell wall-degrading enzymes. Many of the enzymes that degrade plant cell wall polysaccharides are modular proteins, which contain single or multiple copies of both catalytic domain(s) and CBM(s). Cellulolytic microorganisms developed a couple of strategies to break down the complex plant cell wall; secrete free enzymes into their surroundings or produce large multi-component cell-bound structures, which harbour several enzymes displaying complementary activities. In both case, substrate is attacked by different enzymes acting together at different regions, and much more than sum of individual different enzymatic activities, synergism drives the efficiency of such system. Nevertheless, this complex process is still not completely understood. Despite an abundant literature about the role of CBM as an enhancer of the catalytic activity, we miss information about relationship between enzymes, CBMs and modularity *in planta*.

Here we report an original way to easily associate catalytic domains and CBMs to each other. Jo and In are small proteins (10.6 and 16.7 kDa respectively) from *Streptococcus pneumoniae*<sup>1,2</sup>, which are able to spontaneously form one intradomain isopeptide bond. The xylanase Xyn11A from *Neocallimastix patriciarum* and the xylosidase Xyl43 from *Bacillus halodurans* were successfully expressed in *E.coli* and purified as Jo or In tagged proteins. In addition, cellulose specific CBM3a from *Clostridium thermocellum* and xylane specific CBM2b-1 from *Cellulomonas fimi* were also expressed and purified. Various combinations of these proteins were designed as free or solid support associated modular enzymes. Catalytic activities were investigated using chromogenic substrates, beach wood xylan and milled wheat straw.

The fine spatial proximity/synergism relationship in multimodular enzymes within the context of intact plant cell wall is presented in this work.

### Literature

1. Izoré, T. *et al.* Structural basis of host cell recognition by the pilus adhesin from *Streptococcus pneumoniae*. *Structure* **18**, 106–115 (2010).
2. « PEPTIDES APTES À FORMER UN COMPLEXE COVALENT ET LEURS UTILISATIONS » Anne Marie Di Guilmi and Thierry Vernet (FR11 52432, demande internationale N° PCT/EP2012/055333 déposée le 26 mars 2012)

## Development of tailor-made ‘oxidative boosted’ enzyme mixtures for the bioconversion of targeted feed stocks

*Madhu Nair Muraleedharan*<sup>1</sup>, *Anthi Karnaouri*<sup>1</sup>, *Maria Dimarogona*<sup>2</sup>, *Evangelos Topakas*<sup>2</sup>, *Ulrika Rova*<sup>1</sup>, *Paul Christakopoulos*<sup>1</sup>

paul.christakopoulos@ltu.se

1. Biochemical Process Engineering, Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden
2. Biotechnology Laboratory, Department of Synthesis and Development of Industrial Processes, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

The role of *Myceliophthora thermophila* and its enzyme aristocracy in the field of bioconversion of complex carbohydrates is well known today. Several cellulolytic enzymes have been discovered in this species which have been proven to have excellent efficiency on lignocellulosic substrates. With the latest introduction of the new enzyme group Lytic Polysaccharide Monooxygenases (LPMOs) and their proven ‘boosting’ effect on traditional cellulases, the dream of complete bioconversion of those recalcitrant polysaccharides is one more step closer to reality (1). However, the complexity of different feed stocks brings the need of customized enzyme proportions, specific for each of them, to attain maximum degradation without using excess enzyme. The lack of such customized enzyme cocktails results in either enzyme wastage or inefficient conversion of substrates, hence results in higher process cost. In this study, tailor-made enzymes mixtures targeted towards particular agricultural and forest feed stocks were tested for their ability to maximize hydrolysis yields. Various combinations of an LPMO (*MtLPMO9A*), along with a consortium of traditional cellulases and hemicellulases, all originated from *Myceliophthora Thermophila* and heterologously expressed in *Pichia pastoris* were used at various combinations (2,3). The synergistic effect of *MtLPMO* was optimized by varying the ratio of the various enzymatic components as well as the nature and amount of the reducing agent added in the degrading cocktail. Optimal combinations were predicted from suitable statistical models which were able to further increase hydrolysis yields, suggesting that tailor-made enzyme mixtures targeted towards a particular feed stock can help maximize hydrolysis yields. This work helps to lead the situation from ‘one cocktail for all’ to ‘tailor-made cocktails’ for targeted feed stocks, with better hydrolysis yields.

### Literature

1. Biotechnology for Biofuels 5:45
2. Appl Microbiol Biotechnol. 98:231-42
3. PeerJ. 1:e46

## Novel GH130 $\beta$ -mannoside phosphorylases

Hiroyuki Nakai<sup>1</sup>, Takanori Nihira<sup>1</sup>, Kazuhiro Chiku<sup>1</sup>, Erika Suzuki<sup>1</sup>, Mamoru Nishimoto<sup>2</sup>, Motomitsu Kitaoka<sup>2</sup>, Ken'ichi Ohtsubo<sup>1</sup>

nakai@agr.niigata-u.ac.jp

1. Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan

2. National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305-8642, Japan

Glycoside hydrolase family (GH) 130 has been recently established and contains  $\beta$ -mannoside phosphorylases catalyzing the reversible phosphorolysis of  $\beta$ -mannosides to form  $\alpha$ -D-mannose 1-phosphate with an inversion of its anomeric configuration. In this study, we characterized three GH130 homologues derived from *Bacteroides thetaiotaomicron* VPI-5482 (BT1033)<sup>1</sup> and *Thermoanaerobacter* sp. X-514 (Teth514\_1788 and Teth514\_1789)<sup>2</sup> to find novel phosphorylases showing unreported substrate specificity.

Acceptor specificity analysis of BT1033 in synthetic reaction showed that BT1033 utilized *N*-acetyl-D-glucosamine as the suitable acceptor. Furthermore, it was demonstrated that BT1033 catalyzed reversible phosphorolysis of  $\beta$ -1,4-D-mannosyl-*N*-acetyl-D-glucosamine in a typical sequential Bi Bi mechanism. We thus propose 4-*O*- $\beta$ -D-mannopyranosyl-*N*-acetyl-D-glucosamine:phosphate  $\alpha$ -D-mannosyltransferase as the systematic name and  $\beta$ -1,4-D-mannosyl-*N*-acetyl-D-glucosamine phosphorylase as the short name for BT1033. BT1033 gene is located in a gene cluster involved in complex-type N-glycan metabolism, suggesting that it plays a crucial role as intracellular phosphorolysis of  $\beta$ -1,4-D-mannosyl-*N*-acetyl-D-glucosamine liberated from the complex-type *N*-glycan by sequential glycoside hydrolase reactions.

Teth514\_1788 and Teth514\_1789 catalyzed the synthesis of  $\beta$ -1,2-oligomannan using  $\beta$ -1,2-mannobiose and D-mannose as the optimal acceptors, respectively. Kinetic analysis of phosphorolytic reaction toward  $\beta$ -1,2-oligomannan revealed that Teth514\_1788 and Teth514\_1789 prefer  $\beta$ -1,2-oligomannan containing a DP  $\geq 3$  and  $\beta$ -1,2-mannobiose, respectively. These results indicate that these two enzymes are novel phosphorylases that exhibit distinct chain-length specificities toward  $\beta$ -1,2-oligomannan. Here, we propose  $\beta$ -1,2-oligomannan:phosphate  $\alpha$ -D-mannosyltransferase as the systematic name and  $\beta$ -1,2-oligomannan phosphorylase as the short name for Teth514\_1788 and  $\beta$ -1,2-mannobiose:phosphate  $\alpha$ -D-mannosyltransferase as the systematic name and  $\beta$ -1,2-mannobiose phosphorylase as the short name for Teth514\_1789. Based on the sequence analysis, we suggest that the two phosphorylases play a role in the intercellular phosphorolysis of  $\beta$ -1,2-oligomannan to supply  $\alpha$ -D-mannose 1-phosphate for GDP-D-mannose biosynthesis in the bacterium.

### Literature

1. Nihira T., Suzuki E., Kitaoka M., Nishimoto M., Ohtsubo K., Nakai H. "Discovery of  $\beta$ -1,4-D-mannosyl-*N*-acetyl-D-glucosamine phosphorylase involved in the metabolism of *N*-glycans" The Journal of Biological Chemistry (2013) 288, 27366–27374.
2. Chiku K., Nihira T., Suzuki E., Nishimoto M., Kitaoka M., Ohtsubo K., Nakai H. "Discovery of two  $\beta$ -1,2-mannoside phosphorylases showing different chain-length specificities from *Thermoanaerobacter* sp. X-514" PLoS One (2014) 9, e114882.

## Discovery of 1,2- $\beta$ -oligoglucan phosphorylase and large scale preparation of 1,2- $\beta$ -glucan

Masahiro Nakajima<sup>1,3</sup>, Hiroyuki Toyozumi<sup>1</sup>, Koichi Abe<sup>1</sup>, Yuta Takahashi<sup>2</sup>, Naohisa Sugimoto<sup>2</sup>, Hiroyuki Nakai<sup>2</sup>, Hayao Taguchi<sup>1</sup> and Motomitsu Kitaoka<sup>3</sup>

m-nakajima@rs.tus.ac.jp

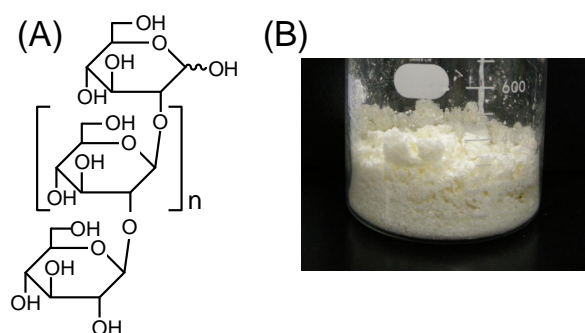
1. Tokyo University of Science

2. Niigata University

3. National Food Research Institute, National Agriculture and Food Research Organization (NARO)

We characterized recombinant Lin1839 protein (Lin1839r) belonging to glycoside hydrolase family 94 from *Listeria innocua*. Lin1839r catalyzed the synthesis of a series of 1,2- $\beta$ -oligoglucans (Sop<sub>n</sub>; n denotes degree of polymerization) using sophorose (Sop<sub>2</sub>) as the acceptor and  $\alpha$ -D-glucose 1-phosphate as the donor. Kinetic analysis of the phosphorolytic reaction towards Sop<sub>3</sub> revealed that Lin1839r followed a sequential Bi Bi mechanism. The kinetic parameters of the phosphorolysis of Sop<sub>4</sub> and Sop<sub>5</sub> were similar to those of Sop<sub>3</sub>, although the enzyme did not exhibit significant phosphorolytic activity on Sop<sub>2</sub>. These results indicate that the Lin1839 protein is a novel inverting phosphorylase that catalyzes reversible phosphorolysis of 1,2- $\beta$ -glucan with a degree of polymerization of  $\geq 3$ . We propose 1,2- $\beta$ -oligoglucan: phosphate  $\alpha$ -glucosyltransferase as the systematic name and 1,2- $\beta$ -oligoglucan phosphorylase (OGP) as the short name for this Lin1839 protein.

We also produced 1,2- $\beta$ -glucan enzymatically from 1.0 M sucrose and 0.5 M glucose by the combination of sucrose phosphorylase and OGP in the presence of 100 mM inorganic phosphate. Accumulation of 1,2- $\beta$ -glucan in 2 L of the reaction mixture reached over 800 mM (glucose equivalent). Sucrose, glucose and fructose were removed after the reaction by yeast treatment. 1,2- $\beta$ -Glucan was precipitated with ethanol to obtain 167 g of 1,2- $\beta$ -glucan from 1 L of the reaction mixture.



**Fig 1.** Structure of 1,2- $\beta$ -glucan (A) and large-scale prepared 1,2- $\beta$ -glucan (B).

### Acknowledgements

We thank Mr. Masao Hiraishi, Dr. Kiyohiko Igarashi and Dr. Masahiro Samejima of The University of Tokyo for their contribution at the early stage of this research. Thanks are also due to the staff of the Instrumental Analysis Center for Food Chemistry of the National Food Research Institute for recording the NMR spectra. The Sugawara Laboratory of the Tokyo University of Science kindly helped us to prepare Sop<sub>n</sub>s.

## Exploring the secretomes of starch degrading fungi

Laura Nekiunaite<sup>1</sup>, Gustav Vaaje-Kolstad<sup>2</sup>, Birte Svensson<sup>1</sup>, Magnus Øverlie Arntzen<sup>2</sup> and Maher Abou Hachem<sup>1</sup>

lanek@bio.dtu.dk

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.
2. Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (UMB), P. O. Box 5003, N-1432 Ås, Norway.

Starch is one of the most abundant renewable biopolymers in nature. Each year more than 2 billion tonnes of starch crops are harvested globally, making it an attractive resource for industrial applications [1,2]. Most applications of starch require the disruption of native granules or starch modification. Thus, developing efficient enzymes that can hydrolyse and modify native starch granules is of interest. The properties of starch granules, especially crystallinity, which varies between 15 to 45%, make complete enzymatic depolymerisation of starch challenging depending on botanic origin and processing [3].

In contrast to lignocellulose matrices [4], much less effort has been imparted on discerning the enzyme cocktails deployed by fungi, both with respect to class, composition and proportion, for the degradation of starch. This insight is likely to have impact on the efficiency of industrial starch processing.

Fungi are renowned for producing an arsenal of carbohydrate active enzymes (CAZymes) [5] to harvest energy from various glycans. The main focus of the present study is to examine the degradation machinery of starch by filamentous *Aspergillus* spp., which exhibit tremendous ecological, biological and metabolic diversity and are used industrially for recombinant enzyme production.

Here we present an *in silico* analyses of the fungal CAZymes plausibly involved in the degradation of starch by fungi. The growth of selected *Aspergillus* strains on starchy substrates of different properties will be assessed and proteomics tools will be used to analyse complex mixture of enzymes that are secreted (i.e. the secretomes) to mediate growth on starch. The latest results from this study will be presented and discussed.

### Acknowledgements

This work is funded by a grant from the Novo Nordisk Foundation in the area “Biotechnology-based Synthesis and Production”.

### Literature

1. Alvani K., *et al.*: *Starch-Stärke*, (2012) 64:297–303.
2. Zeeman S.C., *et al.*: *Annu Rev Plant Biol*, (2010) 61:209–234.
3. Buleon A., *et al.*: *Int J Biol Macromol*, (1998) 23:85–112.
4. Hori, C., *et al.*: *FEMS Microbiol Lett*, (2011) 321:14–23.
5. Cantarel B.L., *et al.*: *Nucleic Acids Res*, (2009) 37:233–238.

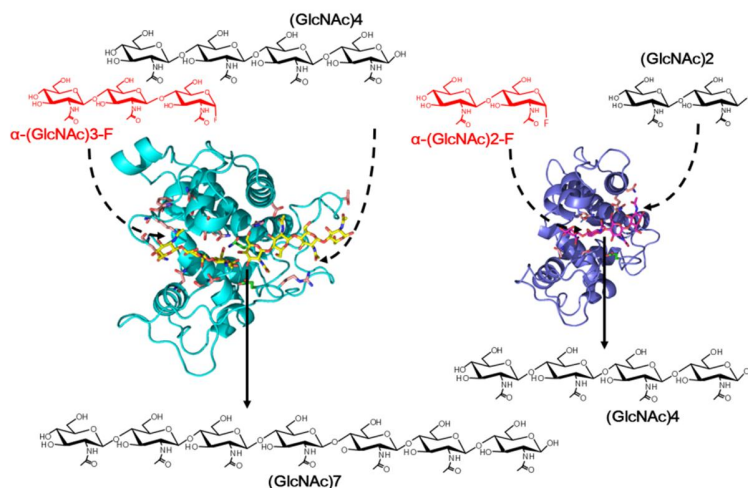
## Chemo-enzymatic synthesis of chitoheptaose using a glycosynthase derived from an inverting chitinase with an extended binding cleft

*Takayuki Ohnuma, Satoshi Dozen and Tamo Fukamizo*

ohnumat@nara.kindai.ac.jp

Department of Advanced Bioscience, Kinki University, 3327-204, Nakamachi, Nara 631-8505, Japan

We successfully created the first glycosynthase derived from a “loopless” family GH19 chitinase from moss (*BcChi-A*), whose oligosaccharide-binding cleft consists of only four subsites -2, -1, +1, and +2 (Ohnuma *et al.*, *Biochem. J.*, 444, 437-443, 2012). However, the chain-lengths of the synthetic products were limited, because the “loopless” enzyme could accept only oligosaccharides with shorter chains as a donor or an acceptor substrate. Here, we report a glycosynthase derived from a “loopful” GH19 chitinase from rye seeds (*RSC-c*) whose binding cleft consists of subsites -4, -3, -2, -1, +1, +2, +3, and +4. When the wild-type *RSC-c* was incubated with  $\alpha$ -(GlcNAc)<sub>3</sub>-F [ $\alpha$ -(GlcNAc)<sub>3</sub> fluoride], (GlcNAc)<sub>3</sub> and hydrogen fluoride were produced through the Hehre resynthesis–hydrolysis mechanism. Then, mutations were introduced into Glu89 acting as a catalytic base and Ser120 holding a nucleophilic water molecule, to produce two single mutants, E89G and S120A. E89G synthesized a small amount of (GlcNAc)<sub>7</sub> from  $\alpha$ -(GlcNAc)<sub>3</sub>-F in the presence of (GlcNAc)<sub>4</sub>, while S120A with the highest F<sup>−</sup>-releasing activity successfully produced a large amount of (GlcNAc)<sub>7</sub>. However, a fraction of (GlcNAc)<sub>7</sub> synthesized by S120A was decomposed by its own residual hydrolytic activity. Thus, we finally produced a double mutant E89G/S120A, which produced a large amount of (GlcNAc)<sub>7</sub> without decomposition. The double mutation completely abolished the hydrolytic activity and maintained moderate F<sup>−</sup>-releasing activity. E89G/S120A, which was the best glycosynthase, can produce chitin oligosaccharides with longer chains.



**Fig 1.** Chemo-enzymatic synthesis of chitin oligosaccharide using a glycosynthase derived from an inverting GH19 chitinase

## A transglycosylation of catalytic nucleophile mutant of GH97 $\alpha$ -galactosidase with an external nucleophile

*Masayuki Okuyama<sup>1</sup>, Kana Matsunaga<sup>1</sup>, Ken-ichi Watanabe<sup>1</sup>, Takayoshi Tagami<sup>1</sup>, Keitaro Yamashita<sup>2</sup>, Haruhide Mori<sup>1</sup>, Min Yao<sup>2,3</sup> and Atsuo Kimura<sup>1</sup>*

okuyama@abs.agr.hokudai.ac.jp

1. Research Faculty of Agriculture, Hokkaido University
2. Graduate School of Life Science, Hokkaido University
3. Faculty of Advanced Life Science, Hokkaido University

$\alpha$ -Galactoside is one of the most important classes of carbohydrates, including such as  $\alpha$ -gal epitope [ $\alpha$ -Gal-(1 $\rightarrow$ 3)- $\beta$ -Gal-(1 $\rightarrow$ 4)-GlcNAc-R or  $\alpha$ -Gal-(1 $\rightarrow$ 3)- $\beta$ -Gal-(1 $\rightarrow$ 3)-GlcNAc-R], globosyl-Gb2 [ $\alpha$ -Gal-(1 $\rightarrow$ 4)- $\beta$ -Gal-R], -Gb3 [ $\alpha$ -Gal-(1 $\rightarrow$ 4)- $\beta$ -Gal-(1 $\rightarrow$ 4)- $\beta$ -Glc-R], and raffinose [ $\alpha$ -Gal-(1 $\rightarrow$ 6)- $\alpha$ -Glc-(1 $\leftrightarrow$ 2)  $\beta$ -Fru]. The  $\alpha$ -gal epitope and Gb3/Gb2 are of major clinical significance and raffinose receives a lot of attention as a prebiotics.

Most, if not all, of  $\alpha$ -galactosides are synthesized by NDP-forming glycosyltransferases, but an  $\alpha$ -galactosidase also catalyzes the formation of  $\alpha$ -galactoside in a transglycosylation reaction. To ensure the maximum yield of the transglycosylation product is the biggest challenge, because the normal hydrolysis reaction would reduce the transfer product. Glycosynthase, having a site-directed mutation at a nucleophile catalyst of a retaining glycosidase, is capable of catalyzing glycosyl transfer from wrong glycosyl fluoride to acceptor alcohols without hydrolysis of the product, and can overcome this problem. However, the glycosyl donor substrate of the glycosynthase formed from the  $\alpha$ -galactosidase is less stable  $\beta$ -galactosyl fluoride, and it is expected to make a low yield of the transglycosylation product. We thus exploit somewhat more stable  $\alpha$ -galactosyl fluoride ( $\alpha$ -GalF) as the glycosyl donor substrate of the catalytic nucleophile mutant of glycoside hydrolase family 97  $\alpha$ -galactosidase, *BtGH97b*, with an external nucleophile, to establish the maximum yield of the product.

The glycosyl transfer from  $\alpha$ -GalF to lactose by the catalytic nucleophile mutant of *BtGH97b*, D415G, occurs with a formate ion as the nucleophile. NMR-analysis indicates that the product is  $\beta$ -lactosyl  $\alpha$ -galactopyranoside [ $\alpha$ -D-Galp-(1 $\leftrightarrow$ 1)  $\beta$ -D-Glcp-(4 $\leftarrow$ 1)  $\beta$ -D-Galp], which is a unique non-reducing sugar. Sodium azide can more effectively restore the catalytic reaction, but no transglycosylation product is detected and an accumulation of  $\beta$ -galactosyl azide is observed. A kinetic analysis suggests that the less reactivity of  $\beta$ -galactosyl azide results in the accumulation. Glucose, xylose, maltose, and cellobiose can also act as acceptor, with the yields of 75% (glucose), 90% (xylose), 90% (maltose), and 75% (cellobiose), respectively, but galactose cannot act as acceptor.

A crystal structure of D415G in complex with  $\beta$ -lactosyl  $\alpha$ -D-galactoside allows to investigate the molecular aspect of substrate binding site of *BtGH97b*. Two aromatic residues at the entrance of the active-site pocket of *BtGH97b* make the acceptor binding site narrow, and this structural feature can be responsible for the formation of  $\alpha$ -Gal-(1 $\leftrightarrow$ 1)- $\beta$ -Glc linkage and for inability to form the  $\alpha$ -galactosyl galactose linkage. The narrow entrance might prohibit the galactose residue having axial OH-4 from binding to the acceptor site.



The abstract has been withdrawn

## Design of a nano-system targeting the tumor micro-environment for the treatment of tumor by inhibition of a specific $\beta$ -endoglycosidase responsible for angiogenesis

*Nicolas Poupard, Nicolas Bridiau, Jean-Marie Piot, Thierry Maugard, Ingrid Fruitier-Arnaudin*

nicolas.poupard@univ-lr.fr

Laboratoire Littoral Environnement et Sociétés (LIENSs), département biotechnologies, équipe Approches Moléculaires, Environnement-Santé (AMES), UMR 7266 CNRS-ULR, Université de La Rochelle, Avenue Michel Crépeau, 17042 La Rochelle, France

Most of the available anticancer agents cannot distinguish between cancerous and healthy cells, leading to systemic toxicity and undesired side effects. The development of nanoscale tumor-targeted delivery systems may resolve these problems. One of the most common strategy involves coupling a nanocarrier with a specific ligand. This ligand should be sensitive to biological and environmental signals such as pH, temperature, and specific enzymes. Highly-interesting are enzyme sensitive systems exploiting over-expressed disease-associated enzymes to trigger drug release or fluorescence. In case of cancer, these tools are perfectly adapted as the tumor micro-environment shows chemical and structural particularities including overexpressed extracellular protease and glycosidase.

