



**HAL**  
open science

**DISCOVERING NOVEL ENZYMES BY  
FUNCTIONAL SCREENING OF PLURIGENOMIC  
LIBRARIES FROM ALGA-ASSOCIATED  
FLAVOBACTERIIA AND  
GAMMAPROTEOBACTERIA**

Marjolaine Martin, Marie Vandermies, Coline Joyeux, Renee Martin, Tristan Barbeyron, Gurvan Michel, Micheline Vandenbol

► **To cite this version:**

Marjolaine Martin, Marie Vandermies, Coline Joyeux, Renee Martin, Tristan Barbeyron, et al.. DISCOVERING NOVEL ENZYMES BY FUNCTIONAL SCREENING OF PLURIGENOMIC LIBRARIES FROM ALGA-ASSOCIATED FLAVOBACTERIIA AND GAMMAPROTEOBACTERIA. Microbiological Research, 2016, 186-187, pp.52-61. 10.1016/j.micres.2016.03.005 . hal-02137941

**HAL Id: hal-02137941**

**<https://hal.science/hal-02137941>**

Submitted on 23 May 2019

**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.

1 DISCOVERING NOVEL ENZYMES BY FUNCTIONAL SCREENING OF PLURIGENOMIC  
2 LIBRARIES FROM ALGA-ASSOCIATED FLAVOBACTERIIA AND GAMMAPROTEOBACTERIA

3  
4 **Marjolaine Martin<sup>1\*</sup>, Marie Vandermies<sup>2</sup>, Coline Joyeux<sup>1</sup>, Renée Martin<sup>1</sup>, Tristan Barbeyron<sup>3</sup>,**  
5 **Gurvan Michel<sup>3</sup>, Micheline Vandebol<sup>1</sup>**

6 <sup>1</sup> Microbiology and Genomics Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des  
7 Déportés 2, 5030 Gembloux, Belgium

8 <sup>2</sup> Microbial Processes and Interactions, Gembloux Agro-Bio Tech, University of Liège, Passage des  
9 Déportés 2, 5030 Gembloux, Belgium

10 <sup>3</sup> Sorbonne Université, UPMC Univ Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models,  
11 Station Biologique de Roscoff, CS 90074, F-29688, Roscoff cedex, Bretagne, France

12  
13 \* Corresponding author : [Marjolaine.martin@ulg.ac.be](mailto:Marjolaine.martin@ulg.ac.be)

14 **ABSTRACT**

15 Alga-associated microorganisms, in the context of their numerous interactions with the host and the  
16 complexity of the marine environment, are known to produce diverse hydrolytic enzymes with  
17 original biochemistry. We recently isolated several macroalgal-polysaccharide-degrading bacteria  
18 from the surface of the brown alga *Ascophyllum nodosum*. These active isolates belong to two  
19 classes: the *Flavobacteriia* and the *Gammaproteobacteria*. In the present study, we constructed two  
20 “plurigenomic” (with multiple bacterial genomes) libraries with the 5 most interesting isolates  
21 (regarding their phylogeny and their enzymatic activities) of each class (Fv and Gm libraries). Both  
22 libraries were screened for diverse hydrolytic activities. Five activities, out of the 42 identified in the  
23 natural polysaccharolytic isolates, were recovered by functional screening: a xylanase (GmXyl7), a  
24 beta-glucosidase (GmBg1), an esterase (GmEst7) and two iota-carrageenases (Fvi2.5 and Gmi1.3).  
25 We discuss here the potential role of the used host-cell, the average DNA insert-sizes and the used

26 restriction enzymes on the divergent screening yields obtained for both libraries and get deeper  
27 inside the “great screen anomaly”. Interestingly, the discovered esterase probably stands for a novel  
28 family of homoserine o-acetyltransferase-like-esterases, while the two iota-carrageenases represent  
29 new members of the poorly known GH82 family (containing only 19 proteins since its description in  
30 2000). These original results demonstrate the efficiency of our uncommon “plurigenomic” library  
31 approach and the underexplored potential of alga-associated cultivable microbiota for the  
32 identification of novel and algal-specific enzymes.