The main strategy used in this project is the production of an inactive glycopeptidic precursor that will be activated when cleaved by a protease hyper-secreted and activated in tumoral microenvironment: the Cathepsin-D<sup>1</sup>. Then, the released glycosidic part is designed to inhibit an endoglycosidase responsible for the angiogenesis<sup>2</sup> phenomenon: the Heparanase.

We are also working on adding a FRET fluorescence system on the peptidic part that will show fluorescence as the cleavage occurs.

The purpose of this project is thus to prepare by a standard procedure, a functional nano-tool to be used for both measurement of cellular activities of biomarkers involved in cancers and the release of a drug.

### Literature

1. Fruitier, I., Garreau, I. & Piot, J.-M. Cathepsin D Is a Good Candidate for the Specific Release of a Stable Hemorphin from Hemoglobin In Vivo: VV-Hemorphin-7,. *Biochemical and Biophysical Research Communications* **246**, 719-724 (1998).
2. Vlodavsky, I. & Friedmann, Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. *J. Clin. Invest.* **108**, 341-347 (2001).

## Diversity of xylan deacetylases of family CE16: action on acetylated aldotetrauronic acid and glucuronoxylan

*Vladimír Puchart*<sup>1</sup>, *Jane Agger*<sup>2</sup>, *Jean-Guy Berrin*<sup>3</sup>, *Anikó Varnai*<sup>2</sup>, *Lin-Xiang Li*<sup>4</sup>, *Alasdair MacKenzie*<sup>2</sup>, *Vincent G.H. Eijsink*<sup>2</sup>, *Bjørge Westereng*<sup>2</sup>, *Peter Biely*<sup>1</sup>

chempuk@savba.sk

1. Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

2. Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences Aas, Norway

3. Aix Marseille University, Polytech Marseille, Marseille, France

4. Youtell Biotech Inc., Bothell, WA 98011, USA

Studies of enzymatic degradation of acetylated hardwood glucuronoxylan showed that microbial acetylxyylan esterases are capable of liberating acetic acid from singly and doubly acetylated xylopyranosyl (Xylp) residues, but not from position 3 of Xylp residues  $\alpha$ -1,2-substituted with 4-*O*-methyl-D-glucuronic acid (MeGlcA) [1-3]. This acetyl group was found to be attacked by family CE16 deacetylases [4,5], but only in aldotetrauronic acid, MeGlcA<sup>3</sup>Xyl<sub>3</sub>, acetylated at the non-reducing end Xylp residue, which is a product of GH10 xylanase degradation of the polysaccharide [6-8]. The monoacetylated aldotetrauronic acid was isolated from an enzymatic aspen acetylglucuronoxylan hydrolysate by a combined action of GH10 xylanase, CE6 acetylxyylan esterase and GH67  $\alpha$ -glucuronidase. The monoacetylated MeGlcA<sup>3</sup>Xyl<sub>3</sub> did not serve as substrate for the GH67  $\alpha$ -glucuronidase. Here we report that in the monoacetylated aldotetrauronic acid the 3-*O*-acetyl group migrates to position 4 which was originally involved in the glycosidic linkage. However, the migration is much slower than in linear xylooligosaccharides acetylated at the non-reducing end Xylp residue [9]. The MeGlcA side residue at position 2 obviously rigidifies the xylopyranosyl ring and hampers the acetyl group migration. Examination of the mode of action of *Trichoderma reesei* CE16 acetylsterase by <sup>1</sup>H-NMR showed that the enzyme liberates acetic acid from the aldotetrauronic acid from both position 3 and 4, and at about the same rate. Surprisingly, another CE16 deacetylase from *Podospira anserina* behaved differently. In the monoacetylated aldotetrauronic acid the *P. anserina* enzyme deacetylated exclusively position 4 although the enzyme deacetylated all positions 2, 3 and 4 on the non-reducing end of methyl  $\beta$ -D-xylotrioside. In contrast to *T. reesei* CE16, the *P. anserina* enzyme also deacetylated all singly and doubly acetylated Xylp residues in the polymeric substrate. These results outline a strategy for complete enzymatic degradation of hardwood acetylglucuronoxylan isolated by non-alkaline extraction procedures. At the same time, they illustrate variation among CE16 deacetylases, emphasizing the need to characterize more members of this interesting enzyme family.

### Literature

1. Biely P. et al. J. Appl. Glycosci. 61, 35-44 (2014)
2. Uhliaríková I. et al. Biochim. Biophys. Acta 1830, 3365-3372 (2013)
3. Biely P. et al. Biochim. Biophys. Acta 1830, 5075-5086 (2013)
4. Poutanen K. et al. Appl. Microbiol. Biotechnol. 33, 506-510 (1990)
5. Li X.-L. et al. Appl. Environ. Microbiol. 74, 7482-7489 (2008)
6. Biely P. et al. Biochim. Biophys. Acta 1840, 516-525 (2014)
7. Koutaniemi S. et al. J. Biotechnol. 168, 684-692 (2013)
8. Neumüller K. G. et al. Biotechnol. Biofuels, in press
9. Puchart V. and Biely P. Appl. Microbiol. Biotechnol., in press; DOI: 10.1007/s00253-014-6160-2

## Conformational studies on trivalent acetylated mannobiose clusters

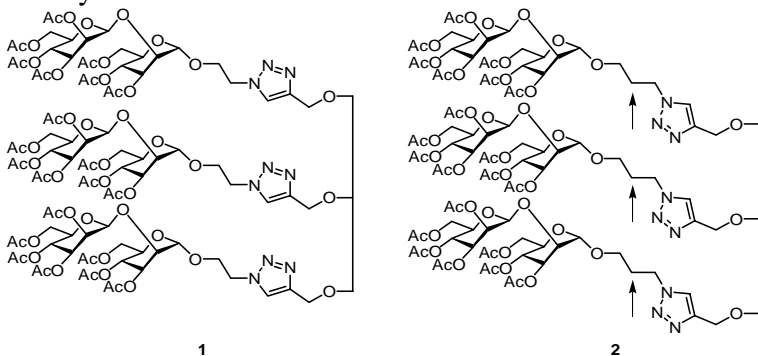
Jani Rahkila,<sup>1</sup> Rajib Panchadhayee,<sup>1</sup> Ana Ardá,<sup>2</sup> Jesús Jiménez-Barbero,<sup>2</sup> and Reko Leino<sup>1</sup>

rleino@abo.fi

1. Laboratory of Organic Chemistry, Åbo Akademi University; Biskopsgatan 8 20540, Turku, Finland

2. Structural Biology Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia Building 801A, 48160 Derio, Spain

The immunostimulatory properties of  $\beta$ -(1 $\rightarrow$ 2)-linked mannosides of natural origin have been studied for some time already<sup>1</sup> and considerable amount of effort has gone into the synthesis<sup>2</sup> and biological evaluation<sup>2,3</sup> of such compounds. One of the underlying reasons for the observed biological activity is believed to be the unique helical conformation resulting from the rare  $\beta$ -(1 $\rightarrow$ 2)-linkage, which is apparent even in molecules as small as tri- and tetrasaccharides. We have previously screened a large number of fully synthetic mannose-based structures for potential use as adjuvants in allergen immunotherapy.<sup>4</sup> The screened molecules have ranged from simple mono- and oligosaccharides to oligovalent clusters containing also acetylated compounds. Among these molecules, the trivalent cluster **1** was found to be a potent candidate for further investigations showing promising activity both *in vitro* and *in vivo*.<sup>4c,5</sup> Similar *in vitro* activity has recently been demonstrated also for the closely analogous structure **2** with the linker elongated by one  $-\text{CH}_2-$  moiety.



**Fig 1.** Trivalent acetylated mannobioses studied in this work.

In the present work, these two molecules were subjected to comprehensive NMR studies and molecular dynamics simulations in order to elucidate their behavior in solution. This provides valuable information about how such compounds may fit into the binding pockets of receptors on cell surfaces which, in turn, is important for understanding their biological activities.

**Acknowledgements** Financial support from the Academy of Finland and the COST Action CM1102 MultiGlycoNano is gratefully acknowledged.

### Literature

1. Li, R.-K.; Cutler, J. E. *J. Biol. Chem.* **1993**, 268, 18293.
2. Mark Nitz; Bundle, D. In *NMR Spectroscopy of Glycoconjugates*; Jesús Jiménez-Barbero, T. P., Ed.; WILEY-VCH Verlag, GmbH & Co. KGaA: Weinheim, 2003; pp. 145–187.
3. See, for example: Jouault, T.; Lepage, G.; Bernigaud, a.; Trinel, P. a.; Fradin, C.; Wieruszeski -, J. M.; Strecker, G.; Poulain, D. *Infect. Immun.* **1995**, 63, 2378.
4. (a) Ranta, K.; Nieminen, K.; Ekholm, F. S.; Poláková, M.; Roslund, M. U.; Saloranta, T.; Leino, R.; Savolainen, J. *Clin. Vaccine Immunol.* **2012**, 19, 1889. (b) Mukherjee, C.; Ranta, K.; Savolainen, J.; Leino, R. *Eur. J. Org. Chem.* **2012**, 2012, 2957. (c) Mukherjee, C.; Mäkinen, K.; Savolainen, J.; Leino, R. *Chem. Eur. J.* **2013**, 19, 7961.
5. Mäkinen, K.; Mukherjee, C.; Leino, M.; Panchadhayee, R.; Lehto, M.; Wolff, H.; Alenius, H.; Leino, R.; Savolainen, J. *submitted for publication* (2015).

## The conformational free-energy landscape of $\beta$ -xylose reveals a two-fold catalytic itinerary for $\beta$ -xylanases

Javier Iglesias-Fernández<sup>1</sup>, Lluís Raich<sup>1</sup>, Albert Ardèvol<sup>2</sup> and Carme Rovira<sup>1,3</sup>

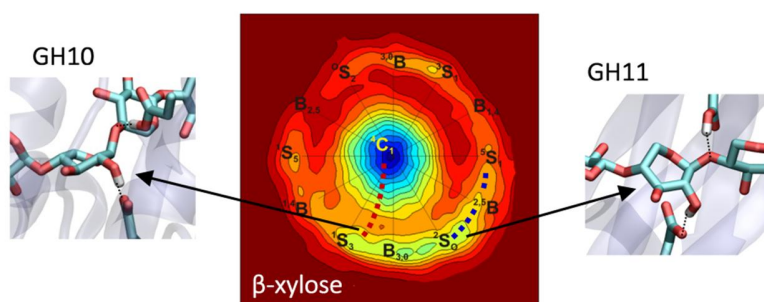
lraich@ub.edu

1. Department of Chemistry. University of Barcelona. Martí i Franquès 1. 08028 Barcelona.

2. ETH Zürich, USI Campus, 6900 Lugano, Switzerland.

3. Institució Catalana de Recerca i Estudis Avançats (ICREA), 08018 Barcelona, Spain.

Unraveling the conformational catalytic itineraries<sup>1</sup> of glycoside hydrolases (GHs) is a growing topic of interest in glycobiology, with major impact in the design of GH inhibitors.  $\beta$ -xylanases are responsible for the hydrolysis of glycosidic bonds in  $\beta$ -xylans, a group of hemicelluloses of high biotechnological interest that are found in plant cell walls. The precise conformations followed by the substrate during catalysis in  $\beta$ -xylanases have not been unambiguously resolved, with three different pathways being predicted from structural analyses. In this work, we compute the conformational free energy landscape (FEL) of  $\beta$ -xylose to predict the most likely catalytic itineraries followed by  $\beta$ -xylanases. The calculations are performed by means of *ab initio* metadynamics, using the Cremer-Pople puckering coordinates as collective variables.<sup>2</sup> The computed FEL supports only two of the previously proposed itineraries,  ${}^2S_0 \rightarrow [{}^{2,5}B]^\ddagger \rightarrow {}^5S_1$  and  ${}^1S_3 \rightarrow [{}^4H_3]^\ddagger \rightarrow {}^4C_1$ , which clearly appear in low energy regions of the FEL. Consistently,  ${}^2S_0$  and  ${}^1S_3$  are conformations preactivated for catalysis in terms of free energy/anomeric charge and bond distances. The results<sup>4</sup> however exclude the  ${}^0E \rightarrow [{}^0S_2]^\ddagger \rightarrow B_{2,5}$  itinerary that has been recently proposed for a family 11 xylanase.<sup>4</sup> Classical and *ab initio* QM/MM molecular dynamics simulations reveal that, in this case, the observed  ${}^0E$  conformation has been enforced by enzyme mutation. These results add a word of caution on using modified enzymes to inform on catalytic conformational itineraries of glycoside hydrolases.



### Literature

1. G. J. Davies, A. Planas and C. Rovira, *Acc. Chem. Res.* **2012**, 45, 308-316.
2. X. Biarnés, A. Ardèvol, A. Planas, C. Rovira, A. Laio and M. Parrinello, *J. Am. Chem. Soc.* **2007**, 129, 10686-10693.
3. J. Iglesias-Fernández, L. Raich, A. Ardèvol and C. Rovira, *Chem. Sci.* **2015**, 6, 1167-1177.
4. Q. Wan, Q. Zhang, S. Hamilton-Brehm, K. Weiss, M. Mustyakimov, L. Coates, P. Langan, D. Graham and A. Kovalevsky, *Acta Crystallogr. D* **2014**, 70, 11-23.

## Structural and biochemical characterization of endo-acting chondroitin AC lyase a family 8 polysaccharide lyase (PsPL8a) from *Pedobacter saltans* DSM 12145

Aruna Rani<sup>1</sup>, Joyeeta Mukherjee<sup>2</sup>, Munishwar N. Gupta<sup>2</sup> and Arun Goyal<sup>1</sup>

r.aruna@iitg.ernet.in

1. Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati – 781 039, India.
2. Department of Chemistry, Indian Institute of Technology Delhi, New Delhi, 110016, Delhi, India

Chondroitin sulphate (CS) is a sulphated glycosaminoglycan (GAG) composed of an unbranched chain of alternating sugars N-acetylgalactosamine and glucuronic acid. CS is the major polymeric component of animal extracellular matrix, acting as cell surface receptor. It is involved in cell signalling, cell proliferation, tumor growth and metastasis (1). The gene encoding PsPL8a (2034 bp) from *Pedobacter saltans* belonging to family 8 polysaccharide lyase was cloned, expressed and purified as soluble protein of molecular size ~77 KDa. Purified PsPL8a showed specificity towards both chondroitin 4 sulphate (C4S) and chondroitin 6 sulphate (C6S), showing it to be chondroitin AC lyase. PsPL8a was found to be endo-acting as determined by Thin Layer Chromatography and Mass Spectrometry analysis of degraded products of C4S. Protein melting studies by UV-Visible spectroscopy showed that protein starts melting at 45°C and completely melts at 60°C. Effect of chaotropic agents like urea and guanidine hydrochloride on structural integrity of PsPL8a was studied by fluorescence spectroscopy and a red shift was observed at 5 M Urea and 3 M GdHCl indicating loss of structural integrity. 3D structure of PsPL8a was developed by homology modeling using Modeller v9.14. PsPL8a sequence was aligned with best matched template of known chondroitin AC lyase with pdb id 1HM2 from *Flavobacterium heparinus* of PL8 family. Modeled structure was refined and energy minimization was carried out at 3D refine protein structure refinement server. The modeled structure of enzyme showed N-terminal domain containing predominantly  $\alpha$ -helices forming ( $\alpha/\alpha$ )<sub>6</sub> toroidal topology, while C-terminal domain contains predominantly  $\beta$  strands arranged in 4 layered  $\beta$ -sheet sandwich. The resulting structure was validated using the tools available on the SAVES and ProSA-web server. Ramachandran plot revealed that 98.5% of the residues lie in the allowed regions, 1.2% lies in the generously allowed regions while only 0.5% of the residues lie in the disallowed regions. Secondary structure elements were predicted by PSIPRED server which showed presence of  $\alpha$  helix (24.92%),  $\beta$  strands (21.68%) and rest, the coils. Far UV Circular Dichroism spectrum of PsPL8a, showed presence  $\alpha$  helix (27.31%),  $\beta$  strand (22.7%) and rest were the coils corroborating the predicted data. PsPL8a is the first chondroitin AC lyase reported from *Pedobacter saltans*.

### Literature

1. Denholm, E. M., Lin, Y. Q. and Silver, P. J. (2001) Anti-tumor activities of chondroitinase AC and chondroitinase B: Inhibition of angiogenesis, proliferation and invasion. Eur. J. Pharmacol. 416, 213-221.

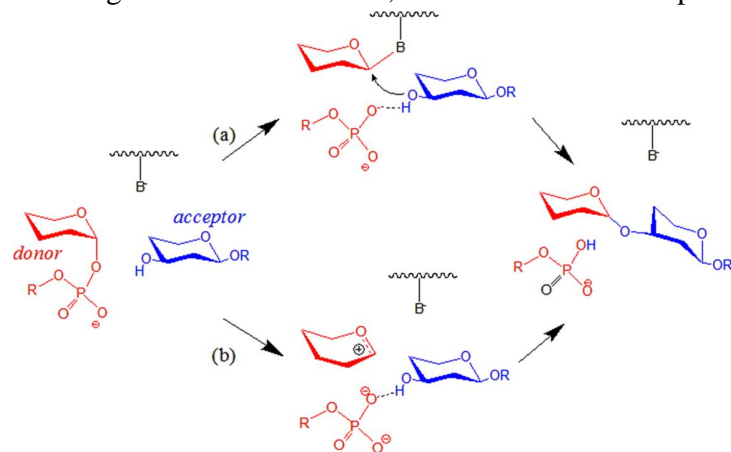
## Molecular mechanisms of retaining glycosyltransferases. Insight from QM/MM metadynamics simulations

Javier Iglesias-Fernández,<sup>1</sup> Albert Ardèvol,<sup>2</sup> Víctor Rojas-Cervellera,<sup>1</sup> Ramón Hurtado-Guerrero<sup>3</sup>, Antoni Planas<sup>4</sup> and Carme Rovira<sup>1,5</sup>

c.rovira@ub.edu

1. Department of Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.
2. ETH Zürich, USI Campus, 6900 Lugano, Switzerland.
3. BIFI - University of Zaragoza & Fundación ARAID. Ed. Pignatelli 36. 50018 Zaragoza, Spain.
4. Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390. 08017 Barcelona, Spain.
5. Institució Catalana de Recerca i Estudis Avançats (ICREA), 08018 Barcelona, Spain.

The catalytic mechanism of nucleotide-sugar dependent glycosyltransferases (GTs), especially those that act with retention of anomeric configuration, remains one of the intriguing unanswered questions in glycobiology. In contrast to the well-characterized mechanistic strategies used by glycoside hydrolases (GHs) to catalyze the cleavage of glycosidic bonds, the mechanisms of retaining GTs remain unclear, with both double displacement (a) and front-face (b) mechanisms



being proposed. By means of QM/MM metadynamics simulations, based on Density Functional Theory (DFT), we initially demonstrated that the front-face mechanism is feasible for trehalose-6-phosphate synthase (OtsA) a GT lacking a putative nucleophile residue on the beta face of the donor sugar, thanks to the formation of a short-lived oxocarbenium-ion-like species.<sup>1</sup> Similar results have been obtained for polypeptide GalNAc-transferase 2 (GalNAc-T2),<sup>3</sup> one of the key enzyme

isoforms controlling human protein O-glycosylation. In contrast, a GT with a putative nucleophile residue, such as  $\alpha$ 3-galactosyltransferase ( $\alpha$ 3GalT), was found to operate via a double-displacement mechanism, with the formation of a glycosyl-enzyme covalent intermediate.<sup>2</sup> A detailed picture of the atomic rearrangement during the complete reaction pathway will be provided and differences between the three enzymes will be analyzed.

### Literature

1. Ardèvol, A.; Rovira, C. *Angew. Chem. Int. Ed.* **2011**, 50, 10897-10901.
2. Rojas-Cervellera, V.; Ardèvol, A.; Boero, M.; Planas, A.; Rovira, C. *Chem. Eur. J.* **2013**, 42, 14018-14023.
3. E. Lira-Navarrete, J. Iglesias-Fernández, W. F. Zandberg, I. Compañón, Y. Kong, F. Corzana, B. M. Pinto, H. Clausen, J. M. Peregrina, D. Vocadlo, C. Rovira, R. Hurtado-Guerrero. *Angew. Chem. Int. Ed.* **2014**, 53, 8206-8210.

## Comparative analysis of transcriptomes and secretomes of the white-rot fungus *Dichomitus squalens* cultured in lignocellulosic substrates

*Johanna Rytioja*<sup>1</sup>, *Miaomiao Zhou*<sup>2,3</sup>, *Kristiina Hildén*<sup>1</sup>, *Marcos Di Falco*<sup>4</sup>, *Outi-Maaria Sietio*<sup>1</sup>, *Adrian Tsang*<sup>4</sup>, *Ronald P. de Vries*<sup>2,3</sup> and *Miia R. Mäkelä*<sup>1</sup>

johanna.rytioja@helsinki.fi

1. Food and Environmental Sciences, University of Helsinki, Helsinki, Finland
2. Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
3. Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands
4. Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada

White-rot fungi are essential organisms in the degradation of plant biomass and thus play an important role in the global carbon cycle. In addition to their ability to mineralize the aromatic lignin polymer, white-rot fungi produce enzymes that degrade plant cell wall polysaccharides into oligosaccharides and monomeric sugars that they can use as carbon source. These enzymes are important tools for biotechnology, as the products of their catalysis can be used as precursors of bio-based fuels and chemicals.

*Dichomitus squalens* is a white-rot basidiomycete capable of efficient cellulose and lignin degradation on softwood. The focus of this work was to dissect the enzyme combinations used by the *D. squalens* in the degradation of different types of plant-derived biomass. For that, *D. squalens* was grown on hardwood, softwood, and residues from monocots and dicots as carbon sources and sampled at two time points for transcriptome and secretome analyses. Extracellular plant biomass degrading enzyme activity profiles were also determined during the growth of *D. squalens* on these plant biomass materials. These analyses provide an in-depth appreciation of the mechanisms used by *D. squalens* in the degradation of diverse plant-derived biomass. Further, these results are also expected to enhance the discovery and use of novel enzymes in biotechnological applications.

### Acknowledgements

Funding from the Finnish Cultural Foundation (to J.R.) is acknowledged.

### Literature

1. Rytioja, J., Hildén, K., Yuzon, J., Hatakka, A., De Vries, R.P., Mäkelä, M.R. Plant-polysaccharide-degrading enzymes from basidiomycetes (2014) Microbiology and Molecular Biology Reviews, 78 (4), pp. 614-649.