33 **KEYWORDS:** alga-associated microflora, marine bacteria, agarase, iota-carrageenase, kappa-  
34 carrageenase, esterase, beta-glucosidase, xylanase, marine enzymes

## 35 **INTRODUCTION**

36 For a long time, researchers have investigated environmental samples to identify novel microbial  
37 biocatalysts. Early efforts focused on discovering novel enzymes of known or newly described  
38 cultivable microorganisms. More recently, (sequence- or function-based) metagenomic screening  
39 has been included among the tools used to mine for novel microbial enzymes (Handelsman, 2004).  
40 Sequence-based metagenomic approaches provide access to non-cultivable microorganisms, but as  
41 enzymes are sought by sequence comparisons with known biocatalysts, only new variants of existing  
42 enzymes are discovered (Simon and Daniel, 2011). In contrast, function-based screening of  
43 metagenomic libraries can lead to discovering completely novel enzymes, while looking only at the  
44 function of the gene and not its similarities with known sequences. Yet searching for a particular  
45 enzyme type in a metagenome, is somewhat like looking for a needle in a haystack. It is fastidious,  
46 and yields are poor (Ekkers et al., 2012; Ferrer et al., 2015). Therefore the old-fashioned culturing  
47 approach, followed by selection of isolates showing activities of interest, still has a place in the hunt  
48 for novel enzymes. Preselection of “active” strains limits the number of genomes to be prospected  
49 and allows more focused work. Furthermore, even though culturing methods restrict the diversity of  
50 the findings, as only a very low percentage of existing microbes are cultivable (estimated at less than

51 1% in most environmental samples), novel enzymes and enzyme families far different from known  
52 ones can still be discovered in underexplored bacterial taxa and environments.

53 Bacteria associated with algae have been shown to produce many polysaccharide hydrolases,  
54 because of their complex and dynamic interactions with their hosts (Holmström et al., 2002; Martin  
55 et al., 2014b). We have recently demonstrated that the cultivable microbiota associated with the  
56 brown alga *Ascophyllum nodosum* is rich in macroalgal-polysaccharide-degrading bacteria (Martin et  
57 al., 2015). Furthermore, many novel species and genera have been identified in alga-associated  
58 microbiotas, suggesting that they constitute an interesting biotic environment for the discovery of  
59 new bacterial taxa and hence, original biocatalysts (Goecke et al., 2013). In the class *Flavobacteriia*  
60 (*Bacteroidetes*), for example, several polysaccharolytic genera were first identified on a macroalga:  
61 for example, *Cellulophaga* on the brown alga *Fucus serratus*, *Formosa* on the brown alga *Fucus*  
62 *evanescens*, and *Zobellia* on the red alga *Delesseria sanguine* (Barbeyron et al., 2001; Ivanova et al.,  
63 2004; Johansen et al., 1999). Novel glycoside hydrolase families have also been discovered in these  
64 genera (Michel and Czjzek, 2013), such as the iota-carrageenase (GH82) and  $\beta$ -porphyranase (GH86)  
65 families in *Z. galactinovorans* (Barbeyron et al., 2000; Hehemann et al., 2010). On the other hand,  
66 few studies have focused on identifying more classical hydrolytic enzymes in alga-associated  
67 bacterial species, such as cellulases, xylanases, beta-glucosidases, or esterases, even though such  
68 enzymes should differ markedly from their terrestrial homologs (Dalmaso et al., 2015). It thus seems  
69 obvious that cultivable alga-associated bacteria constitute an interesting source of a large range of  
70 novel hydrolytic enzymes.

71 In a previous study, we isolated and identified several algal-polysaccharide-degrading bacteria from  
72 the microflora associated with the brown alga *Ascophyllum nodosum* (Martin et al., 2015). All the  
73 isolates, some of which very probably represent new species, were taxonomically assigned to the  
74 classes *Flavobacteriia* and *Gammaproteobacteria*. They display diverse hydrolytic activities. In the  
75 present work, to identify novel enzyme-encoding genes from these original alga-associated bacteria,  
76 we have used an uncommon approach: constructing and screening “plurigenomic” (multiple bacterial

77 genomes) libraries. One library was constructed with the genomes of five *Flavobacteriia* isolates and  
78 one with the genomes of five *Gammaproteobacteria* isolates. Screening was done for agarase, iota-  
79 carrageenase and kappa-carrageenase, beta-glucosidase, endo-cellulase, xylanase, and esterase  
80 activity.