## Biochemical characterization and crystal structure of a novel GH127 $\beta$ -L-arabinofuranosidase

*Rachel Salama*<sup>1</sup>, *Shifra Lansky*<sup>2</sup>, *Ruth Goldschmidt*<sup>1</sup>, *Gil Shoham*<sup>2</sup> and *Yuval Shoham*<sup>1</sup>

salamar@tx.technion.ac.il

1. Faculty of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Haifa, Israel.
2. Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

Arabinan is a branched polysaccharide and is part of pectin, one of the components in the plant cell wall. The arabinan backbone consists of  $\alpha$ -1,5-linked L-arabinofuranosyl units, and is decorated mainly with  $\alpha$ -1,2- and  $\alpha$ -1,3-linked arabinofuranosides. Unlike the widespread  $\alpha$  configuration of arabinofuranosyl,  $\beta$ -arabinofuranosyl residues are relatively rare in native polysaccharides.

*Geobacillus stearothermophilus* T-6 is a thermophilic soil bacterium that possesses an extensive system for the utilization of L-arabinan. The system is composed of five transcriptional units, one of which contains eleven genes (*abnEFJ-abnA-abfBA-araJKLMN*). This operon encodes for an arabino-oligosaccharides transporter system, two  $\alpha$ -L-arabinofuranosidases (Abf51A and Abf51B), and several genes encoding for an alternative arabinose utilization pathway. The last gene in the operon, *ara127N*, encodes for a  $\beta$ -L-arabinofuranosidase and shows activity towards both synthetic and natural substrates containing  $\beta$ -L-arabinofuranoside residues. The  $k_{\text{cat}}$  value of Ara127N towards pNP- $\beta$ -L-arabinofuranoside in pH 6.5 is  $1.5 \text{ sec}^{-1}$ ,  $K_m$  is 0.57 mM and the calculated value of  $k_{\text{cat}}/K_m$  is  $2.6 \text{ sec}^{-1} \cdot \text{mM}^{-1}$ . The crystal structure of Ara127N has recently been determined (at 2.30 Å resolution) and it appears to have a metal-binding motif with  $\text{Zn}^{2+}$  coordinated by glutamate and cysteines in the active site. Inductive coupled plasma (ICP) measurements show that Ara127N contains 0.8 mol of zinc per mol of enzyme. Amino acid sequence analysis revealed three conserved cysteine residues, Cys329, Cys406 and Cys407 that could be involved in metal binding. Preliminary results demonstrated that a single mutation of Cys406Ala abolished metal binding and affected the catalytic activity, suggesting the involvement of the metal in the catalytic mechanism.

### Literature

1. Lansky, S., Salama, R., Dann, R., Shner, I., Manjasetty, B. A., Belrhali, H., Shoham, Y. & Shoham, G. (2014). Cloning, purification and preliminary crystallographic analysis of Ara127N, a GH127 beta-L-arabinofuranosidase from *Geobacillus stearothermophilus* T6. *Acta Crystallogr F Struct Biol Commun* 70, 1038-45.

## Xylooligosaccharides (XOs) from xylan extracted from quinoa (*Chenopodium quinoa*) stalks

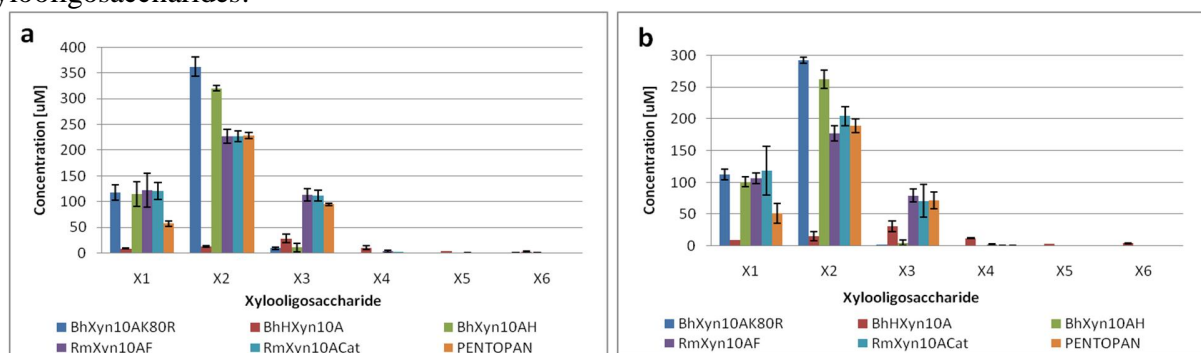
*Daniel Martin Salas-Veizaga*<sup>1,2</sup>; *Javier Linares-Pastén*<sup>1</sup>; *Teresa Álvarez-Aliaga*  
and *Eva Nordberg-Karlsson*<sup>1</sup>

daniel\_m.v\_salas@biotek.lu.se

1. Biotechnology, Department of Chemistry, P.O. Box 124, 22100 – Lund, Sweden

2. Instituto de Investigaciones Fármaco Bioquímicas, UMSA – La Paz, Bolivia

Xylooligosaccharides (XOs) were obtained from xylan, extracted from alkaline pretreated, milled Quinoa stalks through the action of thermostable xylanases. Alkaline pre-treatment with NaOH (2%) resulted in a total yield of 10.853 g of xylan/100 g raw-material, of which 62% and 38 % was obtained between soluble and insoluble fractions respectively. Monosaccharides analysis of the extracted xylan showed the major components to be xylose:arabinose in a molar ratio of 12.3:1. Six different xylanases variants including Xyn10AK80R, HXyn10A, Xyn10AH from *Bacillus halodurans*, Xyn10AF, Xyn10ACat from *Rhodotermus marinus* and PENTOPAN<sup>®</sup> were evaluated for their ability to transform the soluble/insoluble xylan in water or buffer to XOs with degree of polymerization (DP) in the range 2 – 6. The major XOs obtained with 5 of the 6 thermostable xylanases were xylobiose (X2) and xylotriose (X3). The major XOs product using Xyn10AK80R and Xyn10AH was X2, while treatment with Xyn10AF, Xyn10ACat and PENTOPAN<sup>®</sup> resulted X3 from both soluble and insoluble xylan fractions. XOs with DP 4 to 6 were only produced in low amounts, using all enzymes. The enzyme variant HXyn10A produced all XOs, but in low or negligible concentrations. Although XOs production from both soluble and insoluble xylan followed the same trend, there was a significant difference ( $p < 0.05$ ) between the concentration of XOs obtained in the respective xylan fraction, with higher yields of X2 and X3 using soluble xylan. In contrast, reactions run in buffer or water did not show a significant difference ( $p > 0.05$ ), showing that either water or buffer reaction media are good for these enzymes. In conclusion, XOs were successfully obtained using thermostable xylanases acting on xylan extracted from Quinoa stalks; a novel agricultural waste which could be a useful material for production of prebiotic xylooligosaccharides.



**Fig 1.** Xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6) obtained after reaction with thermostable xylanases on soluble (a) and insoluble (b) xylan. *B. halodurans*: Xyn10AK80R, HXyn10A, Xyn10AH; *R. marinus*: Xyn10AF, Xyn10ACat and PENTOPAN<sup>®</sup>.

### Acknowledgements

The Swedish International Development Cooperation Agency (SIDA) is thanked for financial support.

## Conformational study on homoallylic polyol derived from D-mannose

*Tiina Saloranta,<sup>1</sup> Anssi Peuronen,<sup>2</sup> Johannes Dieterich,<sup>3</sup> Manu Lahtinen,<sup>2</sup> Reko Leino<sup>1</sup>*

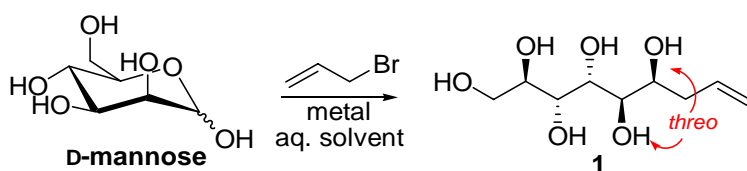
tiina.saloranta@abo.fi

1. Laboratory of Organic Chemistry, Åbo Akademi University, Turku, Finland

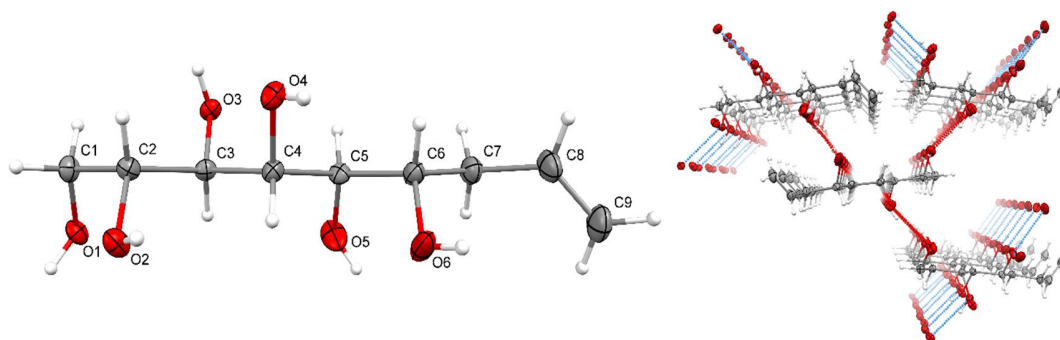
2. Department of Chemistry, University of Jyväskylä, Jyväskylä, Finland

3. Institut für Physikalische Chemie, Universität Göttingen, Göttingen, Germany

Terminally unsaturated and diastereomerically pure polyol derived from D-mannose shows spontaneous aggregation behavior in water solution. In order to clarify this unforeseen phenomenon, conformational study based on NMR spectroscopic studies was pursued. Ab initio geometry optimizations using the COSMO-solvation model were used to quantify the conformational effects observed in the NMR analysis. The recently performed X-ray diffraction studies provided new insights into the conformation and aggregation behavior. In this poster, we will describe our twisting path with polyol **1** from visual observation in the laboratory towards detailed conformational analysis.



**Fig 1.** Synthesis of diastereomerically pure polyol **1** from D-mannose



**Fig 2.** Left: asymmetric unit of crystal structure of **1**. Right: intermolecular hydrogen bonding pattern (dotted lines) observed in crystal structure of **1** and viewed along crystallographic *c*-axis.

## Protein stability engineering by structure-guided chimeragenesis

Mats Sandgren<sup>1</sup>, Nils Mikkelsen<sup>1</sup>, Saeid Karkehadadi<sup>1</sup>, Henrik Hansson<sup>1</sup>, Mikael Gudmundsson<sup>1</sup>, Igor Nikolaev<sup>2</sup>, Sergio Sunux<sup>3</sup>, Amy Liu<sup>3</sup>, Rick Bott<sup>3</sup>, Thijs Kaper<sup>3</sup>

mats.sandgren@slu.se

1. Swedish Agricultural University, Uppsala, Sweden

2. Dupont Industrial Biosciences, Leiden, Netherlands

3. Dupont Industrial Biosciences, Palo Alto, CA, USA

$\beta$ -glucosidases are key enzymes during conversion of plant biomass glucans to the major final degradation product D-glucose. We have identified a GH family 3  $\beta$ -glucosidase from *Fusarium verticilloides* (Fv3C) that displays good performance on a panel of biomass substrates. However, when this enzyme was expressed in *Trichoderma reesei*, this molecule was proteolytically clipped. MS analysis, N-terminal sequencing, and X-ray crystallography characterizations showed that the protein got clipped within the C-terminal third domain of the molecule resulting in a significant loss of activity performance. Attempts to fix the integrity of the Fv3C molecule locally, including site-specific mutations, loop replacement or deletions, linker or disulfide bridge insertions, and removal or introduction of N-linked glycosylation sites, were unsuccessful. Alternatively, we applied a “global” approach via interchanging regions between Fv3C and homologs. Replacing the C-terminal domain of Fv3C repaired the clip site, but surprisingly created another one in a loop upstream of the previous clip site. This loop is specific only to *Fusarium* GH3  $\beta$ -glucosidases, and shortening this sequence to a single Gly residue, or to a corresponding region from a homologous GH3 enzyme led to new and improved molecules that are produced in intact form by *T. reesei*. The three-molecule hybrid performed well and remained stable throughout extended saccharification assays.

## Evaluation of microbial production of exopolysaccharide by *Rhodothermus marinus* strains: potential for industrial biotechnology

Roya R.R. Sardari<sup>1</sup>, Evelina Kulcinskaja<sup>2</sup>, and Eva Nordberg Karlsson<sup>1</sup>

Roya.Rezaei @biotek.lu.se

1. Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, 22100 Lund, Sweden
2. Biochemistry and Structural Biology, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund, Sweden

The formation of extracellular polysaccharides (EPS) by *Rhodothermus marinus* DSM 4252 and *Rhodothermus marinus* 493, two wild type species of thermophilic bacteria has been screened in different culture media. Marine broth containing either 1 or 10 g/l of glucose, sucrose, lactose, and maltose, separately was used. The results showed that these two strains have the ability to produce and release the EPS. Marine broth containing 10 g/l lactose showed the highest production of EPS in both strains. Besides, EPS production was mainly during the stationary phase. The monosaccharide composition of the produced exopolysaccharides was analyzed and quantified. The results suggested a heteropolymer structure for produced EPS of both strains. The most abundant monomers were xylose, arabinose, and mannose in all media in both strains. Also, the presence of glucose, galactose, and raffinose was varied depending on the type of media used for production of EPS. The EPS of *R. marinus* DSM 4252 included high quantity of xylose, while in *R. marinus* 493 arabinose had the highest amount compared to the other produced monosaccharides. The molecular mass of produced EPS by these two strains was determined by size exclusion chromatography technique using Sephacryl S-200 and Sephacryl S-500 columns. These results lead us for further studies aimed at increasing the interest in the application of produced EPS of *Rhodothermus marinus* in food, pharmaceutical, and wastewater treatment industries by optimizing the condition for high production of EPS and characterizing its physicochemical properties.

### Acknowledgements

The authors are grateful to Seabiotek (EU FP7) for the financial support to the project.

## Characterization of two trisaccharides produced in the presence of lactose by *Weissella confusa* dextranucrase

Qiao Shi<sup>1</sup>, Minna Juvonen<sup>1</sup>, Yaxi Hou<sup>1</sup>, Ilkka Kajala<sup>2</sup>, Antti Nyssölä<sup>2</sup>, Ndegwa Henry Maina<sup>1</sup>, Hannu Maaheimo<sup>2</sup>, Liisa Virkki<sup>1</sup>, Maija Tenkanen<sup>1</sup>

qiao.shi@helsinki.fi

1. Department of Food and Environmental Sciences, P.O. Box 27, FI-00014 University of Helsinki, Finland,  
2. VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland

Many lactic acid bacteria are able to synthesize polymeric glucans or fructans from sucrose. Dextran-producing *Weissella* species have received recently increasing attention due to their potential in food fermentations [1], especially in sourdough production. However, in the presence of suitable acceptors, such as disaccharides, oligosaccharides are produced in addition to elongating polysaccharide. These acceptor reactions of *Weissella* dextranucleases have not been investigated except for maltose, which is the best known acceptor. Lactose is naturally present in milk. Thus it is of interest to identify the acceptor products which may be formed during fermentation of milk-based foods by *Weissella* strains.

The main oligosaccharides produced by the recombinant *W. confusa* VTT E-90392 dextranuclease [2] in the presence of lactose and sucrose were studied in this work. Beside the main product, interestingly another oligosaccharide was observed in the HPAEC-PAD chromatogram. The two products were isolated by gel filtration and subject to detailed characterization. They were both trisaccharides, and their structures were elucidated by NMR spectroscopy as 2<sup>Glc</sup>- $\alpha$ -Glc<sub>p</sub>-lactose and isomelezitose (6<sup>Fru</sup>- $\alpha$ -Glc<sub>p</sub>-sucrose), respectively. The products were also analyzed by ESI-MS/MS. The further use of labeled <sup>13</sup>C<sup>Glc</sup> sucrose in the reaction enabled the detailed identification of fragmentation pattern of these two trisaccharides in the positive and negative ion MS<sup>n</sup> analysis.

2<sup>Glc</sup>- $\alpha$ -Glc<sub>p</sub>-lactose has earlier been identified as the lactose acceptor product of *L. mesenteroides* B-512F dextranuclease, and has shown potential as prebiotics. However, the dextranuclease-catalyzed synthesis of isomelezitose has not been reported before. The fragmentation pathways of the two trisaccharides in MS<sup>n</sup> are studied for the first time. The information obtained will be useful for future LC-MS/MS characterization of oligosaccharides of similar structure.

**Acknowledgements** This study was supported by the Academy of Finland (MT) via WISEDextran project, the ABS Graduate School (QS) and the Raisio plc Research Foundation (MJ) in Finland.

### Literature

1. Juvonen R. *et al.* The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (*Daucus carota* L). International Journal of Food Microbiology. In press.
2. Kajala I. *et al.* (2015) Cloning and Characterization of a *Weissella confusa* Dextranuclease and Its Application in High Fibre Baking. PLoS ONE 10(1): e0116418. doi:10.1371/journal.pone.0116418.

## **$\alpha$ -L-Fucosidase from *Fusarium proliferatum* LE1: specificity and transglycosylation abilities**

Svetlana V. Shvetsova<sup>1,2</sup>, Kirill S. Bobrov<sup>1</sup>, Konstantin A. Shabalin<sup>1</sup>, Olga L. Vlasova<sup>2</sup>, Elena V. Eneyskaya<sup>1</sup>, Anna A. Kulminskaya<sup>1,2</sup>

shvetsova@omrb.pnpi.spb.ru

1. National Research Center «Kurchatov Institute», B.P. Konstantinov Petersburg Nuclear Physics Institute, Gatchina, Russia
2. Medical Physics Department, St. Petersburg State Polytechnical University, St. Petersburg, Russia

Oligosaccharidic chains of many L-fucose-containing glycoconjugates involved in many vital biochemical events. Fucosylated glycans play important roles in various physiological and pathological processes [1]. One possible approach to study the role of fucosylation is the release of L-fucose by specific enzyme such as  $\alpha$ -L-fucosidase. Additionally, the ability of some of these enzymes to catalyze the transfucosylation reactions indicates the potential to use them in biotechnology.

Recently, we have discovered and identified a new strain of filamentous fungus *Fusarium proliferatum* LE1 (deposition number in Russian Collection of Agricultural Microorganisms RCAM02409), which is a producer of  $\alpha$ -L-fucosidase [2]. This strain appeared to highly produce  $\alpha$ -L-fucosidase when grown in a medium containing L-fucose, which is the reaction product of the enzyme. As a result of four chromatographic stages, we obtained a purified enzyme with a molecular mass of about 60 kDa. The  $\alpha$ -L-fucosidase exhibits predominant selectivity towards  $\alpha 1 \rightarrow 4$ -O-fucosyl bonds in difucosaccharides with different types of linkages; hydrolyzes *p*-nitrophenyl  $\alpha$ -L-fucopyranoside (*p*NPFuc) with  $K_m = 1.1 \pm 0.05$  mM and  $k_{cat} = 35.7 \pm 1.8$  s<sup>-1</sup>. L-fucose was found to be a competitive inhibitor of hydrolysis of *p*NPFuc with the inhibition constant  $K_I = 0.2 \pm 0.05$  mM. As a result of NMR analysis of the stereochemical course of the enzyme-catalyzed *p*NPFuc hydrolysis, we observed retention mechanism of the  $\alpha$ -L-fucosidase action.  $\alpha$ -L-Fucosidase from *F. proliferatum* LE1 was able to catalyze fucosylation of aliphatic alcohols but no transglycosylation products were found in the reaction with *p*NPFuc as a donor and an acceptor. Analysis of sequenced gene encoding the synthesis of  $\alpha$ -L-fucosidase from *F. proliferatum* LE1 revealed classification of the enzyme to the glycosyl hydrolase family 29 (The Carbohydrate-Active Enzymes database (CAZy; <http://www.cazy.org>) [3].

### **Literature**

1. Becker DJ, Lowe JB. 2003. Fucose: Biosynthesis and biological function in mammals. *Glycobiology*. 13(7):41R-53R.
2. Shvetsova SV, Zhurishkina EV, Bobrov KS, Ronzhina NL, Lapina IM, Ivanen DR, Gagkaeva TY, Kulminskaya AA. 2014. The novel strain *Fusarium proliferatum* LE1 (RCAM02409) produces  $\alpha$ -L-fucosidase and arylsulfatase during the growth on fucoidan. *J Basic Microbiol*. 54, 1-10.
3. Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acid Res*. 42, 490–495.

## Hemicellulases in total hydrolysis of wood-based substrates

Matti Siika-aho<sup>1</sup>, Anikó Várnai<sup>1,2,3</sup>, Jaakko Pere<sup>1</sup>, Kaisa Marjamaa<sup>1</sup> and Liisa Viikari<sup>2</sup>

matti.siika-aho@vtt.fi

1. VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, FI-02044 VTT, Finland
2. Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, 00014 Helsinki, Finland
3. Present address: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, 1432 Aas, Norway

Woody raw materials are greatly potential renewable sources for fuels and chemicals, which can be produced using various chemical and biochemical routes. In recent years there has been increasing global interest to use these feedstocks as raw materials for microbial fermentations in bioproduction. This so called sugar route is based on efficient hydrolysis of natural biopolymers to sugars which is in most cases carried out by enzymes. Developing efficient enzyme mixtures requires knowledge of the limiting and enhancing factors in the action of hydrolytic enzymes. In this work we studied the role of hemicellulose-hydrolyzing enzymes in the mixtures, and simultaneously gained information of the role of hemicellulose when using woody raw materials as source of monosaccharides for sugar route.

Various woody raw materials (spruce chemical pulp, spruce pretreated either by steam explosion or catalytic oxidation, nanofibrillated cellulose and crystalline softwood cellulose) were studied in hydrolysis using designed enzyme mixtures. The enzymes used were purified preparations from fungi *Trichoderma reesei* (TrCel5A, TrCel6A, TrCel7A, TrCel7B, TrMan5A, TrXyn11) or *Aspergillus niger* (a GH3  $\beta$ -glucosidase). The effect of the mixture composition on the release of carbohydrates from the substrates was analyzed. The adsorption of the single components of the mixture on several substrates was also analyzed and will be discussed in relation to hydrolysis efficiency.

The results showed the great importance of hemicellulolytic enzymes in total hydrolysis of wood-based cellulosic substrates. On the other hand, the results also indicated that a part of hemicellulose is located deeply embedded in the cellulosic matrix, and evidently prevents efficient enzymatic saccharification of wood-based cellulose, unless processed by additional corresponding enzymes. Both cellulose and lignin fraction of biomass seemed to adsorb hemicellulases during hydrolysis.

**Acknowledgements:** Funding from Tekes – the Finnish Funding Agency for Innovation (for SugarTech project) and Finnish Cultural Foundation (for A. Várnai's PhD work) is greatly acknowledged.

### Literature:

1. Várnai, A; Huikko, L.; Pere, J; Siika-aho, M; Viikari, L. 2011. Synergistic action of xylanase and mannanase improves the total hydrolysis of softwood. *Bioresource Technology*, vol. 102, 19, ss. 9096-9104, doi:10.1016/j.biortech.2011.06.059
2. Várnai, A; Viikari, L; Marjamaa, K; Siika-aho, M. 2010. Adsorption of monocomponent enzymes in enzyme mixture analyzed quantitatively during hydrolysis of lignocellulose substrates. *Bioresource Technology*, vol. 102, 2, ss. 1220-1227, doi:10.1016/j.biortech.2010.07.120.



## Structure-function relationships in the active site of the blue mussel $\beta$ -mannanase *MeMan5A*

*Johan Svantesson Sjöberg<sup>1</sup>, Viktoria Bågenholm<sup>1</sup> and Henrik Stålbrand<sup>1</sup>*

johan.svantesson\_sjoberg@biochemistry.lu.se

1. Department of Biochemistry and Structural Biology, Center for Molecular Protein Science, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

Hemicelluloses are important components of lignocellulosic biomass, and their enzymatic degradation has great promise for substituting fossil-based products with renewable bio-based alternatives. A variety of enzymes are required to efficiently degrade these hemicelluloses. One such enzyme type is  $\beta$ -mannanases, which hydrolyze the backbone of  $\beta$ -1,4-mannans. Some  $\beta$ -mannanases are also able to synthesize new mannosidic bonds or transfer other molecules onto manno oligosaccharides, through a process called transglycosylation. In addition to their innovative potential, studying  $\beta$ -mannanases is also important from a biological perspective, since many organisms use  $\beta$ -mannanases in order to utilize mannans as a source of nutrients. The blue mussel *Mytilus edulis* is one such organism, having a  $\beta$ -mannanase (*MeMan5A*) in its digestive tract. Previous work [1] suggests that *MeMan5A* is highly efficient in degrading mannan-based substrates, and that it may also be able to perform transglycosylation. The +2 sugar-binding subsite seems to be particularly important for these properties. In the current work, two tryptophans in the +2 subsite of *MeMan5A* were substituted with alanines, leading to a reduction in both hydrolytic efficiency and transglycosylation capacity, as well as affecting substrate binding in the active site. This is consistent with studies of other glycoside hydrolases, where aglycone (+) subsites were also found to be important for hydrolysis and transglycosylation [2, 3]. The effect of substituting both tryptophans was greater than either residue by itself, possibly suggesting some degree of cooperativity between these two residues in the +2 subsite of *MeMan5A*. Future work intends to study the structural basis of the observed effects using nuclear magnetic resonance (NMR). Such studies of structure-function relationships in  $\beta$ -mannanases could enable fine-tuning enzyme properties to create the most efficient  $\beta$ -mannanase for any given application.

### Literature

1. Larsson AM, Anderson L, Xu B, Muñoz IG, Usón I, Janson JC, Stålbrand H, Ståhlberg J. Three-dimensional crystal structure and enzymic characterization of  $\beta$ -mannanase Man5A from blue mussel *Mytilus edulis*. *J Mol Biol.* 2006; 357: 1500-1510.
2. Rosengren A, Hägglund P, Anderson L, Pavon-Orozco P, Peterson-Wulff R, Nerinckx W, Stålbrand H. *Biocatal Biotransform.* 2012; 30: 338-352.
3. Armand S, Andrews SR, Charnock SJ, Gilbert HJ. Influence of the aglycone region of the substrate binding cleft of *Pseudomonas* xylanase 10A on catalysis. *Biochemistry.* 2001; 40: 7404-7409.

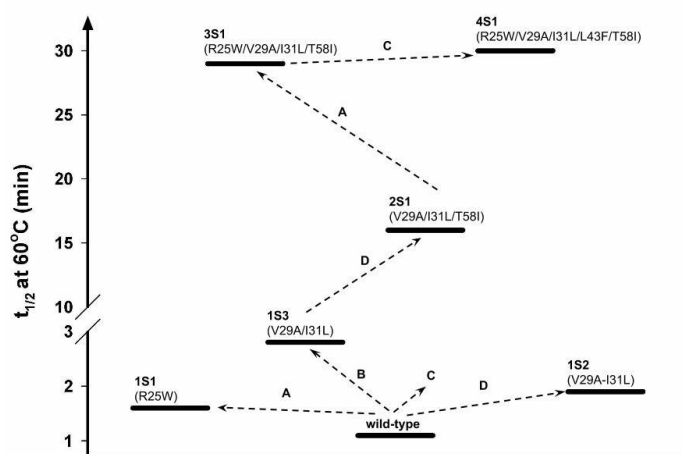
## Engineering a thermostable fungal GH10 xylanase, importance of N-terminal amino acids

*Letian Song*<sup>1,2</sup>, *Adrian Tsang*<sup>1</sup> and *Michel Sylvestre*<sup>2</sup>

michel.sylvestre@iaf.inrs.ca

1. Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke St. W., Montreal, QC H4B 1R6, Canada
2. Institut National de la Recherche Scientifique, INRS-Institut Armand-Frappier, Laval, QC H7V 1B7, Canada

Xylanases are used in many industrial processes including pulp bleaching, baking, detergent and the hydrolysis of plant cell wall in biofuels production. In this work we have evolved a single domain GH10 xylanase, Xyn10A\_ASPNG, from *Aspergillus niger* to improve its thermostability. We introduced a rational approach involving as the first step a computational analysis to guide the design of a random mutagenesis library in targeted regions which identified thermal important residues that were subsequently randomly mutagenized through rounds of iterative saturation mutagenesis (ISM). Focused on five mutational positions, four rounds of ISM have generated a quintuple mutant 4S1 (R25W/V29A/I31L/L43F/T58I) which exhibited thermal inactivation half-life ( $t_{1/2}$ ) at 60°C that was prolonged by 30 folds in comparison with wild-type enzyme. Furthermore, the mutant melting temperature ( $T_m$ ) increased by 17.4°C compared to the wild type. Each of the five mutations in 4S1 was found to contribute to thermoresistance, but the dramatic improvement of enzyme thermoresistance of 4S1 was attributed to the synergistic effects of the five mutations. Comparison of biochemical data and model structure between 4S1 and the wild-type enzyme suggested that the N-terminal coil of the enzyme is important in stabilizing GH10 xylanases structure. Based on model structure analyses, we propose that enforced hydrophobic interactions within N-terminal elements and between N- and C-terminal ends are responsible for the improved thermostability of Xyn10A\_ASPNG.



**Fig 1.** Stepwise increase in Xyn10A\_ASPNG thermostability ( $t_{1/2}$  60°C) along with the process of iterative saturation mutagenesis. The letters A, B, C and D correspond to saturation mutagenesis randomized at position 25, 29/31, 43 and 58, respectively. The best hits arising from each mutant library were indicated in the figure.

## Development of mannuronan C-5 epimerases to perform *in vitro* tailoring and upgrading of alginates

Annalucia Stanisci<sup>1</sup>, Finn Lillelund Aachmann<sup>1</sup>, AnneTøndervik<sup>2</sup>, Håvard Sletta<sup>2</sup>, Gudmund Skjåk-Bræk<sup>1</sup>

annalucia.stanisci@ntnu.no

1. NTNU, Department of Biotechnology
2. SINTEF, Materials and Chemistry, Department of Bioprocess Technology

Alginate is a linear polysaccharide composed of  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) and it has a wide range of industrial, medical and pharmaceutical applications. The bacterium *Azotobacter Vinelandii* encodes a family of seven secreted and calcium ion-dependent mannuronan C-5 epimerases (algE1-algE7) that convert mannuronic acid residues (M) in the alginate chain to guluronic acid residues (G). These epimerases consist of two types of structural modules, A-module which contains the catalytic site of the enzyme and R-module which is involved in substrate and calcium binding, increasing the activity of the enzyme. Through the engineering of new hybrid mannuronan C-5 epimerases constituted by the A-module from algE6 and the R-module from algE4, new and different epimerization patterns are observed. Defined manipulation with the sequences of new enzymes and modifying the linkage zone between the A- and R- module of these innovative epimerases lead to a better comprehension of the 'mode of action' of the epimerases and open up greater avenues for developing more efficient epimerases that can be used to generate new alginate structures of relevant interest for different applications.

### Acknowledgements

RCN BioTek2021 MARPOL # 221576 for financial support

## Using alginate milk protein complexes for model foods to investigate how food structure affects satiety

*Emil G. P. Stender<sup>1</sup>, Maher Abou Hachem<sup>1</sup>, Per Häggglund<sup>1</sup>, Richard Ipsen<sup>2</sup> and Birte Svensson<sup>1</sup>*

emigst@bio.dtu.dk

1. Technical University of Denmark (DTU) – Department of Systems Biology, Enzyme and Protein Chemistry, Søtoft Plads, Building 224, DK-2800 Kgs. Lyngby
2. University of Copenhagen - Department of Food Science, Rolighedsvej 30, DK-1958 Frederiksberg C

Obesity is an increasing problem in modern society. Food designed for weight management usually has reduced energy content as compared to their conventional counterparts. The structure of food may increase intraluminal viscosity affecting subjective satiety and energy intake. (1) Here it is hypothesized that foods with similar energy content (isoenergetic) but with different structures can have different influence on satiety. To investigate this hypothesis, the macromolecular interactions of pure whey proteins and alginate will be characterized using Isothermal Titration Calorimetry, Differential Scanning Calorimetry, Surface Plasmon Resonance, Size Exclusion Chromatography and Dynamic Light Scattering. Alginate is an indigestible polysaccharide composed of (1→4)-β-D-mannuronic acid and α-L-guluronic acid (2) that is extensively used in the food and pharmaceuticals industry for its gelling and stabilising properties, as well as its high viscosity. (3;4) The results obtained will be used to design model foods that will be tested by *in vitro* digestion. The goal is to create a toolbox of very well characterized isoenergetic model foods both in terms of the macromolecular interactions in the food matrix, texture and microstructure as well as *in vitro* digestion pattern. These model foods will then subsequently be used in the project StrucSat (<http://strucsat.ku.dk/>) to clarify how food structure *per se* influences satiety and energy uptake.

### Literature

1. Lawton, C. L., Walron, J., Hoyland, A., Howarth, E., Allan, P., Chesters, D., and Dye, L. (2013) *Nutrients* 5, 1436-1455
2. Haug, A., Larsen, B., and Smidrod, O. (1966) *Acta Chemica Scandinavia* 20, 183-&.
3. Ci, S., Huynh, T., Luie, L., Yang, A., Beals, B. J., Ron, N., Tsang, W. G., Soonshiong, P., and Desai, N. P. (1999) *Journal of chromatography A* 864, 199-210.
4. Johnson, F. A., Craig, D. Q. M., Mercer, A. D., and Chauhan, S. (1997) *International Journal of Pharmaceutics* 159, 35-42.

## Structural enzymology and engineering of $\beta$ -mannanases and $\alpha$ -galactosidases for galactomannan modification

Anna Rosengren, Evelina Kulcinskaja, Johan Svantesson Sjöberg, Sumitha Reddy, Anna Aronsson, Viktoria Bågenholm, Oskar Aurelius, Derek Logan, and Henrik Stålbrand

Henrik.stalbrand@biochemistry.lu.se

Biochemistry and Structural Biology, Lund university, Sweden

$\beta$ -Mannans are abundant, notably present as the major soft-wood hemicellulose (galactoglucomannan, GGM) which can be isolated from industrial sidestreams (1). Another  $\beta$ -mannan is dietary galactomannan (GM) from guar applied as a thickener in food. We are studying the structure, function and applications of enzymes that are responsible for the hydrolysis of GGM and GM (2, 3, 4, 5). These enzymes are the retaining glycoside hydrolases (GHs)  $\alpha$ -galactosidase and  $\beta$ -mannanase. The enzymes in the study originate from different environments such as soil bacteria and fungi, marine organisms and human gut bacteria. The different origins and cellular locations reflect the enzyme function (e.g. mode of attack, heteroglycan recognition and product profiles). We have recently discovered and characterized  $\beta$ -mannanases and  $\alpha$ -galactosidases from gut bacteria. A cell-attached GH family 26  $\beta$ -mannanase from *Bifidobacterium adolescentis* carries a GM-binding carbohydrate-binding module (CBM) of family 23 (5). The abundance of bifidobacteria increased in mice and rats fed with partially hydrolysed GM, showing the bifidogenic effect of this type of processed dietary fiber (6, 7). A loop deletion in a GH36  $\alpha$ -galactosidase appear to be responsible for the unusually efficient hydrolysis of galactose substituents from GM. This is potentially applicable to alter the properties of mannan-based hydrogels (8) for drug-delivery applications.

$\beta$ -Mannanase catalysed transglycosylation reactions can also be used to modify  $\beta$ -mannans. The transglycosylation efficiency, however, varies between homologous  $\beta$ -mannanases of the TIM-barrel clan GH-A. Certain  $\beta$ -mannanases are efficient in synthesising alkyl glycoside conjugates (9) with potential applications as environmentally friendly tensides. Our investigations include structure-based protein engineering to alter the functionality of  $\beta$ -mannanases as well as the development of novel isotope labelling/mass spectrometry methods to analyse product formation (4). Systematic studies of clan GH-A  $\beta$ -mannanases show the importance and specificity of acceptor binding, notably in the the +2 subsite. We are thus determining the structural characteristics for acceptor-binding of  $\beta$ -mannanases which are efficient in synthesising alkyl glycosides and others that are efficient in the synthesis of oligosaccharides (9) potentially applicable as prebiotics. Ongoing work focus on protein engineering of  $\beta$ -mannanases for specific synthetic applications.

### Literature

1. Andersson A, Persson T et al. Appl Bioch Biotech 137 971 (2007)
2. Larsson A, Anderson L et al. J Mol Biol 357 1500 (2006)
3. Couturier M, Roussel A, Rosengren A et al JBC 79 133 (2013)
4. Rosengren A, Hägglund P, Anderson L et al Biocat Biotransf 30 338 (2012)
5. Kulcinskaja E, Rosengren A et al Appl Env Microbiol 79 133 (2013)
6. Berger K, Falck P et al J Agr Food Chem 62 8169 (2014)
7. Fåk F, Jakobsdottir G, Kulcinskaja E et al submitted (2015)
8. Andersson-Roos A, Edlund U et al Biomacromol 9 2104 (2008)
9. Rosengren A, Reddy S, Sjöberg JS Appl Microb Biotech 98 10091 (2014)

## Structural and biochemical studies of sugar beet $\alpha$ -glucosidase exhibiting high specificity for long-chain substrates

*Takayoshi Tagami*<sup>1</sup>, *Keitaro Yamashita*<sup>2</sup>, *Masayuki Okuyama*<sup>1</sup>, *Haruhide Mori*<sup>1</sup>, *Min Yao*<sup>2, 3</sup>, and *Atsuo Kimura*<sup>1</sup>

t-tagami@rakuo.ac.jp

1. Research Faculty of Agriculture, Hokkaido University

2. Graduate School of Life Science, Hokkaido University

3. Faculty of Advanced Life Science, Hokkaido University

$\alpha$ -Glucosidase is an exo-type enzyme catalyzing the hydrolysis of  $\alpha$ -glucosidic linkage at the non-reducing termini of substrate molecules. A majority of  $\alpha$ -glucosidases exhibits preference for disaccharides and trisaccharides as substrates. In contrast, several plant  $\alpha$ -glucosidases are known to display high substrate specificity for long-chain substrates. Among them, an  $\alpha$ -glucosidase from sugar beet exhibits low  $K_m$  and high  $k_{cat}/K_m$  for maltoheptaose and soluble starch, even though the active site structure of the enzyme is typical pocket shape as well as other  $\alpha$ -glucosidases [1]. To comprehend the binding of long-chain substrates to sugar beet  $\alpha$ -glucosidase, a series of long-chain pseudo-saccharides, acarviosyl-maltooligosaccharides (AC5–AC10, where the numerals represent DP), were employed. AC5–AC10 have longer maltooligosaccharide parts than the maltose unit of acarbose (AC4), which is a transition-state analog for  $\alpha$ -glucosidases. We had synthesized AC5–AC10 from AC4 and maltotetraose using a disproportionating enzyme [2]. In the current study, the potential of the acarviosyl-maltooligosaccharides for the inhibition of sugar beet  $\alpha$ -glucosidase was evaluated, and their complex structure analysis was performed [3]. Kinetic analyses showed that AC4–AC10 were competitive inhibitors for sugar beet  $\alpha$ -glucosidase and their  $K_i$  values were decreased with increasing in DP of the inhibitor. The plots of  $\log K_i$  for AC4–AC7 against  $\log(K_m/k_{cat})$  for the hydrolysis of maltotetraose–maltoheptaose showed linear correlation with correlation coefficient of  $r=0.96$ , suggesting that the acarviosyl-maltooligosaccharides mimic the transition state as well as AC4. The complex structure of sugar beet  $\alpha$ -glucosidase bound with AC8 was determined at 1.5 Å resolution. The electron density of AC8 was observed only at the active site, which was formed by a long loop (N-loop) protruding from the N-terminal sandwich domain and the catalytic  $(\beta/\alpha)_8$ -barrel domain containing two short insertions, subdomain b1 and b2. Two residues of AC8 at the non-reducing end were buried in the active-site pocket, while the maltohexaose part of AC8 at the reducing side attached to the surface of the N-loop and subdomain b2. The substrate binding at a distance from the active-site pocket was maintained largely by van der Waals interactions, with the five glucose residues at the reducing terminus retaining a left-handed single-helical conformation. These results indicate that sugar beet  $\alpha$ -glucosidase ingeniously uses the self-stabilizing property of amylose and soluble starch to form stable Michaelis complexes, which is responsible for lower  $K_m$  and higher  $k_{cat}/K_m$  for these long-chain substrates.

### Literature

1. T. Tagami, *et al.*, (2013) *J. Biol. Chem.*, **288**, 19296-19303.
2. T. Tagami, *et al.*, (2013) *Biosci. Biotechnol. Biochem.*, **77**, 312-319.
3. T. Tagami, *et al.*, *J. Biol. Chem.*, In press.

## Transcriptional and functional analysis of polysaccharide utilization loci reveals novel mechanisms of carbohydrate foraging by uncultivated gut bacteria

*Alexandra Tauzin<sup>1,2,3</sup>, Elisabeth Laville<sup>1,2,3</sup>, Stéphanie Heux<sup>1,2,3</sup>, Sébastien Nouaille<sup>1,2,3</sup>, Pascal Le Bourgeois<sup>1,2,3</sup>, Jean-Charles Portais<sup>1,2,3</sup>, Magali Remaud-Simeon<sup>1,2,3</sup>, Gabrielle Potocki-Véronèse<sup>1,2,3</sup> and Florence Bordes<sup>1,2,3</sup>*

Florence Bordes, [bordes@insa-toulouse.fr](mailto:bordes@insa-toulouse.fr)

1 Université de Toulouse, INSA/UPS/INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France

2 CNRS, UMR5504, F-31400 Toulouse, France

3 INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France

Humans rely on their symbiotic microorganisms to provide the enzyme machinery required for the breakdown of complex glycans, mostly provided by dietary fibers. In particular, to face the structural complexity of plant cell wall polysaccharides, gut bacteria have evolved sophisticated strategies consisting of gene clusters encoding for multi-functional enzyme complexes. The first extensive activity-based metagenomics study focused on CAZyme discovery from the human gut microbiome revealed that numerous uncultivated bacteria, which represent more than 70% of the gut microbiota, possess a very complete glycan degrading enzymatic arsenal [1]. Involving mostly glycoside hydrolases, it is encoded by various multigenic clusters acquired by horizontal gene transfers, and annotated as putative Polysaccharide Utilisation Loci (PULs).

In order to exploit these novel catabolic pathways for biorefinery and synthetic biology, a PUL functional characterization strategy was developed, based on their rational engineering, metabolic and transcriptional analysis in *E. coli*. This approach was applied to the characterization of a multigenic system encoding for putative proteins involved in the binding, the degradation and the import of prebiotic xylo-oligosaccharides (XOS) [2] by an uncultivated bacterium assigned to the *Bacteroides* genus. Transcriptional analyses revealed that in *E. coli*, these genes are not transcribed as a polycistronic mRNA. Moreover, a series of deletions was generated to investigate function of the different components of the PUL specific of XOS catabolism. In particular, this work has demonstrated the specificity of a novel carbohydrate transporter for XOS of polymerization degree up to 4.

This strategy allows circumventing the difficulties of the PUL characterization in native strains and provides a promising framework to better understand their component functionalities, especially those of carbohydrate transporters, which are key elements of catabolic pathways.

### Literature

1. Tasse, L. *et al.* Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Res.* 20, 1605-1612 (2010).
2. Cecchini, D. *et al.* Functional Metagenomics Reveals Novel Pathways of Prebiotic Breakdown by Human Gut Bacteria. *PLoS ONE* 8(9): e72766 (2013).

## Is the metabolic preference for specific $\beta$ -galactosides established by enzymes or by uptake systems in gut adapted bacteria?

Mia Christine Theilmann<sup>1</sup>, Morten Ejby<sup>1</sup>, Birte Svensson<sup>1</sup> and Maher Abou Hachem<sup>1</sup>

mchth@bio.dtu.dk

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Denmark

A diversity of  $\beta$ -galactosides of different monosaccharide composition and glycosidic linkages is abundantly present in human diet, e.g. in fruits, vegetables and milk (1, 2). With the exception of lactose,  $\beta$ -galactosides are indigestible by human enzymes presenting an attractive nutritional resource to human gut adapted bacteria. Oligomeric  $\beta$ -galactosides are reported to preferentially stimulate the growth of health promoting members of the gut microbiota, especially those from the *Bifidobacterium* genus. A degree of species-specific  $\beta$ -galactosides utilization by bifidobacteria is observed, but the possible contribution of transport systems to this remains unexplored.

A  $\beta$ -galactoside utilization gene cluster from *Bifidobacterium animalis* subsp. *lactis* BI-04 encodes a transcriptional regulator, an ATP-binding cassette (ABC) importer and an intracellular  $\beta$ -galactosidase of glycoside hydrolase family 42 (GH42) (3). The latter enzyme has been shown to exhibit broad specificity hydrolyzing  $\beta$ -1,6,  $\beta$ -1,3 and  $\beta$ -1,4 galactosidic bonds with only a modest preference for  $\beta$ -1,6 bonds (4). The objective of the present study is to investigate the ligand preference of the solute binding protein associated with the ABC transporter responsible for  $\beta$ -galactosides uptake in this locus.

The results of the present study represent a unique case allowing the comparison of selectivities of a transport protein and a glycoside hydrolase presumably involved in the metabolism of the same glycan type. These findings will be presented and discussed to highlight the role of ABC-mediated glycan transport in establishing the metabolic preference in an important group of the gut microbiota.

### Acknowledgements

The project is funded by the Danish Research Council for Independent Research | Natural Sciences (DFF, FNU) by a Research Project 2 grant and by a FøSu grant from the Danish Strategic Research Council to the project 'Gene discovery and molecular interactions in pre/probiotics systems. Focus on carbohydrate prebiotics'

### Literature

1. Vincken *et al.* (2003) *Plant Physiol.* 132:1781-1789
2. Han *et al.* (2012) *Biotechnol. Adv.* 30:1268-1278
3. Andersen *et al.* (2013) *BMC Genomics* 14: 312
4. Viborg *et al.* (2014) *Mol. Microbiol.* 94:1024-1040



## Chitin hydrolysis by *Chitinbacter tainanensis* enhancing via explosive puffing

Min-Lang Tsai<sup>1</sup>, Too Shen Tan<sup>1</sup> and Chao-Lin Liu<sup>2</sup>

tml@mail.ntou.edu.tw

1. Department of Food Science, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan, R.O.C.
2. Department of Chemical Engineering, Ming Chi University of Technology, 84 Gungjuan Rd., Taishan Dist. New Taipei City 24301, Taiwan R.O.C.