## 81 **MATERIALS AND METHODS**

### 82 **Construction of plurigenomic libraries with *Flavobacteriia* and *Gammaproteobacteria* strains**

83 Diverse algal-polysaccharide-degrading bacteria were isolated from the brown alga *Ascophyllum*  
84 *nodosum*, as described in our previous study (Martin et al., 2015). These bacteria were assigned to  
85 the classes *Flavobacteriia* and *Gammaproteobacteria*. Two plurigenomic libraries were constructed,  
86 one for each class. Five isolates per class were used. The *Flavobacteriia* used were the *Cellulophaga*  
87 isolates An8, An9, and An20 (16s rRNA sequence accession numbers: LN881186, LN881202,  
88 LN881252), the *Zobellia* isolate An14 (LN881227), and the *Maribacter* isolate An21 (LN881276). The  
89 *Gammaproteobacteria* used were the *Shewanella* isolates An4 and An36 (LN881152, LN881379), the  
90 *Pseudoalteromonas* isolate An33 (LN881360), the *Colwellia* isolate An23 (LN881284), and the  
91 *Paraglaciecola* isolate An27 (L881305). These ten isolates were selected on the basis of their original  
92 phylogeny and the intensity and/or diversity of their activities (Table 1). They were grown for 24 to  
93 48 hours at 20°C in 3 ml Zobell medium (Difco Marine Broth). Genomic DNA from each isolate was  
94 extracted as described by Cheng and Jiang (2006). DNA quantity and quality were checked,  
95 respectively, with the Qubit fluorometer (Invitrogen) and by gel electrophoresis through a 0.8%  
96 agarose gel. The genomic DNA of each strain was tested for restriction by *Sau3AI* or its isoschizomer  
97 *DpnII*. We then pooled, on the one hand, 3 µg genomic DNA of each *Flavobacteriia* isolate, and on  
98 the other hand, 3 µg genomic DNA of each *Gammaproteobacteria* isolate. The pool of genomic DNA  
99 from the *Flavobacteriia* isolates was partially restricted for 1 to 3 minutes with 0.2 U/µg DpnII (NEB),  
100 and the *Gammaproteobacteria* pool for 1-2 minutes with 0.3 U/µg *Sau3AI* (Roche). After elimination  
101 of small DNA inserts by size-selective polyethylene glycol-NaCl precipitation as described by Biver

102 and Vandenberg (2012), the DNA was purified by migration through a 1% low-melting-point agarose  
103 gel (Promega). DNA inserts exceeding 5 kb were recovered by beta-agarase digestion (NEB). The  
104 cloning vector *pHT01* (MoBiTec, Germany) was linearized with *BamHI* (Roche) and dephosphorylated  
105 (Dephos and Ligation kit, Roche). A vector:insert ratio of 1:3 was used to ligate the DNA inserts of  
106 each library into the *pHT01* cloning vector at 16°C overnight (T4 DNA ligase, Roche).  
107 Electrocompetent Electromax<sup>TM</sup> *Escherichia coli* DH10B cells (Life Technologies) were transformed  
108 with 1 µl ligation products. The average DNA insert size in each plurigenomic library was estimated  
109 by isolation and purification of 20 randomly chosen plasmids.

#### 110 **Screening the plurigenomic libraries for diverse enzymatic activities**

111 Almost 12600 clones of the *Gammaproteobacteria* (Gm) library and 15000 clones of the  
112 *Flavobacteriia* (Fv) library were isolated in 96-well plates and grown overnight in 2xYT liquid culture  
113 medium at 37°C. The liquid cultures were then replicated onto the various screening media and  
114 incubated at room temperature. Agarase, iota-carrageenase and kappa-carrageenase activities were  
115 detected on minimal medium (1 g/l Yeast Extract, 5 g/l Bacto-tryptone (MP, Biomedicals), 5 g/l NaCl  
116 (Merk)) containing, respectively, 1.5% agar, 2% iota-carrageenan, or 1% kappa-carrageenan (Sigma).  
117 Clones showing hydrolytic activity (a hole in the jellified medium for agarase or κ-carrageenase  
118 activity or complete liquefaction of the medium for ι-carrageenase activity) were considered positive.  
119 Minimal medium containing 1.5% agar and AZCL-HE-cellulose, -amylose or -xylan (birchwood)  
120 (Megazyme) was used to detect endocellulase, alpha-amylase, or endo-1,4-β-D-xylanase activity,  
121 respectively. A blue halo around a colony was indicative of hydrolase activity. Lipolytic activity was  
122 detected on minimal medium containing 1.5% agar and 3% Difco Lipase Reagent (Difco). Positive  
123 clones were detected by a clear halo around the colony. Beta-glucosidase activity was detected as  
124 described by Matteotti *et al.* (2011) on minimal medium containing 0.5% esculin and 0.1%  
125 ammonium iron (III) citrate (Sigma-Aldrich). A clone was identified as positive when a brown  
126 precipitate appeared around its colony. As no natural strain used to construct either library showed  
127 any amylase activity (Table 1), the libraries were not screened for this activity. As isolates An27 and