The purpose of this study is to investigate enhancement of chitin hydrolyzed to N-acetyl-D-glucosamine by *Chitinbacter tainanensis* using explosive puffing to modify structure of chitin. The raw and puffed chitins were prepared and were used as substrate for *C. tainanensis* to study the influence of molecular chain and spatial structure of chitin on degradation. Chitin was tempered to 75%, 50% and 35% moisture content and equilibrated at 25°C then being puffed in a puffing gun. The structure of chitin was modified by explosive puffing which were analyzed by XRD, SEM and density. The bulk densities of puffed chitins were significantly decreased with the decreased of puffed moisture content. The crystal structure of puffed chitin was strongly destroyed with lower moisture content. The holes on surface of puffed chitins were became larger and more with the decreased of puffed moisture content. The puffed chitin was more easily utilized by *C. tainanensis* than the unpuffed chitin. The *C. tainanensis* cultivated in puffed chitin with 75% moisture content had the highest yield of N-acetyl-D-glucosamine after 72 hours.

**Table 1.** The density, crystallinity index and yield of hydrolysis (Brix, %) of puffed chitin with different moisture contents

Chitin	Tapped density (g/cm <sup>3</sup> )	Crystallinity index (CrI <sub>020</sub> , %)	Brix (%)
Raw	0.1806	78.76	0.9
75%	0.1686	77.09	1.4
50%	0.1572	72.40	1.1
35%	0.1504	69.57	1.0

### Acknowledgements

The authors wish to express their appreciation for the financial support from National Science Council, R.O.C. (NSC 100-2313-B-019-004-MY3 and MOST 103-2313-B-019-003-MY3).

## Supressing transglycosylation to improve hydrolysis of cellobiose to glucose

Sasikala Anbarasan<sup>1</sup>, Tommi Timoharju<sup>1</sup>, Janice Barthomeuf<sup>1</sup>, Ossi Pastinen<sup>1</sup>, Juha Rouvinen<sup>2</sup>, Matti Leisola<sup>1</sup> and Ossi Turunen<sup>1</sup>.

Ossi.Turunen@aalto.fi

1.Aalto University, School of Chemical Technology, Department of Biotechnology and Chemical Technology, P.O. Box 16100, FI-00076 Aalto, Finland

2. University of Eastern Finland, Department of Chemistry, P.O. Box 111, FI-80101 Joensuu, Finland

The surface properties of the active site chamber or cleft are important factors in shaping the kinetic behavior of enzymes. We showed earlier that changing the shape of active site can improve the reaction of xylose isomerase with a promiscuous substrate, L-arabinose (Karimäki et al., 2004). Not unexpectedly, the mutated active site side chain can also impose via a water link its effect on stabilizing another promiscuous substrate, L-ribose, into the active site of xylose isomerase, and thus improving the reaction rate (Santa et al., 2005). The kinetic properties, reaction balance and product pattern have a key role in determining the efficiency of multienzyme reaction series, as in the hydrolysis starch and cellulose and in synthetic biological reaction pathways.

In cellulose hydrolysis to glucose, especially in high substrate and product concentrations, the end-product inhibition can be a significant factor in determining the product yield. Also other concentration-dependent factors than end-product inhibition, affect the yield. High substrate concentration is known to trigger the reverse reaction, transglycosylation, in glycoside hydrolases. This behavior is utilized biotechnologically to produce galacto-oligosaccharides from lactose to be used in food applications. In the hydrolysis of starch and cellulose to glucose such property of hydrolytic enzymes decreases the yield (e.g., Bohlin et al., 2013).

Beta glycosidase (BGAL\_SULAC) from *Sulfolobus acidocaldarius* is highly thermostable enzyme (optimum at 90-95°C with cellobiose as substrate) cleaving efficiently lactose and cellobiose. We studied how the active site mutations affect the kinetic behavior of the enzyme (Anbarasan et al., 2015) and observed an effect on the formation of trisaccharide in high concentration of cellobiose. When cellobiose was the substrate in 200 g/L concentration, BGAL\_SULAC produced 40-60% amount of trisaccharide (probably cellotriose) in relation to produced glucose. A mutation in the active site close to the catalytic residues reduced remarkably the trisaccharide formation, and therefore, increased the glucose yield.

### Literature

1. Anbarasan, S., Timoharju, T., Barthomeuf, J., Pastinen, J., Rouvinen, J., Leisola, M. and Turunen, O. (2015) Effect of active site mutation on pH activity and transglycosylation of *Sulfolobus acidocaldarius* beta-glycosidase. Submitted.
2. Bohlin, C.H., Westh, P., Baumann, M.J., Præstgaard, E., Borch, K., Praestgaard, J. and Rune N.M. (2013) A comparative study of hydrolysis and transglycosylation activities of fungal beta-glucosidases. *Appl. Microb. Biotechnol.* 97:159-169.
3. Karimäki, J., Parkkinen, T., Santa, H., Pastinen, O., Leisola, M., Rouvinen, J. and Turunen, O. (2004) Engineering the substrate specificity of xylose isomerase. *Protein Eng. Des. Sel.* 17: 861–869.
4. Santa, H., Kammonen, J., Karimäki, J., Leisola, M. and Turunen, O. (2005) Stochastic Boundary Molecular dynamics simulation of L-ribose in the active site of *Actinoplanes missouriensis* xylose isomerase and its Val135Asn mutant with improved reaction rate. *BBA – Proteins Proteomics* 1749:65-73.

## Evaluation of the secretomes of cellulolytic and chitinolytic microorganisms

*Tina R. Tuveng<sup>1</sup>, Magnus Ø. Arntzen<sup>1</sup>, Oskar Bengtsson<sup>1</sup>, Gustav Vaaje-Kolstad<sup>1</sup>, Vincent Eijsink<sup>1</sup>*

tina.tuveng@nmbu.no

<sup>1</sup> Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, 1432 Aas, Norway

Cellulose and chitin are the two most abundant biopolymers in nature and several microorganisms are known to degrade these biopolymers (e.g. [1, 2]). Investigation of proteins secreted by microorganisms growing on different chitin or cellulose substrates potentially allows identification of novel proteins involved in biomass degradation. We use here a novel agar-plate method that enables isolation of secretomes with low contamination of intracellular proteins.

Microorganisms were grown on agar-plates with a filter included in the center of the plate. Different chitin or cellulose substrates were used as the sole carbon source and proteins were isolated, followed by trypsinization and identification by bottom-up mass spectrometry using a QExactive (Thermo) coupled to a nanoHPLC (Dionex). Relative protein abundances were calculated using label-free quantification with the MaxQuant software.

Several carbohydrate-active enzymes (CAZymes) were identified, including substrate specific enzymes like chitinases and cellulases. In addition, we found several uncharacterized proteins, which provide leads towards the identification of hitherto non-identified elements in the enzymatic machineries used by microorganisms when degrading different biomasses.

### Literature

1. Gardner, J.G., et al., Systems biology defines the biological significance of redox-active proteins during cellulose degradation in an aerobic bacterium. *Molecular Microbiology*, 2014. **94**(5): p. 1121-1133.
2. Vaaje-Kolstad, G., et al., The chitinolytic machinery of *Serratia marcescens*--a model system for enzymatic degradation of recalcitrant polysaccharides. *FEBS J*, 2013. **280**(13): p. 3028-49.

## Functional metagenomics boosts enzyme discovery for plant cell wall polymer breakdown

*Lisa Ufarté<sup>1,2</sup>, Elisabeth Laville<sup>1,2</sup>, Diego Morgavi<sup>3</sup>, Guillermina Hernandez-Raquet<sup>1,2</sup>, Sophie Bozonnet<sup>1,2</sup>, Claire Dumon<sup>1,2</sup>, Patrick Robe<sup>4</sup>, Bernard Henrissat<sup>5</sup>, and Gabrielle Potocki-Veronese<sup>1,2</sup>*

veronese@insa-toulouse.fr

1. Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse
2. UMR5504, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, CNRS, INRA, F-31400 Toulouse
3. INRA, UR1213, Herbivores, F-63122 Saint-Genès-Champanelle, France
4. LibraGenSA; Bat. Canal Biotech. I, 3 rue des Satellites, F-31400 Toulouse
5. Architecture et Fonction des Macromolécules Biologiques, UMR6098, CNRS, Universités Aix-Marseille I & II, 163 Avenue de Luminy, F-13288 Marseille

To face the structural diversity of plant polysaccharides and polyaromatic compounds composing their main carbon source in many ecosystems, microorganisms have evolved sophisticated multi-functional enzyme complexes. In order to unlock them from the vast world of uncultured microorganisms, and to explore their extraordinary functional potential, a high-throughput function-based metagenomic approach was developed to boost the discovery of enzymes involved in complex polymer breakdown.

Metagenomic libraries from the highly diverse bovine rumen microbiome, totalising 1.5 Gbp of DNA, were screened for glycoside-hydrolase, esterase, and oxidase activities. Several hundreds of positive clones were found active on the screening substrates. In a second step, positive clones were characterised for their abilities to degrade resistant plant cell wall substrates. The metagenomic DNA of the best performing clones were sequenced using the MiSeq technology, and thus functionally and taxonomically annotated, highlighting very original sequences, including novel CAZy modules.

This work allowed the identification of dozens of novel enzymes of high potential for numerous biotechnological processes, as they are highly efficient, alone or acting synergically, to breakdown plant cell wall polysaccharides and polyaromatic compounds.

## **Oligosaccharides production using a glucansucrase from a lactic acid bacteria strain in its free and immobilized form**

*Simon Johansson, Gilles Bourdin, Charlotte Gancel and Christina Vafeiadi*

Christina.Vafeiadi@rdls.nestle.com

Nestlé Research Center, Vers-Chez-Les-Blanc, Lausanne, Switzerland

Glucansucrases, also known as glucosyltransferases (GTFs) are extracellular enzymes that synthesize high molecular weight glucans or in the presence of acceptors molecules, low molecular weight oligosaccharides, using sucrose as a donor. In the present study, a glucansucrase from a lactic acid bacteria strain was applied at highly concentrated sucrose solutions. Reaction parameters were optimized and the synthesized oligosaccharides were quantified and partially characterized. Glycosidic linkages were analysed in the crude reaction mixtures using DOSY NMR. The enzyme was used in its free (soluble) and immobilized form, after its immobilization into a polyvinylalcohol matrix in the form of LentiKat's.

### **Acknowledgements**

The authors would like to thank LentiKat's Biotechnology for the immobilization of the enzyme and Spectral Service for the DOSY NMR analysis.

## Factors affecting enzymatic cellulose hydrolysis in ionic liquid solutions

Ronny Wahlström<sup>1</sup>, Jenni Rahikainen<sup>1</sup>, Kristiina Kruus<sup>1</sup> and Anna Suurnäkki<sup>1</sup>

ronny.wahlstrom@vtt.fi

1. VTT – Technical Research Centre of Finland, Tietotie 2, Espoo, FI-02044 VTT, Finland

Certain ionic liquids (ILs, salts with melting points < 100 °C) dissolve cellulose and in some cases even wood [1]. It has been shown that pretreating lignocellulose with ILs increases the feedstock's digestibility in enzymatic hydrolysis. However, the cellulases typically used for cellulose hydrolysis are sensitive to inactivation by even low amounts of IL, and therefore a water-intensive washing step is needed between the IL pretreatment and hydrolysis steps. Developing compatible cellulase-IL systems for one-pot hydrolysis is an intensively studied topic, but achieving this objective exerts a need to elucidate the factors governing IL-induced cellulase inactivation in detail, which is the topic of this work.

The hydrolytic action of *Trichoderma reesei* Cel5A was compared in 20 % of different ILs during 72 h hydrolysis at 45 °C using 1 % microcrystalline cellulose as substrate [2]. The hydrolysis yields were below 2.5 %, compared to 7.7 % in the buffer reference system. The differences in hydrolysis yields were generally small between the different ILs. In another experiment, the endoglucanase stability of a *T. reesei* cellulase cocktail was measured in two pure ILs, 1-ethyl-3-methylimidazolium acetate ([EMIM]AcO) and 1,3-dimethylimidazolium dimethylphosphate ([DMIM]DMP) [3]. In [EMIM]AcO, complete inactivation was observed in 4 h whereas the endoglucanase activity decreased only slowly in [DMIM]DMP. This result suggests that cellulase inactivation in IL solutions may not be the only reason for observed low hydrolysis yields, as hydrolysis yields in 20 % [EMIM]AcO and [DMIM]DMP were similar, but the endoglucanase stability differed greatly in the two ILs. The effect of [EMIM]AcO and [DMIM]DMP on cellulase substrate binding and the role of the carbohydrate-binding module (CBM) were also studied using the intact (core domain + CBM) and core domain (lacking CBM) proteins of *T. reesei* Cel5A and Cel7A [4]. The results showed increasing IL concentrations to inhibit substrate binding and especially binding through the CBM was affected.

### Acknowledgements

VTT Graduate School and the Finnish Bioeconomy Cluster's (FIBIC) Future Biorefinery (FuBio) program are gratefully acknowledged for supporting this study.

### Literature

1. I. Kilpeläinen, H. Xie, A. King, M. Granström, S. Heikkinen and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2007, **55**, 9142–9148.
2. R. Wahlström, A. King, A. Parviainen, K. Kruus and A. Suurnäkki, *RSC Adv.*, 2013, **3**, 20001–20009.
3. A) G. Ebner, P. Vejdovsky, R. Wahlström, A. Suurnäkki, M. Schrems, P. Kosma, T. Rosenau and A. Potthast, *J. Mol. Cat. B*, 2014, **99**, 121–129; B) R. Wahlström, PhD Thesis, *VTT Science*, 52, VTT - Technical Research Centre of Finland, 2014.
4. R. Wahlström, J. Rahikainen, K. Kruus and A. Suurnäkki, *Biotechnol. Bioeng.*, 2014, **111**, 726–733.

## Structural-functional analysis reveals a specific domain organization in family GH20 hexosaminidases

*Cristina Val-Cid, Xevi Biarnés, Magda Faijes and Antoni Planas.*

cristinavalc@iqs.url.edu

Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, 08017 Barcelona, Spain.

GH20 hexosaminidases are widely distributed in nature catalyzing the hydrolysis of *N*-acetyl-hexosaminyl residues in oligosaccharides and glycoconjugates. They present several accompanying domains with a diverse organization [1]. We performed structural and sequence analysis of the known GH20 enzyme structures that lead us to propose two different domain organization models: model A, containing at least a non-catalytic GH20b domain and the catalytic GH20 always followed by an extra alpha-helix, and model B, with only the catalytic GH20. In addition, the GH20b-GH20- $\alpha$  topology of model A appeared not always to be the minimal functional unit. We see that for GH20 model A enzymes, the substrate-binding cavity always involved a remote element which is provided by a long loop of the catalytic domain, or, when the loop is short, by an accompanying domain of the same protein or of a dimer partner.

The large *B. bifidum* lacto-*N*-biosidase (LnbB) was used as a model protein due to its biotechnological interest and structural complexity [2,3]. LnbB enzyme is involved in the degradation of human milk oligosaccharides, specifically the hydrolysis of tetrasaccharide Gal $\beta$ 1,3GlcNAc $\beta$ 1,3Gal $\beta$ 1,4Glc to lacto-*N*-biose (Gal $\beta$ 1,3GlcNAc) and lactose. It consists of five domains GH20b-GH20-lectin-CBM32-Iglike and the truncated protein GH20b-GH20- $\alpha$  proved not to be active. Here we report evidences of the requirement of a remote element for the protein to be catalytically active. *In vitro* complementation experiments of the inactive truncated protein and the C-terminal lectin domain restores activity due to protein-protein interactions that locates the remote element in the active conformation. This analysis provides new insights for further enzyme engineering for biotechnological applications.

CV acknowledges Instituto Danone for financial support.

### Literature

1. Vocadlo, D.J. and Withers, S.G. (2005) Biochemistry. 44, 12809-12818.
2. Wada, J., Ando, T., Kiyohara, M., Ashida, H., Kitaoka, M., Yamaguchi, M., Kumagai, H., et al. (2008). Appl.Environ. Microbiol. 74, 3996-4004.
3. Ito,T.; Katayama,T.; Hattie,M.; Sakurama,H.; Wada,J.; Suzuki,R.; Ashida,H.; Wakagi,T J.Biol.Chem, 288,11795-11806.

## **Novel carbohydrate targeting mechanisms by the human gut symbiont *Bacteroides thetaiotaomicron***

Alicia Lammerts van Bueren<sup>1</sup> Eric Martens<sup>2</sup> and Lubbert Dijkhuizen<sup>1</sup>

a.lammerts.van.bueren@rug.nl

1. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands
2. Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Over 30% of our resident gut microbiota are from the *Bacteroidetes* family (1). These gram-negative, obligate anaerobes are well armored with a repertoire of carbohydrate degrading enzymes to battle the gut environment for competing carbohydrate nutritional resources. One species, *Bacteroides thetaiotaomicron*, devotes 10% of its genome to carbohydrate foraging. Discrete loci within its genome are upregulated in response to carbohydrates dependent on the source (2). These sources arise from either dietary (eg: plant polysaccharide) or mammalian (eg: mucins) origin. Using a combination of techniques we have discovered novel aspects of *B. thetaiotaomicron* degradation of unique polysaccharide sources. By combining anaerobic growth, cultivation, gene expression, mass spectrometry and enzymatic characterization techniques, we are able to view overall novel carbohydrate degradation mechanisms and identify new mechanisms which are responsible for making *B. thetaiotaomicron* a prominent and well established human gut symbiont.

### **Literature**

- 1 Arumugam, M et. al. (2011) Nature 473, 174-180.
- 2 Sonnenburg, JL et. al. (2005) Science 307(5717), 1955-1959.



## Insight into structural, biochemical and *in silico* determinants of ligand binding specificity of family 6 carbohydrate binding module (CtCBM6) from *Clostridium thermocellum*

Anil Kumar Verma<sup>1\*</sup>, Pedro Bule<sup>2</sup>, Teresa Ribeiro<sup>2</sup>, Joana L. A. Brás<sup>2</sup>, Joyeeta Mukherjee<sup>3</sup>, Munishwar N. Gupta<sup>3</sup>, Carlos M.G.A. Fontes<sup>2</sup> and Arun Goyal<sup>1</sup>

ak.verma@iitg.ernet.in

1. Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati, 781039, Assam, India.
2. CIISA-Faculdade de Medicina Veterinária, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal.
3. Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India.

CtCBM6 is a part of modular glucuronoxylan-xylanohydrolase (CtXynGH30) from *Clostridium thermocellum* [1-2]. CtCBM6 was cloned, expressed and purified as a soluble ~14 kDa protein. CtCBM6 showed binding with polysaccharides containing xylan main chain as well as decorated with arabinose or glucuronic acid as side chains. Quantitative binding analysis with soluble polysaccharides by affinity electrophoresis and ITC revealed that CtCBM6 displays higher affinity towards wheat- and rye-arabinoxylans than beechwood-, birchwood- and oat spelt-xylan. Site-directed mutagenesis revealed that Tyr28 and Phe84 in Cleft A play major role in ligand binding. Protein melting study displayed thermostable nature of CtCBM6 and Ca<sup>2+</sup> ions did not affect its structure stability and binding affinity. CtCBM6 model was developed by homology modelling and refined by molecular dynamic simulation. Structure analysis indicated the presence of predominantly  $\beta$ -sheets forming a jelly roll fold. CtCBM6 displayed two potential binding clefts. Cleft A, is situated between two loops connecting  $\beta$ 4 to  $\beta$ 5 and  $\beta$ 8 to  $\beta$ 9 strands. Tyr28 and Phe84 present on these loops made a planar hydrophobic ligand binding surface to accommodate flat sugar ring. Cleft B, is located on the concave surface of  $\beta$ -sandwich fold. Tyr34 and Tyr104 make a planar hydrophobic platform, inaccessible to ligand due to hindrance by Pro68. CD spectrum of CtCBM6 showed only  $\beta$ -strands and random coils and supported *in silico* structure prediction.

### Literature

1. Anil K. Verma, Arun Goyal, Freire F., Bule P., Venditto I., Bras J. L. A., Santos H., Cardoso V., Bonifacio C., Thompson A., Romao M. J., Prates J. A. M., Ferreira L. M. A., Fontes C. M. G. A., and Najmudin S. (2013) Overexpression, crystallization and preliminary X-ray crystallographic analysis of glucuronoxylan-xylanohydrolase (Xyn30A) from *Clostridium thermocellum*, Acta Crystallogr. F 69, 1440-1442
2. Anil K. Verma and Arun Goyal (2014) *In silico* structural characterization and molecular docking studies of first glucuronoxylan-xylanohydrolase (Xyn30A) from family 30 glycosyl hydrolase (GH30) from *Clostridium thermocellum*. Molecular Biology, 48, 278-286.

## Diversity in $\beta$ -galactosidase specificities within *Bifidobacterium*: towards an understanding of $\beta$ -galactoside metabolism in the gut niche

Alexander Holm Viborg<sup>1,2,4,5</sup>, Maher Abou Hachem<sup>2</sup>, Takane Katayama<sup>3</sup>, Leila Lo Leggio<sup>4</sup>, Motomitsu Kitaoka<sup>5</sup>, Shinya Fushinobu<sup>1</sup>, and Birte Svensson<sup>2</sup>

ahv@ahv.dk

1. Dept. of Biotech., Graduate School of Agricultural and Life Sciences, The University of Tokyo

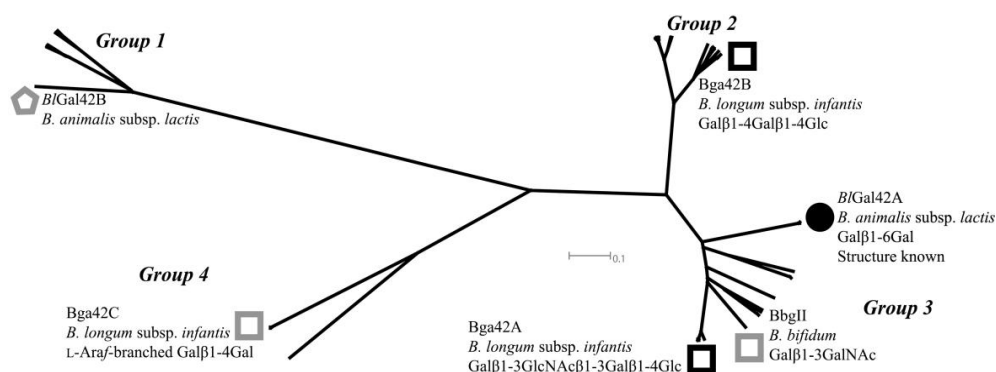
2. Dept. of Systems Biology, Technical University of Denmark

3. Host-Microbe Interaction Research Laboratory, Ishikawa Prefectural University

4. Dept. of Chemistry, University of Copenhagen

5. National Food Research Institute, National Agriculture and Food Research Organization

The *Bifidobacterium* genus harbors several health promoting members of the gut microbiota, which display metabolic specialization by preferentially utilizing dietary or host-derived  $\beta$ -galactosides. To approach a deeper understanding of the  $\beta$ -galactoside catabolism in the gut niche, we investigated the bioinformatic, biochemical and structural properties of glycoside hydrolase family 42 (GH42)  $\beta$ -galactosidases within *Bifidobacterium* associated with the human gut. GH42  $\beta$ -galactosidases segregate according to function in a phylogenetic analysis (Fig. 1) and display a large variety of sub-specificities (1–3) in accordance with the diversity and complexity of  $\beta$ -galactosides available in the gut. The first function-structure insights in GH42 illustrate that diversity manifested in sub-specificities, correlates to subtle changes in loop regions in the near vicinity of the site of catalysis (1). We are currently pursuing further studies in sub-specificities and structures to advance the understanding of the evolution of GH42 that at the molecular level support metabolic specialization in the gut niche.