128 An33 showed beta-glucosidase activity, the *Gm* library was additionally screened for this activity  
129 (Table 1). Positive clones were isolated and their plasmids purified. Activity was confirmed by  
130 transforming other *E. coli* strains (DH5 $\alpha$ ) with the purified plasmid and testing recombinant clones on  
131 the screening medium corresponding to the observed activity.

### 132 **Sequencing, identification, and subcloning of the gene(s) putatively responsible for the observed** 133 **activities**

134 The DNA inserts of confirmed positive candidates were sequenced by Sanger sequencing (Germany)  
135 at GATC Biotech, and open reading frames (ORFs) were identified with ORF Finder (NCBI) and  
136 SnapGene<sup>TM</sup>. All putative proteins corresponding to ORFs identified in the inserts were analyzed with  
137 BlastP (NCBI) against the non-redundant GenBank database and the curated Swissprot database. The  
138 genes putatively conferring the observed activities (as judged by sequence similarity) were  
139 subcloned. Genes for subcloning were amplified with primers chosen to anneal in the 200-basepair  
140 region located upstream (forward primer) or downstream (reverse primer) from the coding  
141 sequence. A *Bam*HI restriction site was added on each primer. PCR amplifications were done with the  
142 Phusion<sup>®</sup> High-Fidelity DNA Polymerase according to the manufacturer's instructions (NEB, New  
143 England Biolabs). After amplification, the PCR products were restricted with *Bam*HI (Roche). The  
144 vector *pHT01* was also linearized with *Bam*HI. Vector dephosphorylation and ligations were done  
145 with the Roche Dephos&Ligation kit. *E. coli* DH5 $\alpha$  cells were transformed with the ligation products.  
146 Subclones were tested on the screening medium corresponding to the expected activity. The DNA  
147 insert of each positive subclone was checked at GATC. In order to know from which alga-associated  
148 strain the contig derived, we looked at the Blast results of the different ORFs constituting the contig  
149 (Table 2) and verified our hypotheses by PCR amplification (Taq polymerase, Roche) of part of each  
150 candidate insert in the genomic DNA of the bacterial isolate from which it was assumed to derive.

### 151 **Additional bioinformatic analyses**

152 Conserved domains and family motifs were sought in the Conserved Domain Database (NCBI), the  
153 Pfam database (Finn et al., 2014), and the CAZyme database using the CAZyme Analysis Toolkit (CAT)

154 (Lombard et al., 2014; Park et al., 2010). Operons were predicted with the Softberry FGENESB  
155 software (Solovyev and Salamov, 2011). Protein sequences were aligned with MAFFT (Kato et al.,  
156 2002). The sequence alignment with the iota-carrageenases were manually edited with Bioedit (©  
157 Tom Hall), based on the protein sequence of CgiA\_Af from *Alteromonas fortis*. Sequence alignment  
158 figures were arranged with EsPript3 (<http://escript.ibcp.fr>, Robert and Gouet, 2014). The maximum  
159 likelihood method with bootstrap values was used to construct phylogenetic trees with MEGA6  
160 (Tamura et al., 2013).