**Fig 1. Phylogenetic relationship of GH42  $\beta$ -galactosidases within *Bifidobacterium***

### Acknowledgements

Viborg is an International Research Fellow of the Japan Society for the Promotion of Science.

### Literature

1. Viborg *et al.* (2014) *Mol. Microbiol.* **94**, 1024–40
2. Viborg *et al.* (2014) *Glycobiology.* **24**, 208–16
3. Yoshida *et al.* (2012) *Glycobiology.* **22**, 361–8

## Mining anaerobic digester consortia metagenomes for secreted carbohydrate active enzymes

*Casper Wilkens<sup>1</sup>, Peter Kamp Busk<sup>1</sup>, Bo Pilgaard<sup>1</sup>, Rasmus Kirkegaard<sup>2</sup>, Mads Albertsen<sup>2</sup>, Per Halkjær Nielsen<sup>2</sup> and Lene Lange<sup>1</sup>*

cwi@bio.aau.dk or lla@adm.aau.dk

1. Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Copenhagen, Denmark
2. Center for Microbial Communities, Section for Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark

Anaerobic digesters (ADs) are one of several ways to produce renewable energy, which in the case of ADs is in the form of methane. Several microbial groups are involved in anaerobic degradation of organic wastes such as animal manures and wastewater, and solid organic wastes such as sludge, crop, and food wastes (Alvarado *et al.*, 2014).

The processes and the roles of the microorganisms that are involved in biomass conversion and methane production in ADs are still not fully understood. We are investigating thermophilic and mesophilic ADs that use wastewater surplus sludge for methane production. To gain insight into both the degradation of the carbohydrates and the various roles of the microbes in the ADs we have mined metagenomes from both types of ADs for glycoside hydrolases, carbohydrate esterases, polysaccharide lyases, auxiliary activities, and carbohydrate binding modules. The mining was done with the Peptide Pattern Recognition (PPR) program (Busk and Lange, 2013), which is a novel non-alignment based approach that can predict function of *e.g.* CAZymes. PPR identifies a set of short conserved sequences, which can be used as a finger print when mining genomes for novel enzymes. In both thermophilic and mesophilic ADs a wide variety of carbohydrate active enzyme functions were discovered in the metagenomic sequencing of the microbial consortia. The most dominating type of glycoside hydrolases were  $\beta$ -glucosidases (up to 27%),  $\alpha$ -amylases (up to 10%),  $\alpha$ -glucosidases (up to 8%),  $\alpha$ -galactosidases (up to 9%) and  $\beta$ -galactosidases (up to 7%). For carbohydrate esterases the by far most dominating type was acetylxytan esterases (up to 59%) followed by feruloyl esterases (up to 16%). Less than 15 polysaccharide lyases were identified in the different metagenomes and not surprisingly no polysaccharide monooxygenases were identified. For the carbohydrate binding modules one of the most dominating families were CBM48 (up to 16%).

### Acknowledgements

This work is supported by the Villum Foundation.

### Literature

1. Alvarado *et al.* (2014) Microbial trophic interactions and *mcrA* gene expression in monitoring of anaerobic digesters. *Front. Microbiol.*, 5, 597, doi: 10.3389/fmicb.2014.00597
2. Busk, P. and Lange, L. (2013) Function-based classification of carbohydrate-active enzymes by recognition of short, conserved peptide motifs. *Appl. Environ. Microbiol.*, 79, 3380-3391

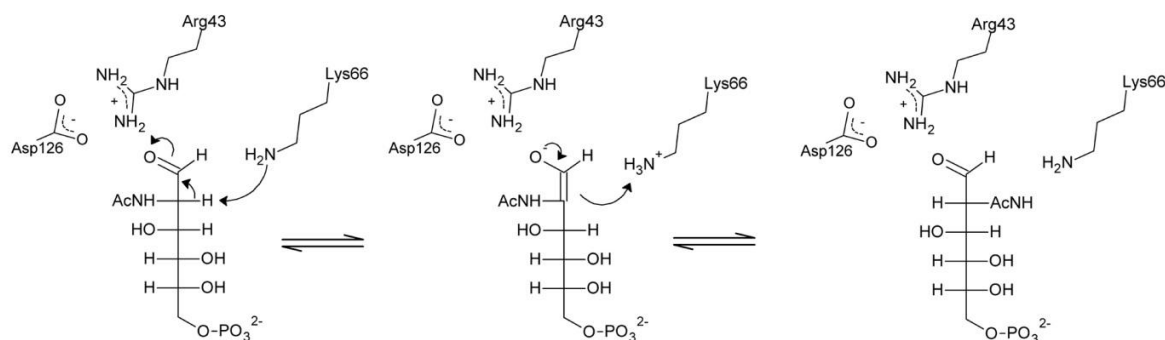
## Structural and functional characterization of the *Clostridium perfringens* N-acetylmannosamine-6-phosphate 2-epimerase essential for the sialic acid salvage pathway

Marie-Cécile Pélissier<sup>1,2</sup>, Corinne Sebban-Kreuzer<sup>3</sup>, Françoise Guerlesquin<sup>3</sup>, James A. Brannigan<sup>4</sup>, Yves Bourne<sup>1,2</sup> and Florence Vincent<sup>1,2</sup>

florence.vincent@afmb.univ-mrs.fr

1. Aix-Marseille University, AFMB UMR7257, 163 avenue de Luminy 13288 Marseille, France
2. CNRS, AFMB UMR7257, 163 avenue de Luminy 13288 Marseille, France
3. Laboratoire d'Ingénierie des Systèmes Macromoléculaires, CNRS UMR7255, Aix-Marseille Université, 31 chemin Joseph Aiguier, 13402 Marseille Cedex 20, France
4. Structural Biology Laboratory, Department of Chemistry University of York, Heslington, York, YO10 5DD, UK

Pathogenic bacteria are endowed with an arsenal of specialized enzymes to convert nutrient compounds from their cell hosts. The essential N-acetylmannosamine-6-phosphate 2-epimerase (NanE) belongs to a convergent glycolytic pathway for utilization of the three amino sugars, GlcNAc, ManNAc and sialic acid. The crystal structure of ligand-free NanE from *Clostridium perfringens* reveals a modified TIM ( $\beta/\alpha$ )<sub>8</sub> barrel in which a stable dimer is formed by exchanging the C-terminal helix. By retaining catalytic activity in the crystalline state, the structure of the enzyme bound to the GlcNAc-6P product identifies the topology of the active site pocket and points to invariant residue Lys66 as a putative single catalyst, supported by the structure of the catalytically inactive Lys66Ala mutant in complex with substrate ManNAc-6P. <sup>1</sup>H-NMR-based time course assays of native NanE and mutated variants demonstrate the essential role of Lys66 for the epimerization reaction with participation of neighboring Arg43, Asp126 and Glu180 residues. These findings unveil a one-base catalytic mechanism of C2 deprotonation/reprotonation via an enolate intermediate and provide the structural basis for the development of new antimicrobial agents against this family of bacterial 2-epimerases.



**Fig 1.** Once the C2 proton is abstracted by the base catalyst Lys66, a negatively charged intermediate is stabilized by the salt bridge and Glu180. A proton is donated by the same Lys66 catalyst to the enolate intermediate to yield the GlcNAc-6P product.

## Discovering novel glycan utilization loci in probiotic bacteria

Jens Vogensen<sup>1</sup>, Quanhui Wang<sup>2</sup>, Maher Abou Hachem<sup>1</sup>, Siqi Liu<sup>2</sup>, Birte Svensson<sup>1</sup>

jenvo@bio.dtu.dk

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Denmark
2. Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, 100101, China

Consumption of host-indigestible glycans is essential for the maintenance of a healthy population of gut microbes. The thriving of probiotic bacteria, *Bifidobacterium* and *Lactobacillus* being the most commercially exploited strains, necessitates the intake of prebiotics<sup>1</sup>. Mannan-oligosaccharides (MOS) are one such potential prebiotic which is present in nature in different structures such as galactomannan and glucomannan<sup>2</sup>. Xyloglucan utilization loci have also recently been identified and characterized in several *Bacteroidetes* strains<sup>3</sup>. Not much is known about either MOS or xyloglucan utilization by probiotics, even less on their roles as prebiotics.

Identification of putative loci for MOS or xyloglucan utilization has been pursued through genome-mining in different strains of probiotic bacteria. Utilization of either glycan will be investigated in these probiotic taxa through bacterial growth assays. Identification of upregulated genes when the bacteria grow in media with or without oligosaccharides, including novel glycoside hydrolases (GHs) genes, will be carried out through transcriptomics and proteomics. These identified GHs will be functionally characterized including crystal structure determination. This investigation will broaden the knowledge of the different strategies gut microbes have evolved in the crowded and competitive gut environment to utilize varying carbohydrate sources. Preliminary results will be presented.

### Acknowledgments

This research project is supported by the Sino-Danish Center (SDC) and Technical University of Denmark (DTU)

### Literature

1. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J Nutr* 125:1401–12.
2. Moreira LRS (2008) An overview of mannan structure and mannan-degrading enzyme systems. *Appl Microbiol Biot* 79:165–78.
3. Larsbrink J, Rogers TE, Hemsworth GR, McKee LS, Tauzin AS, Spadiut O, Klintner S, Pudlo NA, Urs K, Koropatkin NM, Creagh AL, Haynes CA, Kelly AG, Cederholm SN, Davies GJ, Martens EC, Brumer H (2014) A discrete genetic locus confers xyloglucan metabolism in select human gut *Bacteroidetes*. *Nature* 506:498–502

## Activity-based probing of $\alpha$ -L-fucosidase

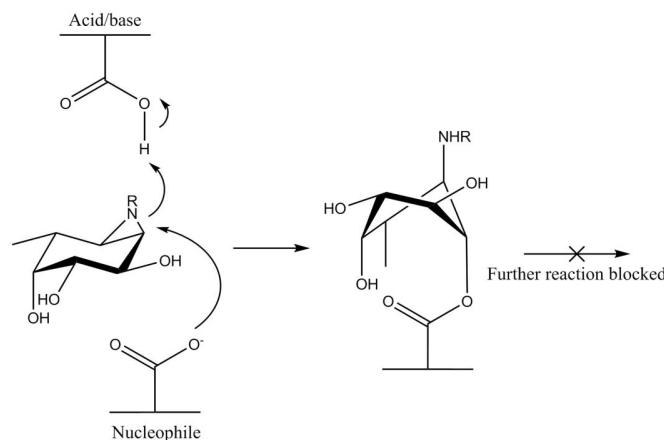
Daniel Wright<sup>1</sup>, Jianbing Jiang<sup>2</sup>, Wouter Kallemijn<sup>3</sup>, Johannes Aerts<sup>3</sup>, Herman Overkleeft<sup>2</sup>, Gideon Davies<sup>1</sup>

dww501@york.ac.uk

1. York Structural Biology Laboratory, Department of Chemistry, University of York, YO10 5DD, United Kingdom
2. Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands
3. Department of Medical Biochemistry, Academic Medical Centre, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

$\alpha$ -L-Fucosidases (GH29, GH95) catalyse the hydrolysis of fucose from fucosylated glycans in an exo-fashion. GH29 fucosidases are medically relevant due to, for example, the differential regulation they have in a number of cancers (they have been shown to be potent biomarkers for a number of cancer types<sup>1</sup>), and links they have to the virulence of pathogens such as *Helicobacter pylori*.<sup>2</sup> Deficiency in GH29 activity also causes the severe lysosomal storage disorder fucosidosis.<sup>3</sup>

Activity-based probes (ABPs) exploit the catalytic mechanisms of enzymes to allow the specific trapping of active enzymes by the formation of irreversible covalent ABP complexes.<sup>4</sup> Herein we report the use of ABPs for probing  $\alpha$ -L-fucosidases (**Fig 1.**), allowing quantification of  $\alpha$ -L-fucosidase activity levels *in vitro* and *in vivo*, and prove covalent attachment of an ABP scaffold to a bacterial  $\alpha$ -L-fucosidase through X-ray crystal structure.



**Fig 1.** Schematic for covalent trapping of  $\alpha$ -L-fucosidase with an aziridine ABP.

### Literature

- 1 Listinsky, J. J., Siegal, G. P. & Listinsky, C. M. The emerging importance of alpha-L-fucose in human breast cancer: a review. *Am J Transl Res* **3**, 292-322 (2011).
- 2 Liu, T. W. *et al.* Role for alpha-L-fucosidase in the control of *Helicobacter pylori*-infected gastric cancer cells. *Proc Natl Acad Sci USA* **106**, 14581-14586 (2009).
- 3 Michalski, J. C. & Klein, A. Glycoprotein lysosomal storage disorders: alpha- and beta-mannosidosis, fucosidosis acid alpha-N-acetylgalactosaminidase deficiency. *Bba-Mol Basis Dis* **1455**, 69-84 (1999).
- 4 Heal, W. P., Dang, T. H. T. & Tate, E. W. Activity-based probes: discovering new biology and new drug targets. *Chem Soc Rev* **40**, 246-257 (2011).

## Gene synthesis, expression and characterization of a thermostable endo- $\beta$ -1, 4-mannanase

Yawei Wang<sup>1</sup>, Wei Zhang<sup>1</sup>, Zhengding Su<sup>2</sup>, Ying Zhou<sup>3</sup>, Ossi Turunen<sup>4\*</sup>, Hairong Xiong<sup>1</sup>

ossi.turunen@aalto.fi (O. Turunen); xionghr@mail.scuec.edu.cn (H. Xiong)

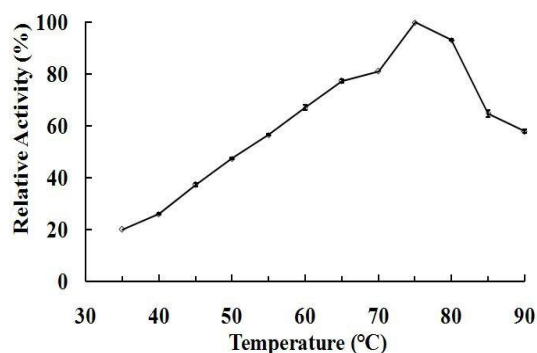
1 College of Life Science, South-central University for Nationalities, Wuhan, 430074, China

2 Hubei University of Technology, Wuhan, 430068, China

3 Wuhan Sunhy Biology Co., Ltd, Wuhan, 430074, China

4 School of Chemical Technology, Aalto University, FI-00076 Aalto, Finland

Thermostable mannanase ManAd3 from *Caldibacillus cellulovorans* was produced as codon optimized version in *Pichia pastoris* strain GS115 from pPIC9k vector (recombinant strain ManAhr-GS115). The protein was secreted to the culture medium as a sole visible protein with a molecular mass about 30 kDa as shown in SDS-PAGE. The enzymatic properties were determined and the optimal reaction temperature and pH were 75°C and pH 6.0, respectively, and the specific activity was up to 3200 U/mg. Moreover, the enzyme retained over 90% of its activity after 30 min treatment at 75°C, pH 6.0.



**Fig 1.** Temperature-dependent activity profile of mannanase ManAhr at pH 4.5 in 10-min assay.

Hubei provincial foreign cooperation project (2013BHE016) and Hubei collaborative innovation center for industrial fermentation were acknowledged.

### Literature

W Zhang, H Xiong, etc. Gene Synthesis, Expression and characterization of a Thermostable mannanase. China Biotechnology, 2014, 34(8):41-46.

## Expression a hyperthermostable *Thermotoga maritima* xylanase 10B in *Pichia pastoris* GS115 and its tolerance to ionic liquids

Yawei Wang<sup>1</sup>, Kubra Telli<sup>2,4</sup>, Tianyi Yu<sup>1</sup>, Ying Zhou<sup>3</sup>, Sasikala Anbarasan<sup>4</sup>, Baris Binay<sup>2</sup>, Michael Hummel<sup>4</sup>, Herbert Sixta<sup>4</sup>, Ossi Turunen<sup>4\*</sup>, Hairong Xiong<sup>1\*</sup>

ossi.turunen@aalto.fi (O. Turunen); xionghr@mail.scuec.edu.cn (H. Xiong)

1 South-central University for Nationalities, College of Life Science, Wuhan, 430074, China

2 Arel University, Faculty of Science and Letters, Istanbul, Turkey

3 Wuhan Sunhy Biology Co., Ltd, Wuhan, 430074, China

4 Aalto University, School of Chemical Technology, FI-00076 Aalto, Finland

The hyperthermostable xylanase 10B from *Thermotoga maritima* (TmXYN10B) was overexpressed as codon optimized in *Pichia pastoris* strain GS115. The expressed xylanase was about 40 kDa, and showed the optimal temperature at 100 °C in 60-min assay. Also, it showed 92% of maximal activity at 105 °C. The half life of inactivation was over 120 min at 100 °C. Thermostability is thought to protect against inactivation by biomass-dissolving ionic liquids. We tested how TmXYN10B tolerates [EMIM]OAc, [EMIM]DMP and [DBNH]OAc. At 80 °C, 15% ionic liquids caused about 25-45% inactivation and 35% ionic liquids about 74-86% inactivation. Although the activity loss in TmXYN10B by the ionic liquids was higher than in the most ionic liquid-tolerant xylanases, TmXYN10B was able to retain partial activity (49%) even at 90 °C in 35% [EMIM]DMP during 24-hour reaction. In the same conditions, the 15% [EMIM]DMP caused only 13% loss in the activity. In conclusion, when partial activity in quite long reactions at very high temperatures in aqueous ionic liquid solutions is needed, the TmXYN10B enzyme showed promising performance.



## Tailor-made potato starch

*Xuan Xu, Richard G.F Visser and Luisa M. Trindade*

luisa.trindade@wur.nl

Wageningen UR – Plant Breeding, Wageningen University and Research Center, P.O. Box 386. 6700 AJ Wageningen. The Netherlands

Starch, the most important storage carbohydrate, is widely used in food and non-food industry. Native starches have shortcomings that restricts the end uses, hence starch modification is a prerequisite for some industrial applications (Xu *et al.*, 2014). With a better understanding of starch biosynthesis and degradation pathways, new genetic tools have become available for the modification of starch composition and to produce starches with novel functionalities *in planta*. This approach has the enormous potential for tailoring starches with less energy expenditure and without pollutant chemical, thereby broaden starch applications in an economically and environmentally-friendly way. We developed the so-called Starch Binding Domain (SBD) technology to target effector proteins to starch granules during the biosynthesis process in potato (Ji *et al.*, 2003). By using this SBD technology, several heterologous proteins were successfully incorporated into starch granules and resulted in the modification of potato starches in the following aspects: (1) the morphology of starch granule; i.e., expression of an *E. coli* maltose acetyltransferase (MAT) (Nazarian Firouzabadi *et al.*, 2007), a truncated mutansucrase (GTFICAT) (Kok-Jacon *et al.*, 2005), an *E. coli* branching enzyme (GLGB) (Huang *et al.*, 2013); (2) the size of starch granule; i.e., expression of multiple appended SBDs (Ji *et al.*, 2004), a *Neisseria polysaccharea* amylosucrase (Huang *et al.*, 2014); (3) the phosphate content of starch granule; i.e., expression of human phosphatase (Iaforin). Moreover, the effect on starch compositions and properties were investigated. Ultimately, transcription profiling of genes in those transformants were analyzed to get better understanding of starch biogenesis in tuberous storage starch.

### Literature

1. Huang, X.-F., Nazarian-Firouzabadi, F., Vincken, J.-P., Ji, Q., Suurs, L.C.J.M., Visser, R.G.F., and Trindade, L.M. (2013). Expression of an engineered granule-bound Escherichia coli glycogen branching enzyme in potato results in severe morphological changes in starch granules. *Plant Biotechnology Journal* 11, 470-479.
2. Huang, X.F., Nazarian-Firouzabadi, F., Vincken, J.P., Ji, Q., Visser, R.G., and Trindade, L.M. (2014). Expression of an amylosucrase gene in potato results in larger starch granules with novel properties. *Planta* 240, 409-421.
3. Ji, Q., Oomen, R.J., Vincken, J.P., Bolam, D.N., Gilbert, H.J., Suurs, L.C., and Visser, R.G. (2004). Reduction of starch granule size by expression of an engineered tandem starch-binding domain in potato plants. *Plant Biotechnol J* 2, 251-260.
4. Ji, Q., Vincken, J.P., Suurs, L.C., and Visser, R.G. (2003). Microbial starch-binding domains as a tool for targeting proteins to granules during starch biosynthesis. *Plant Mol Biol* 51, 789-801.
5. Kok-Jacon, G.A., Vincken, J.P., Suurs, L.C., and Visser, R.G. (2005). Mutan produced in potato amyloplasts adheres to starch granules. *Plant Biotechnol J* 3, 341-351.
6. Nazarian Firouzabadi, F., Kok-Jacon, G.A., Vincken, J.P., Ji, Q., Suurs, L.C., and Visser, R.G. (2007). Fusion proteins comprising the catalytic domain of mutansucrase and a starch-binding domain can alter the morphology of amylose-free potato starch granules during biosynthesis. *Transgenic Res* 16, 645-656.
7. Xu, X., Visser, R.G.F., and Trindade, L.M. (2014). "Starch Modification by Biotechnology: State of Art and Perspectives," in *Starch Polymers: From Genetic Engineering to Green Applications*. Elsevier B.V., 79-104.



## NMR spectroscopic methods in engineering of sugar acid pathways in yeast

*Hannu Maaheimo, Martina Andberg, Yvonne Nygård, Peter Richard, David Thomas, Jonas Excell, Harry Boer, Mervi Toivari, Laura Ruohonen, Anu Koivula and Merja Penttilä*

hannu.maaheimo@vtt.fi

VTT Technical Research Centre of Finland, Espoo, Finland

In our projects on engineering sugar acid related pathways, the structural analysis of the products by NMR has been a major task, because in addition to the pyranose and furanose ring structures, these molecules may also form lactone rings, and some of the molecules we have been interested in have also keto groups, potentially leading to enol type structures in some conditions<sup>1</sup>. An additional challenge is the symmetry of molecules like mucic acid, in which cases positional <sup>13</sup>C labelling has been used to turn the molecules unsymmetrical from NMR point of view.

The product of a sugar acid forming enzymatic reaction is often a lactone which is not very stable. Therefore, following the reaction in real time has proven an invaluable tool in identification of the initial product of the enzyme and the rate of its spontaneous opening<sup>1,2</sup>. This method is also a way to follow enzyme kinetics and, using modern water suppression methods, the enzyme reaction can be carried out even in 100 % H<sub>2</sub>O buffer (instead of the normal D<sub>2</sub>O). This is important, when the water itself participates the reaction or if the enzyme preparation (a cell extract) has a low activity.