## 161 **RESULTS**

162 To identify novel hydrolases from marine bacteria, two plurigenomic libraries were constructed and  
163 screened for enzymatic activities. Pooled genomic DNA from the five Fv isolates or the five Gm  
164 isolates (Table 1) was restricted and inserted into the *pHT01* cloning vector. The DNA extraction  
165 protocol of Cheng and Jiang (2006) enabled us to extract 3 to 8 µg genomic DNA from 3 ml of each  
166 individual bacterial culture. The genomic DNA of the five Fv isolates was restricted with *DpnII* (as  
167 restriction with *Sau3AI* was unsuccessful) and that of the five Gm isolates was restricted with *Sau3AI*.  
168 The average insert size was estimated at 6.5 kb for the Fv library and 9 kb for the Gm library.  
169 Screening of the libraries for agarase, iota- and kappa-carrageenase, esterase/lipase, xylanase,  
170 endocellulase, and beta-glucosidase activity yielded five contigs containing functional genes from  
171 three of the ten bacterial isolates (An8, An33, and An27) (Table 2, Table S1).

### 172 ***Flavobacteriia* library**

#### 173 One Fv Iota-carrageenase candidate

174 In the Fv library only one activity (iota-carrageenase) was observed, two months after inoculation of  
175 the screening medium. The corresponding clone was Fvi2 (Table S1). Six ORFs were identified on the  
176 Fvi2 contig (Table 2). Only the protein encoded by *Fvi2\_5*, showing sequence identity to a  
177 hypothetical protein, could be responsible for the observed activity, as the other ORFs display  
178 sequence identity to characterized proteins that are not iota-carrageenases. Furthermore, the

179 second-best hit for the sequence of the protein encoded by *Fvi2\_5* is an iota-carrageenase of a  
180 *Cellulophaga sp.* (90% identity) (Table S2). The subclone with the *Fvi2\_5* gene was able to hydrolyze  
181 iota-carrageenans (Table 2, Table S1). The iota-carrageenase Fvi2.5 belongs to the GH82 family.

## 182 ***Gammaproteobacteria* library**

### 183 Nineteen *Gm* xylanase candidates

184 Nineteen clones were found to degrade xylan (Table S1). Their DNA inserts showed similar  
185 sequences. With the DNA insert sequences of the 19 clones, we were able to reconstitute a 12.4-kb  
186 GmXyl contig containing 10 ORFs, originating from the *Pseudoalteromonas sp.* An33 (Table 2, Figure  
187 1). Two complete ORFs (*GmXyl\_7* and *GmXyl\_9*) coding for proteins close to known endo- $\beta$ 1,4-  
188 xylanases were identified on the 19 clone inserts. The last gene locus (*GmXyl\_10*), closely related to  
189 other xylanases, was found only on some plasmids from positive clones and was always incomplete.  
190 We were able to retrieve the complete *GmXyl\_10* gene from the genomic DNA of the  
191 *Pseudoalteromonas sp.* An33 by PCR amplification (a reverse primer was designed on the basis of a  
192 sequence alignment with the ten closest proteins). Each putative xylanase-encoding gene (*GmXyl\_7*,  
193 *GmXyl\_9*, *GmXyl\_10*) was subcloned separately (Table S1). Only the subclone containing the *GmXyl\_7*  
194 gene could hydrolyze xylan. The GmXyl7 protein was found to be a GH8-family xylanase. GmXyl9 and  
195 GmXyl10 belong to the GH10 family, composed essentially of endoxylanases.

### 196 Ten *Gm* Iota-carrageenase candidates

197 Ten clones of the Gm library were able to hydrolyze iota-carrageenans. Their DNA insert sequences  
198 were found to contain identical ORFs and were used to construct a 13.5-kb contig (Table 2, Table S1,  
199 Figure 1). This contig, called Gmi1, also originates from the An33 *Pseudoalteromonas sp.* isolate. No  
200 gene on this contig was found to be closely related to a known iota-carrageenase, but three ORFs  
201 (*Gmi1\_2*, *Gmi1\_3*, *Gmi1\_5*) appeared to code for proteins closely related to uncharacterized  
202 hypothetical proteins and one (*Gmi1\_4*) for a protein closely related to an uncharacterized GH16-  
203 family protein (Table 2). As *Gmi1\_2* wasn't found on the DNA inserts of all ten positive clones, we  
204 discounted it as potentially responsible for the iota-carrageenase activity. The three other ORFs were

205 subcloned, and the subclone containing the *Gmi1\_3* gene was the only one found to hydrolyze iota-  
206 carrageenans. This iota-carrageenase (Gmi1.3) was assigned to the only known GH family containing  
207 iota-carrageenases: GH82.

#### 208 One *Gm* beta-glucosidase candidate

209 The GmBg contig was identified on the basis of beta-glucosidase activity. The protein encoded by its  
210 first gene showed low sequence identity to beta-glucosidases (Table 2). As the protein encoded by its  
211 second gene showed low sequence identity to endo-1,4- beta-glucanases (endocellulases), the  
212 corresponding clone was also tested on AZCL-cellulose, but no activity was observed under our  
213 screening conditions. The beta-glucosidase activity of *GmBg\_1* was confirmed by subcloning (Table 2,  
214 Table S1). The protein GmBg1 was classified in the GH3 CAZyme family.

#### 215 One *Gm* lipolytic candidate

216 Lastly, one clone was found to hydrolyze tributyrin. The sequence of its DNA insert revealed 8 ORFs  
217 with no sequence identity to any known lipolytic enzyme (Table 2). Nevertheless, the sequences of  
218 the proteins encoded by *GmEst\_6* and *GmEst\_7* were found to contain an  $\alpha/\beta$ -hydrolase domain  
219 (found in lipolytic enzymes). Subcloning of these two ORFs showed that only *GmEst\_7* was  
220 responsible for the esterase activity (Table S1). The subclones were also tested for lipase activity on  
221 minimal medium containing olive oil and trioctanoate, but proved unable to degrade these  
222 substrates. The GmEst contig was found, by PCR amplification, to originate from the *Paraglaciecola*  
223 *sp.* isolate An27.

## 224 **DISCUSSION**

### 225 **Functional screening of plurigenomic libraries: probing the “great screen anomaly”**

226 Functional metagenomic screening has emerged as the trendy approach to discovering novel  
227 enzymes. Yet its yield is generally poor, and this has led to intense discussion of its challenges. The  
228 studied environment as well as the host cells, expression systems, DNA extraction methods, DNA  
229 insert sizes, and screening methods used have all been pinpointed as bias-creating factors (Ferrer et  
230 al., 2015; Liebl et al., 2014; Uchiyama and Miyazaki, 2009). Here we have constructed plurigenomic

231 libraries, which can be viewed as “small-scale” metagenomic libraries. Knowing which  
232 microorganisms contributed their genomic DNA to our libraries and which enzymatic activities they  
233 displayed, we can get a closer look at the so-called “great screen anomaly” (Ekkers et al., 2012). Five  
234 *Flavobacteriia* isolates were used to construct one library, and five *Gammaproteobacteria* isolates to  
235 construct the other. *E. coli* cells transformed with these libraries were screened for hydrolytic  
236 enzyme activities and the inserts of positive clones were analyzed. This has enabled us to attribute  
237 functions to five genes, three of which (Fvi2\_5, Gmi1\_3, GmEst\_7) were not previously known to  
238 confer the observed activity. Yet only five activities were recovered, out of the 42 observed prior to  
239 screening (Table 1) for these 10 isolates: the iota-carrageenase activity of An8, the iota-carrageenase,  
240 xylanase, and beta-glucosidase activities of An33, and the esterase activity of An27. This screening  
241 yield seems rather low, especially since the bacterial isolates were preselected as displaying the  
242 activities for which we screened. Nevertheless, the yield is definitely higher than those generally  
243 obtained in less restricted studies using functional metagenomics (Ferrer et al., 2015; Uchiyama and  
244 Miyazaki, 2009). It is noteworthy that the yields of the two screens were not equal: only one active  
245 clone (1 pos/97 Mb screened) was detected in the Fv library, under our screening conditions, versus  
246 31 (1 pos/3.6 Mb screened) in the *Gammaproteobacteria* library. A first obvious explanation could  
247 be the host chosen for cloning and screening the genomic DNA. *E. coli*, the host used here, is a  
248 gammaproteobacterium. It is therefore probably best equipped genetically (in terms of promoter  
249 recognition, transcription, translation, and post-translational modifications such as protein folding  
250 and secretion) to express genes of other *Gammaproteobacteria* (Liebl et al., 2014). This hypothesis is  
251 supported by our previous functional metagenomic study of the microbiota associated with *A.*  
252 *nodosum*, where the esterase and glycoside hydrolase genes identified were mostly from *Alpha*- and  
253 *Gammaproteobacteria* (Martin et al., 2014a). Furthermore, even within each library screened here,  
254 the genes of different bacterial genera do not seem to have been equally expressed. In the *Gm*  
255 library, for instance, no genes from the two *Shewanella* isolates were identified on the basis of  
256 expression in *E. coli*, whilst of the four contigs retrieved from this library, three were from the single