If the aimed product has several molecular forms that can spontaneously be converted to one another (like a lactone to an open chain sugar acid), it is of interest, whether this conversion takes place already intracellularly and which one of the forms is exported from the cells. Therefore, we have used *in vivo* <sup>1</sup>H NMR of engineered, overproducing yeasts to follow the production and exportation rates of xylonolactone and xylonic acid<sup>3</sup>. A bonus achieved by these experiments is that intracellular pH can also be determined, because the <sup>1</sup>H signals of the proton close to the carboxylic group respond to the pH. This is important data, because pH drop due to the acid production can often compromise the viability of the cells.

In addition, the so called ligand-based NMR methods on ligand-protein interactions can be very useful in identification of the binding geometry of the ligand to a particular enzyme<sup>4</sup>. These methods do not need labelled proteins and the protein concentration can be very low.

### Literature

1. Boer H. *et al.*, *Appl Microbiol Biotechnol*, **2009**, 86, 901-9.
2. Andberg M, *et al.*, *J. Biol. Chem.*, **2012**, ;287, 17662-71.
3. Nygård Y, *et al.*, *Metab. Eng.*, **2014**, 25, 238-247
4. Maaheimo, *et al.*, *Biochemistry* **2000**, 39, 12778-88.



# Author Index

## A

Aachmann Finn L.	102, 185
Abe Koichi	163
Abot Anne	34
Abou Hachem Maher	39, 70, 82, 90, 146, 164, 186, 190, 200, 203
Adlercreutz Patrick	84, 119, 147, 150
Adrien Amandine	77
Aerts Johannes	204
Agger Jane	169
Aguiar Tatiana Q.	78
Alalouf Onit	143
Albertsen Mads	201
Albesa-Jové David	48, 88
Almdal Kristoffer	90
Álvarez-Aliaga Teresa	176
Alvira Pablo	79
An Eunbae	131
Anbarasan Sasikala	80, 192, 206
Andberg Martina	43, 81, 209
Andersen Susan	82
Ando Toshio	56
Antonopoulou Io	83
Aragunde Hugo	48, 88
Arakawa Takatoshi	40
Archer David	54
Ardá Ana	170
Ardevol Albert	171, 173
Arnal Gregory	79
Arntzen Magnus Ø.	164, 193
Aronsson Anna	84, 187
Ates Ozlem	85
Auer Lucas	34
Aurelius Oskar	187
Auvinen Petri	140

## B

Bägenholm Viktoria	183, 187
Bahrim Gabriela	55
Bai Yuxiang	41, 86
Balzer Simone	102
Barthomeuf Janice	192
Beccia Maria Rosa	112
Belova Olga	105
Benešová Eva	121, 135, 148
Bengtsson Oskar	193
Bennati-Granier Chloé	51, 112
Berrin Jean-Guy	51, 60, 87, 112, 169
Biarnés Xevi	48, 88, 197
Bidiau Nicolas	77
Biely Peter	169
Bignon Christophe	87

Binay Baris	206
Birch Johnny	90
Bitetti Federica	101
Bjerrum Morten J.	111
Blennow Andreas	65
Blythe Martin	54
Bobrov Kirill	91, 181
Boer Harry	209
Bøjstrup Marie	39
Bolam David	73
Bordes Florence	189
Borisova Anna	91, 92
Bott Rick	178
Bouchoux Antoine	93
Bourdin Gilles	195
Bourne Yves	202
Bozonnet Sophie	79, 194
Brannigan James A.	202
Brás Joana L.A.	94, 199
Brewer M. Kathryn	66
Bridiau Nicolas	168
Bromley Jennifer	58
Brown Steven P.	58
Brugger Dagmar	53
Bruggink Cees	95
Bryant David	123
Buchtová Patricie	135
Bule Pedro	199
Busk Peter Kamp	201
Busse-Wicher Marta	58

## C

Cannella David	96, 111
Carciofi Massimiliano	65
Cardoso Vania	101
Carvalho Ana L.	94
Chai Wengang	94
Chakraborty Soumyadeep	97
Champion Charlotte	51, 112
Chang Ya-Chih	98
Chen Jeen-Kuan	149
Chen Jin-Ting	149
Cheng Ting-Jen Rachel	98
Cheng Wei-Chieh	98
Cherviakova Daria	91
Chiku Kazuhiro	162
Cho Jaeyoung	131
Chong Sun-Li	59, 99
Christakopoulos Paul	83, 106, 161
Christensen Lars H.	47
Christison Terri	95
Cioci Gianluca	100
Clarke Anthony J.	45
Claverie Marion	61, 100
Cobucci-Ponzano Beatrice	101, 122
Codera Victoria	108
Collic-Jouault S.	62, 145
Comino Natalia	67
Courtade Gaston	102
Crost Emmanuelle H	71
Cuskin Fiona	73

Czabany Tibor	46
Czjzek Mirjam	68

## D

Daly Paul	54
Daniellou Richard	118
Das Debasish	115
Davies Gideon	37, 109, 116, 204
De Maria Leonardo	47, 109
De Santi Concetta	103
de Vos Willem M	71
de Vries Ronald P.	36, 105, 152, 174
Delbarre-Ladrat C.	62, 145
Delmas Stephane	54
Derba-Maceluch Marta	59, 99
Devlamynck Tim	104
di Cola Corinna Schiano	101
Di Falco Marcos	174
Dianteill Lucie	93
Dieterich Johannes	177
DiGuilmi Anne-Marie	160
Dijkhuizen Lubbert	41, 86, 104, 157, 158, 198
Dilokpimol Adiphol	105
Dimarogona Maria	92, 106, 161
Dobrurowska Justyna M.	86, 157, 158
Dols-Lafargue M.	145
Domingues Lucilia	78
Dozen Satoshi	165
Duffieux Delphine	68
Dukhande Vikas	66
Dumon Claire	34, 79, 93, 123, 194
Dupree Paul	54, 58, 109
Dupree Ray	58

## E

Eijsink Vincent G. H.	92, 102, 144, 169, 193
Ejby Morten	70, 190
Ekholm Filip S.	107
Eneyskaya Elena	91, 181
Excell Jonas	209

## F

Faijes Magda	108, 197
Fák Frida	72
Falck Peter	147
Faryar Reza	147
Fauré Régis	79, 93
Feizi Ten	94
Felby Claus	96, 111
Felice Alfons	136
Fernandez-Fuentes Narcis	123
Ferreira Luís M.A.	94
Ficko-Blean Elizabeth	68
Fontes Carlos M.G.A.	94, 101, 114, 199
Forsberg Zarah	102
Foumani Maryam	52
Frandsen Kristian E.H.	109
Fredslund Folmer	70
Freire Filipe	114

Fruitier-Arnaudin Ingrid	168
Fujimoto Zui	126
Fujiwara Takaaki	49
Fukamizo Tamo	133, 165
Fukuda Kenji	110
Fushinobu Shinya	40, 200

## G

Gaenssle Aline L.	111
Gama Francisco M.	78
Gancel Charlotte	195
Gandla Madhavi Latha	59
Garajová Soňa	51, 112
Garrigues Christel	90
Gentry Matthew S.	66
Gerwig Gerrit J.	158
Gherbovet Olga	79
Giglio Rosa	101
Gilbert Harry J.	73
Gimbert Isabelle	51, 112
Giocci Gianluca	69
Goldschmidt Ruth	175
Gomes Thiago	58
Gómez Hansel	42
Gonaus Christoph	53
Gor Jayesh	129
Goyal Arun	97, 113, 114, 115, 172, 199
Grantham Nicholas J.	58
Gregory Rebecca	116
Grimaud Florent	61
Grimberg Noam	117
Grisel Sacha	51, 87, 112
Groisillier Agnes	68
Gruber Karl	46
Gruet Antoine	87
Gudmundsson Mikael	178
Guerin Marcelo	48, 67, 88
Guerlesquin Françoise	202
Guigliarelli Bruno	112
Guillotin Laure	118
Gulshan Ara Kazi Zubaida	119
Gupta Ashutosh	115
Gupta Munishwar N.	172, 199

## H

Hägglund Per	186
Hahn Johnnie	120
Hakulinen Nina	43
Haltrich Dietmar	53, 136
Hansen Morten Ejby	146
Hansson Henrik	178
Haon Mireille	51, 87, 112
Harðarson Hörður Kári	90
Hardy-Goddard Jemma	71
Harris Paul	120
Hasani Sumati	120
Haydon Ian	120
Hebelstrup Kim H.	65
Heiskanen Annamari	107
Helin Jari	107



Henriksen Anette	39
Henrissat Bernard	34, 69, 101, 109, 194
Hernandez-Raquet Guillermina	34, 194
Heu Tia	120
Heux Stéphanie	189
Hildén Kristiina	105, 140, 152, 174
Hindsgaul Ole	39, 82
Hinz Sandra	55
Hlat-Glembová Katarína	121
Hou Yaxi	180
Hsieh Chia-wen C.	96
Huang Linya	98
Huang Shi-Hsien	98
Hummel Michael	80, 206
Hurtado-Guerrero Ramón	173
Hurum Deanna	95
Husodo Satrio	66
Hüttner Silvia	134

## I

Iacono Roberta	101, 122
Ibrahim Romany	72
Igarashi Kiyohiko	38, 56
Iglesias-Fernández Javier	171, 173
Ioannou Eleni	123
Ipsen Richard	90, 186
Isaksen Trine	92
Ishida Takuya	38
Itävaara Merja	153
Ivanen Dina	91

## J

Jaito Nongluck	49, 124
Jakobsdóttir Greta	72
Janecek Stefan	125
Jensen Detlef	95
Jensen Jan H.	47
Jiang Jianbing	204
Jiménez-Barbero Jesús	170
Johansen Katja S.	109
Johansson Simon	195
Jones Aubrey	120
Jönsson Jonas	119
Jönsson Leif	59
Jørgensen Henning	96
Juge Nathalie	71
Juvonen Minna	180

## K

Kajala Ilkka	180
Kakutani Ryo	44
Kallemeijn Wouter	204
Kamerling Johannis P.	86, 157, 158
Kamino Kei	126
Kaneko Satoshi	126
Kanelli Maria	106
Kaper Thijs	178
Karkehadadi Saeid	178
Kärkönen Anna	127

Karlsson Eva Nordberg	84, 147, 150, 179
Karnaouri Anthi	161
Katayama Takane	200
Kell Laura	45
Kellock Miriam	128
Kempainen Katariina	138
Khan Sanaullah	129
Kharade Sampada S.	144
Kim Doman	131
Kim Jiyoun	131
Kim Seonghun	130
Kimura Atsuo	139, 166, 188
Kirkegaard Rasmus	201
Kitamura Yoshiaki	126
Kitaoka Motomitsu	40, 132, 162, 163, 200
Kitaoku Yoshihito	133
Klaubauf Sylvia	134
Koivula Anu	43, 56, 209
Kokolski Matthew	54
Koropatkin Nicole	64, 144
Kotiranta Titta	107
Koutaniemi Sanna	55, 99, 127
Kováčová Veronika	148
Koval Tomáš	135
Kovalová Terézia	135
Kracher Daniel	136
Kračun Stjepan K.	35
Králová Blanka	121
Krejzová Jana	137
Křen Vladimír	137
Krondorfer Iris	53
Kruczewicz Katarzyna	65
Kruus Kristiina	56, 128, 138, 196
Kubo Akiko	44
Kuchtova Andrea	125
Kulcinskaja Evelina	72, 179, 187
Kulik Natalia	137
Kulminkaya Anna	91, 181
Kumagai Yuya	139
Kuriki Takashi	44
Kuuskeri Jaana	140
Kwiatkowski Kurt J.	144

## L

Labourdette Delphine	34
Ladevèze Simon	69
Lafite Pierre	118
Lahtinen Manu	177
Laine Pia	140
Lamarre Sophie	34
Lamsa Michael	120
Lang Weeranuch	139
Lange Lene	201
Lansky Shifra	141, 142, 143, 175
Larkin Jennifer	57
Larocque Robert	68
Larsbrink Johan	144
Lauro Federico M.	101
Laville Elisabeth	69, 189, 194
Lawoko Martin	59
Lazewska Ida	155

Lazuka Adèle	34
Le Bourgeois Pascal	189
Le Gall Gwenaelle	71
Lebaz Noureddine	93
Lebellenger Lou	62, 145
Leberre Véronique Anton	34
Leino Reko	154, 170, 177
Leisola Matti	192
Leonov Laura	55, 83
Leth Maria Louise	146
Li Lin-Xiang	169
Lilley Kathryn S.	58
Lin Janine	120
Linares-Pastén Javier A.	119, 147, 176
Linder Markus	63
Lipovová Petra	135, 148
Liu Amy	178
Liu Chao-Lin	149, 191
Liu Fang	120
Liu Siqi	203
Liu Yan	94
Liu Yuan	132
Lluch José M.	42
Lo Leggio Leila	47, 109, 200
Logan Derek	147, 187
Lombard Vincent	101
Lowe Elisabeth	73
Ludwig Roland	136
Luley-Goedl Christiane	46
Lundell Taina	140
Lundemo Pontus	119, 150
López Cesar A.	157

## M

Maaheimo Hannu	180, 209
MacCormick Benjamin	52
MacKenzie Alasdair	144, 169
Maehara Tomoko	126
Mai-Gisondi Galina	151
Maina Ndegwa Henry	180
Mäkelä Miia R.	105, 140, 152, 174
Malla Sunir	54
Mangan David	156
Mansouri Sadegh	105, 152
Marjamaa Kaisa	153, 182
Martens Eric	73, 198
Marungruang Nittaya	72
Masgrau Laura	42
Master Emma	52, 151, 159
Matsui Hirokazu	49
Matsumoto Shinpei	110
Matsunaga Kana	166
Maugard Thierry	77, 168
Mavrynsky Denys	154
McBride Mark J.	144
McCleary Barry	57, 82, 155, 156
McGeough Páraic	155
McKie Vincent	57, 156
Meekins David A	66
Mellerowicz Ewa	59, 99
Mendoza Maria Fernanda	42

Meng Xiangfeng	104, 157, 158
Michel Gurvan	68
Mikkelsen Nils	178
Møller Marie S	39
Mollerup Filip	159
Monsan Pierre	61, 100
Montanier Cédric	93, 160
Moracci Marco	101, 122
Morchain Jérôme	93
Morel Sandrine	61
Morgavi Diego	194
Mori Haruhide	49, 124, 139, 166, 188
Mortimer Jenny C.	58
Moulis Claire	61, 100
Mukherjee Joyeeta	172, 199
Mullikin Ronald	120
Mulloy Barbara	129
Muraleedharan Madhu Nair	161
Musilová Šárka	148
Muto Hirohiko	49

## N

Najmudin Shabir	114
Nakai Hiroyuki	162, 163
Nakajima Masahiro	163
Nakamura Akihiko	38, 56
Nam Young-Woo	40
Navarro David	87
Ndeh Dider	73
Nekiunaite Laura	164
Nguyen Thi Thanh Hanh	131
Nidetzky Bernd	46, 50
Nielsen Morten Munch	65
Nielsen Per Halkjær	201
Niemelä Ritva	107
Nihira Takanori	162
Nikerel Emrah	208
Nikolaev Igor	178
Nikolaivits Efstratios	106
Nikolovski Nino	58
Nishimoto Mamoru	40, 132, 162
Nordberg-Karlsson Eva	119, 176
Nouaille Sébastien	189
Numata Tomoyuki	133
Nygård Yvonne	209
Nyman Margareta	72
Nyyssölä Antti	180

## O

O'Donohue Michael	34, 79, 123, 160
Ohnuma Takayuki	133, 165
Ohtsubo Ken'ichi	162
Oksanen Ilona	140
Okuyama Masayuki	139, 166, 188
Oliveira Carla	78
Olsson Lisbeth	134
Olsson Mats H. M.	47
Oner Ebru Toksoy	85, 208
Overkleeft Herman	204
Owen C David	71

## P

Paananen Arja	63
Paës Gabriel	60
Palcic Monica	39, 65
Palma Angelina S.	94
Panchadhayee Rajib	170
Parikka Kirsti	159
Parkkinen Tarja	43
Passerini Delphine	61
Pastinen Ossi	192
Paulin Lars	140
Pavkov-Keller Tea	46
Pawar Prashant Mohan-Anupama	59
Payne Christina M.	92
Pélissier Marie-Cécile	202
Penttilä Merja	33, 43, 81, 209
Pere Jaakko	182
Perkins Stephen J.	129
Peterbauer Clemens	53
Petersen Bent O.	82
Peuronen Anssi	177
Pijning Tjaard	41, 157, 158
Pilgaard Bo	201
Piot Jean-Marie	168
Pitkänen Virve	107
Planas Antoni	48, 88, 108, 173, 197
Pope Phil B.	144
Porkka Kaija	127
Portais Jean-Charles	189
Potocki-Véronèse Gabrielle	34, 69, 189, 194
Poulsen Jens-Christian N.	47, 109
Poupard Nicolas	168
Prates José A.M.	94
Preims Marita	136
Puchart Vladimír	169
Pullan Steven	54
Puranen Terhi	56
Pynnönen Henna	107

## R

Rahikainen Jenni	56, 128, 196
Rahkila Jani	170
Raich Lluís	171
Rani Aruna	172
Raththagala Madushi	66
Ratiskol Jacqueline	62, 145
Rebuffet Étienne	68
Record Eric	51, 112
Reddy Sumitha	72, 187
Remaud-Siméon Magali	61, 100, 189
Ribeiro Diana	94
Ribeiro Teresa	199
Ribitsch Doris	46
Richard Peter	209
Robe Patrick	194
Rogniaux Hélène	51
Rogowski Artur	73
Røhr Aasmund K.	92
Rohrer Jeff	95
Rojas-Cervellera Victor	173

Rosengren Anna	72, 187
Rouvinen Juha	43, 192
Rova Ulrika	83, 161
Rovira Carme	171, 173
Ruohonen Laura	209
Rychkov Georgy	91
Rytioja Johanna	174

## S

Saarinen Juhani	107
Saburi Wataru	49, 124
Sadahiro Juri	139
Salama Rachel	141, 142, 175
Salas-Veizaga Daniel Martin	176
Salavirta Heikki	140, 153
Saloheimo Markku	81
Saloranta Tiina	177
Samejima Masahiro	38
Sandgren Mats	92, 106, 178
Sardari Roya R.R.	179
Sato Mayo	40
Satoma Tero	107
Schmölzer Katharina	46
Schols Henk	55
Schückel Julia	35, 127
Schwab Helmut	46
Sebban-Kreuzer Corinne	202
Sepúlveda Goreti	78
Séverac Childéric	93
Severac Etienne	61, 100
Shabalin Konstantin	91, 181
Shaik Shahnoor Sultana	65
Sharma Kedar	113
Shi Qiao	180
Shoham Gil	141, 142, 143, 175
Shoham Yuval	117, 141, 142, 143, 175
Shvetsova Svetlana V.	181
Shwartzhtien Omer	141
Sietio Outi-Maaria	174
Siika-aho Matti	138, 182
Sim Lyann	39
Simmons Thomas J.	58
Sinquin Corinne	62, 145
Sixta Herbert	80, 206
Sjöberg Johan Svantesson	84, 183, 187
Skaf Munir	58
Skjåk-Braek Gudmund	185
Skjot Michael	47
Slámová Kristýna	137
Sletta Håvard	185
Slotboom Dirk Jan	70
Smalås Arne Oskar	103
Smola Miroslav	148
Smolander Olli-Pekka	140
Soetaert Wim	104
Solomon Hodaya V.	142, 143
Song Letian	184
Sorieul Mathias R.	58
Sørli Morten	92
Spiwok Vojtěch	121, 148
Ståhlberg Jerry	92

Stålbrand Henrik	72, 84, 183, 187
Stanisci Annalucia	185
Stender Emil G. P.	186
Stoica Leonard	53
Storgårds Erna	153
Stott Katherine	58
Strazzulli Andrea	101, 122
Stringer Maria A.	109
Su Zhengding	205
Sugimoto Naohisa	163
Suh Ye-seul	131
Sunner Hampus	134
Sunux Sergio	178
Suurnäkki Anna	196
Suzuki Erika	162
Svensson Birte	39, 70, 82, 90, 164, 186, 190, 200, 203
Sychantha David	45
Sylvestre Michel	184

## T

Taberman Helena	43
Tagami Takayoshi	166, 188
Taguchi Hayao	163
Tailford Louise E	71
Taira Toki	133
Takaha Takeshi	44
Takahashi Yuta	163
Tan Too Shen	191
Tanaka Yuka	124
Tarquis Laurence	69
Tauzin Alexandra	189
Taylor Garry L	71
te Poele Evelien	104
Telli Kubra	206
Tenkanen Maija	55, 59, 99, 151, 159, 180
Teter Sarah	120
the CESBIC consortium	37
Theilmann Mia Christine	190
Thomas David	209
Thygesen Lisbeth G.	96
Timoharju Tommi	192
Toivari Mervi	209
Tøndervik Anne	185
Topakas Evangelos	83, 106, 161
Torpenholt Søs	47
Tovborg Morten	109
Toyoizumi Hiroyuki	163
Tranier Samuel	69
Trindade Luisa M.	207
Trouilh Lidwine	34
Tsai Min-Lang	191
Tsang Adrian	174, 184
Turunen Ossi	80, 192, 205, 206
Tuveng Tina R.	193

## U

Uchihashi Takayuki	56
Uchiyama Taku	56
Ufarté Lisa	194
Urashima Tadasu	110

## V

Vaaje-Kolstad Gustav	102, 164, 193
Vafeiadi Christina	195
Val-Cid Cristina	197
van Bueren Alicia Lammerts	198
Van Calsteren Marie-Rose	90
van der Kaaij Rachel M.	86
van Munster Jolanda	54
Vander Kooi Craig W.	66
Varjosalo Markku	140
Várnai Anikó	92, 169, 182
Verma Anil Kumar	113, 114, 199
Vernet Thierry	160
Viborg Alexander Holm	200
Vidal-Melgosa Silvia	127
Viikari Liisa	182
Vikman Minna	153
Vilkman Anja	107
Vincent Florence	202
Virkki Liisa	180
Visser Jaap	55
Visser Richard G.F	207
Vlasova Olga L.	181
Vogensen Jens	203
Voutilainen Sanni	63
Vuillemin Marlène	61, 100
Vujicic-Zagar Andreja	70
Vuong Thu	52

## W

Wahlström Ronny	196
Walshaw John	71
Walton Paul	109, 116
Wang Quanhui	203
Wang Yawei	205, 206
Watanabe Ken-ichi	166
Weber Hansjörg	46
Westereng Bjørge	169
Westh Peter	47
Widner William	120
Wiebenga Ad	152
Wilkens Casper	82, 201
Willassen Nils-Peder	103
Willats William G. T.	35, 127
Williamson Adele	103
Windahl Michael S.	39
Wong Chi-Huey	98
Woortman Albert	86
Wright Daniel	204

## X

Xiong Hairong	205, 206
Xu Jin	101
Xu Xuan	207

## Y

Yamashita Keitaro	166, 188
Yanase Michiyo	44

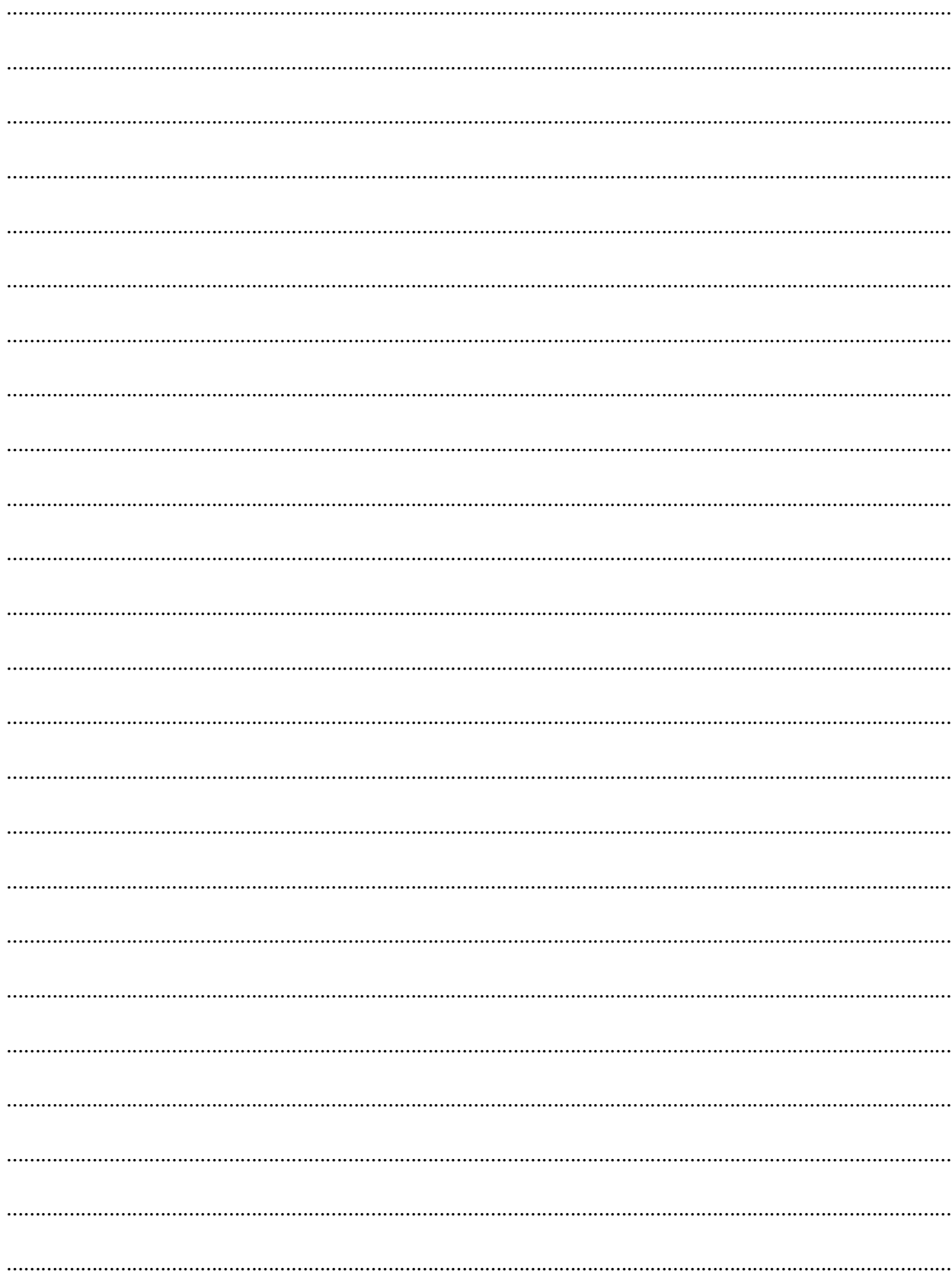


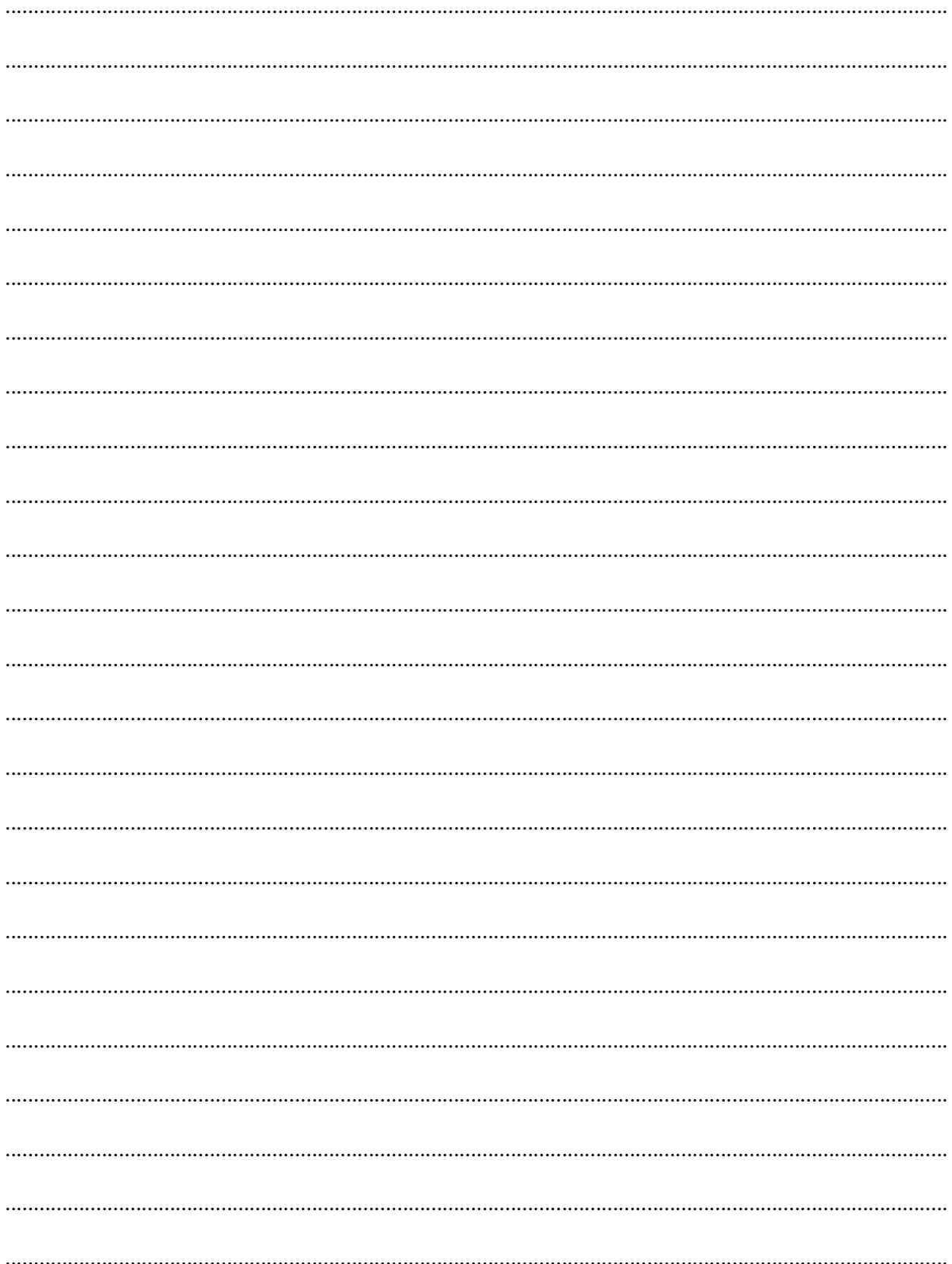
Yao Min	49, 166, 188
Yeh Chao-Hsien	149
Yildiz Songul Yasar	208
Yu Shin-Hye	131
Yu Tianyi	206
Yu Xiaolan	54, 58

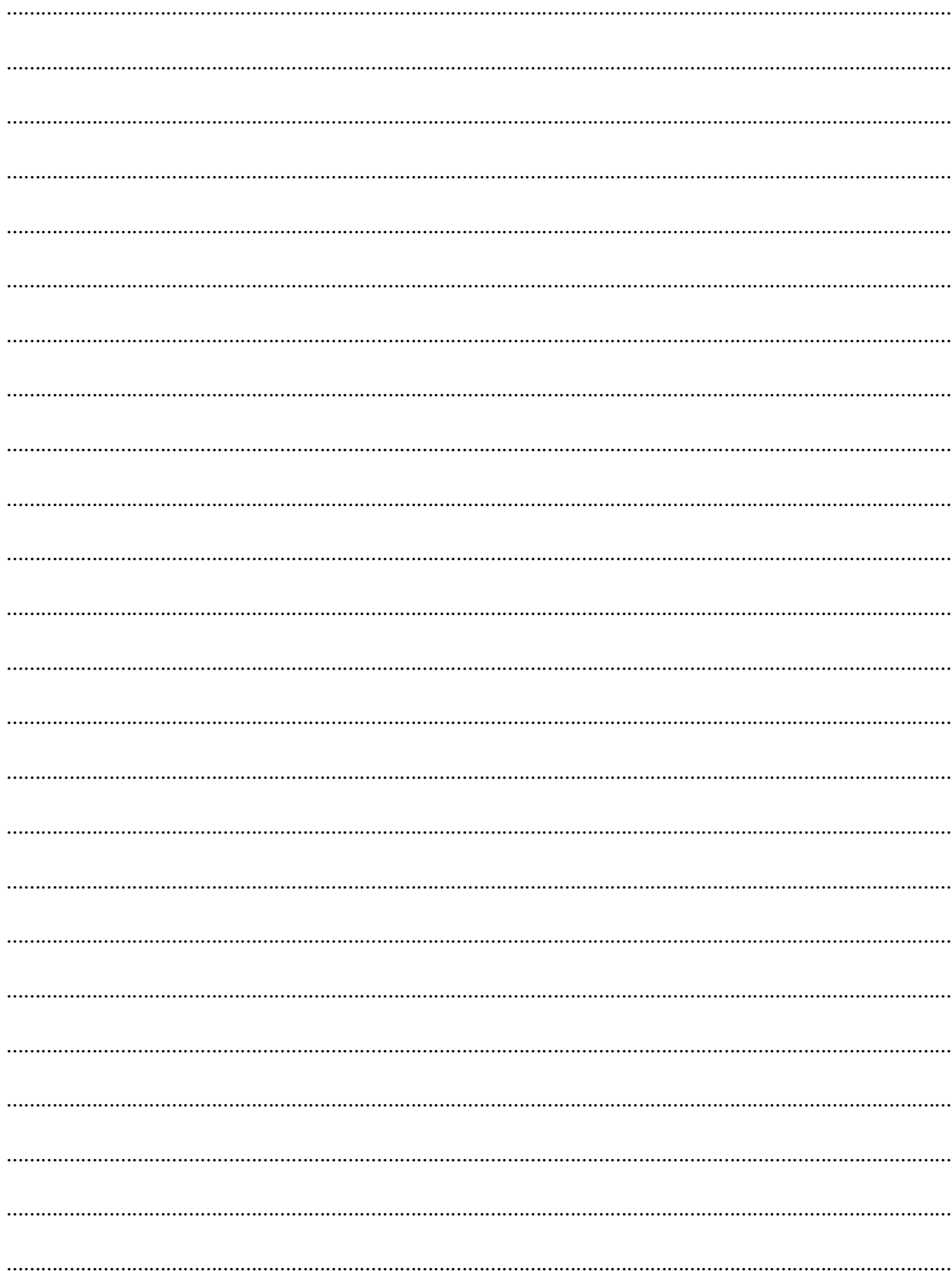
## **Z**

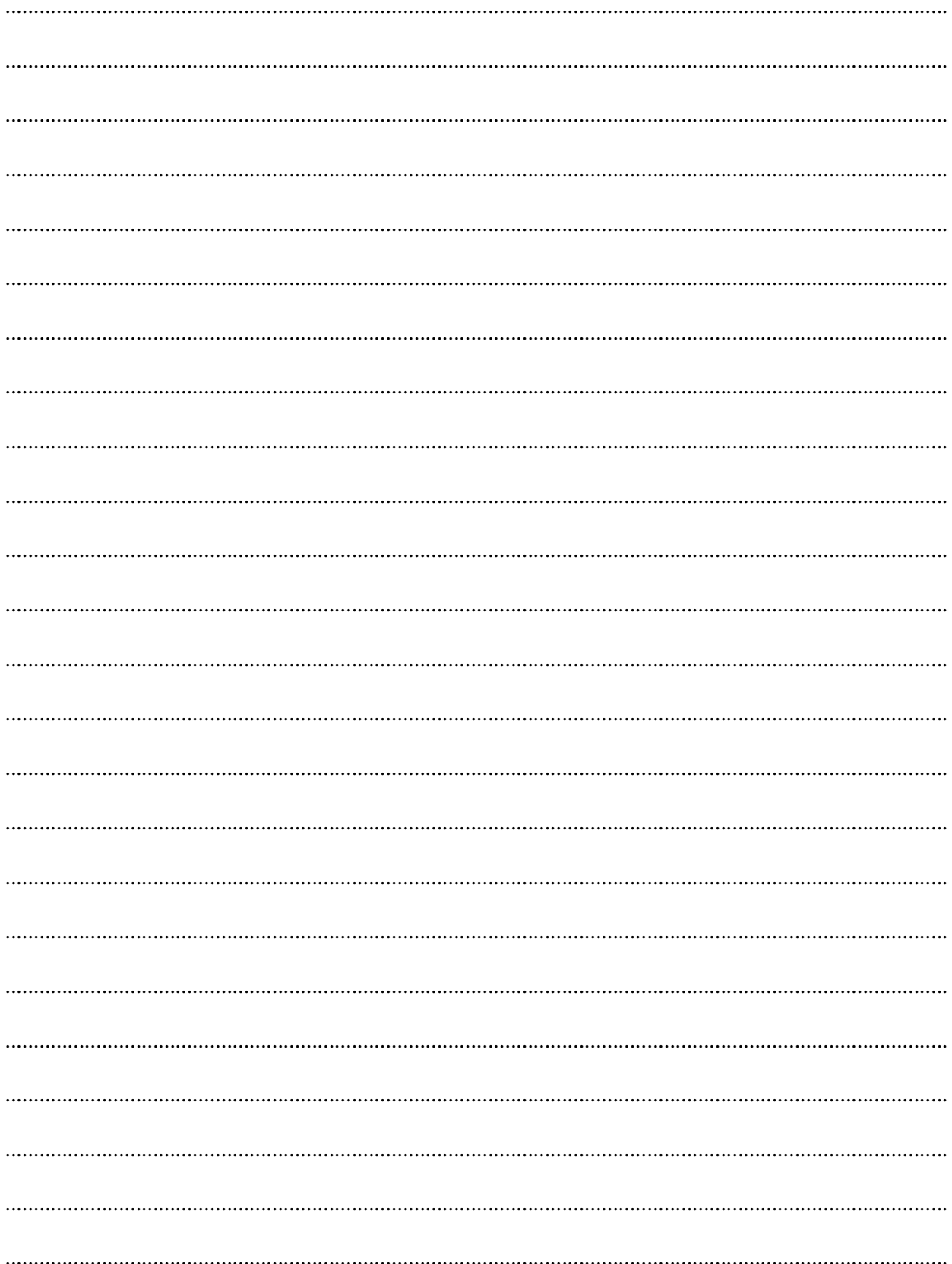
Zeidi Amal	93
Zhang Wei	205
Zhou Miaomiao	174
Zhou Simeng	112
Zhou Ying	205, 206
Zhou Yizhuang	101
Zhu Yongtao	144
Znameroski Elizabeth	120
Zykwinska Agata	62, 145

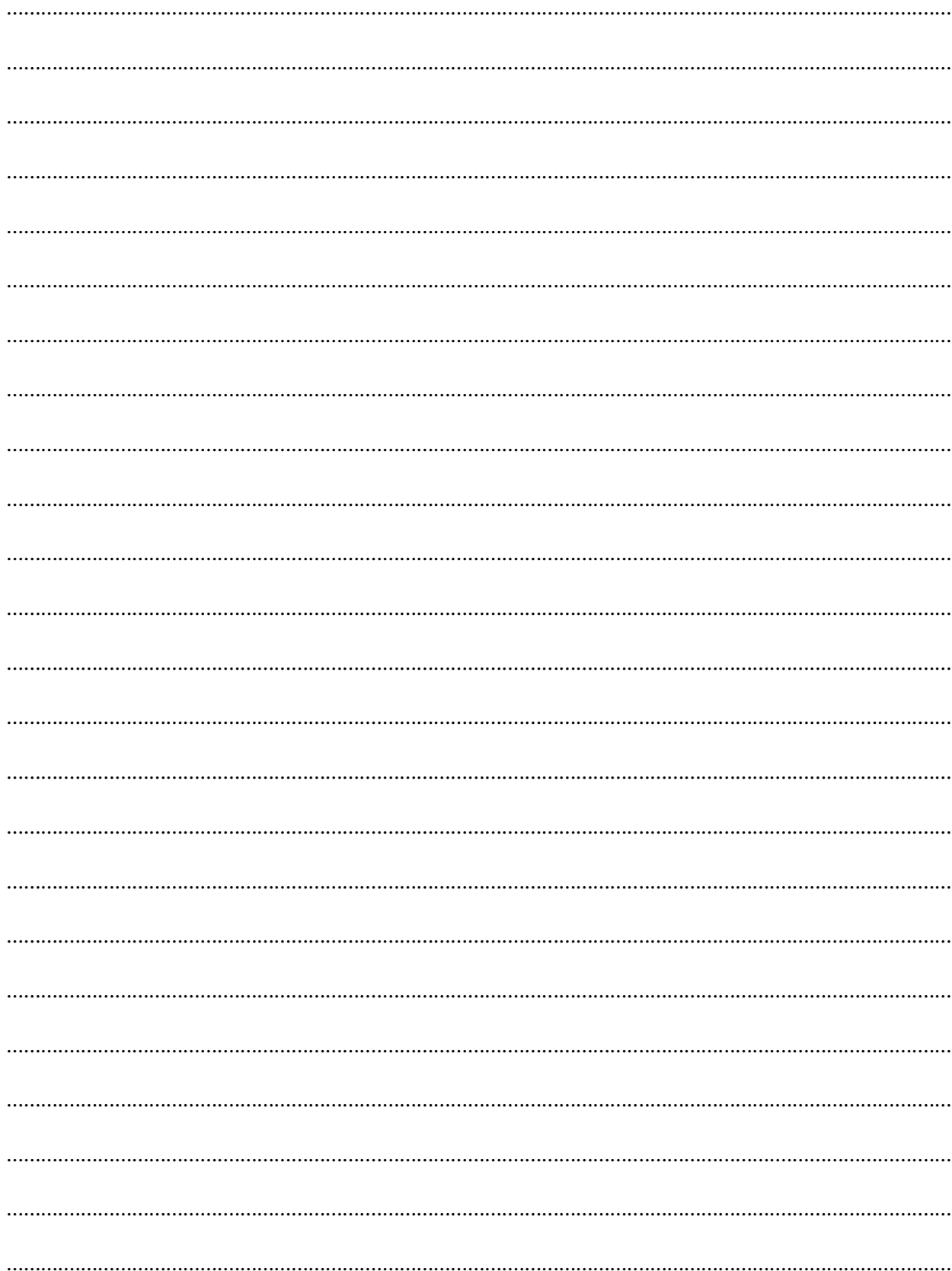


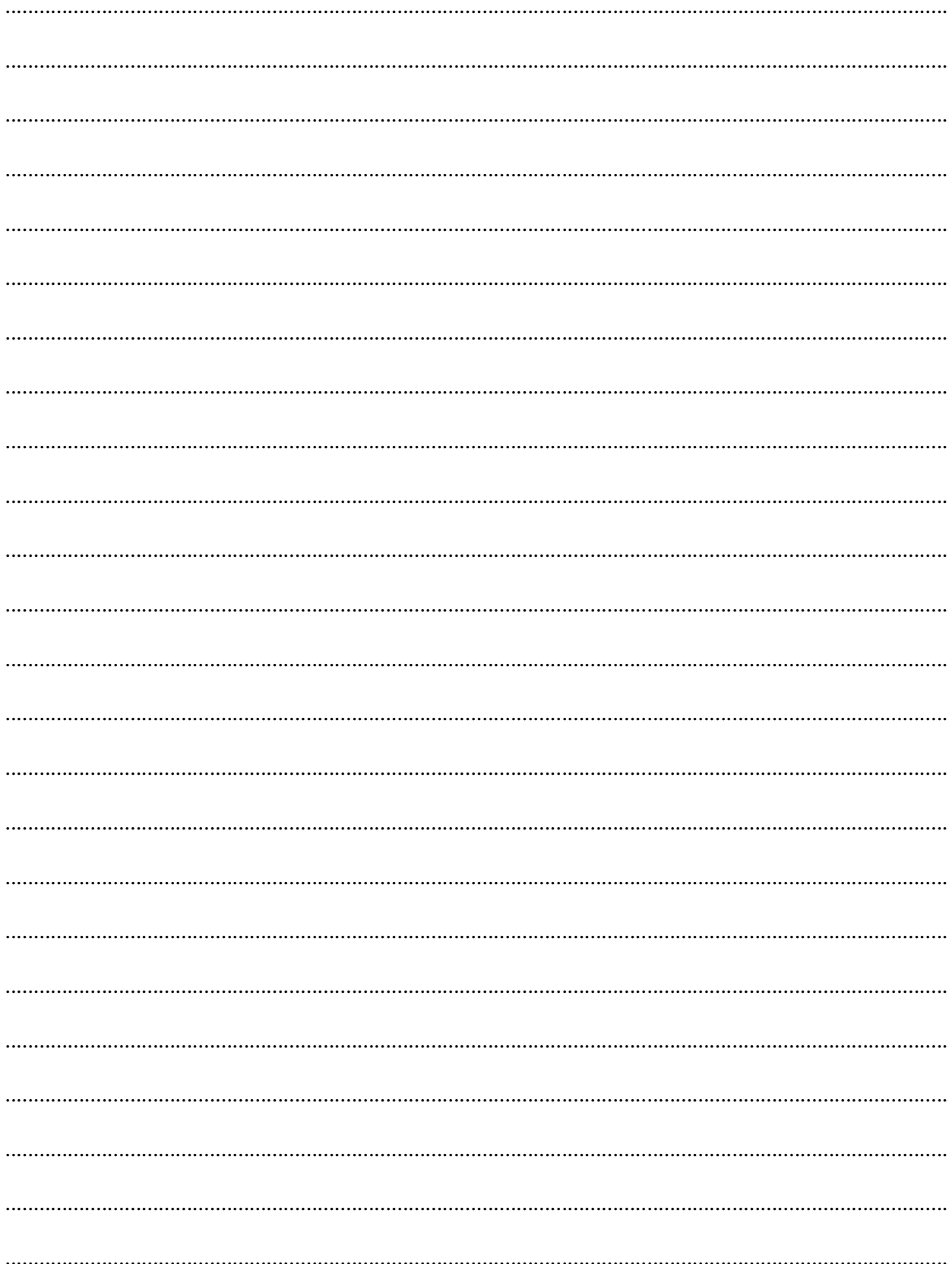
















Glico

novozymes  
Rethink Tomorrow