257 *Pseudoalteromonas* isolate. In the *Fv* library, a contig was retrieved only from the *Cellulophaga*  
258 isolate An8, even though the other four *Flavobacteriia* used were active against most of the tested  
259 substrates. Assuming that the level of heterologous expression increases when the donor of the  
260 foreign DNA is closely related to the expression host, it might be possible to solve these expression  
261 problems by using a marine host. *Pseudomonas antartica*, for example, appears to be an excellent  
262 psychrophilic expression host; it has few interfering natural enzymatic activities and is easily  
263 transformable by electroporation (see for review Liebl et al., 2014). *Rhodobacter capsulatus* is  
264 another promising host for producing functional membrane-bound enzymes from heterologous  
265 genes (Liebl et al., 2014). Yet these bacteria are both *Proteobacteria*, and we have failed to find any  
266 *Bacteroidetes* member (liable to better express flavobacterial genomes) that has already been used  
267 for library constructions.

268 Another explanation for our different screening yields might be the different average DNA insert  
269 sizes of our two plurigenomic libraries (*Fv*: 6.5 kb, *Gm*: 9 kb). Even though the number of megabases  
270 screened was the same for both libraries, the presence of smaller inserts reduces the probability of  
271 having an entire gene or operon, complete with upstream promoter and downstream terminator,  
272 expressed (Ekkers et al., 2012). Functional screening of metagenomic libraries, constructed in  
273 plasmids from similar environmental samples, has been found to have a better yield (expressed in 1  
274 positive/Mb screened) when the insert size is greater (for a review see Uchiyama and Miyazaki,  
275 2009).

276 A last issue worth mentioning is the choice of the restriction enzyme used to generate the library  
277 inserts. In a previous study focusing on the microbiota associated with *A. nodosum*, we found the  
278 extracted DNA to be much more easily restricted with *DpnII* than with *Sau3AI* (Martin et al., 2014a).  
279 We therefore constructed and screened a metagenomic library containing only *DpnII* restriction  
280 fragments as inserts. Here, however, we see that *DpnII* fails to restrict the genomic DNA from some  
281 marine bacteria. This suggests that in our previous study, some genes were probably not inserted

282 into the DNA library and thus not screened. To our knowledge, this particular source of bias has  
283 never been mentioned in relation to the poor yields of functional metagenomics.

284 **Cultivable macroalgal-polysaccharide-degrading bacteria are specialized in the hydrolyzation of**  
285 **sugars**

286 Marine macroalgae contain various sulfated and non-sulfated polysaccharides. According to their  
287 cell-wall composition and phylogeny, they are divided into three phyla: red, brown, and green  
288 seaweeds. Brown algae mainly contain alginates (uronic acids) , fucans (sulfated polysaccharides),  
289  $\beta$ 1,3 -  $\beta$ 1,4 mixed linkage glucans, cellulose, and xylan or arabinoxylan (Deniaud-Bouët et al., 2014;  
290 Popper et al., 2011). In terrestrial environments, specific cellulolytic and hemicellulolytic bacteria are  
291 known to be specialized in the hydrolyzation and mineralization of plant polymers (DeAngelis et al.,  
292 2010; Gibson et al., 2011; Leung et al., 2015). Similar observations have been made on seaweed-  
293 associated microbiotas, where certain bacterial groups have emerged as being specialized in the use  
294 of algal polysaccharides (Martin et al., 2014b; Michel and Czjzek, 2013). Here we have found our  
295 macroalgal-polysaccharide-degrading isolates to hydrolyze other sugars as well: alongside their  
296 ability to degrade algal polysaccharides, they exhibit xylanase, endocellulase, and beta-glucosidase  
297 activities. Furthermore, on the five sequenced genome contigs from such bacteria we have identified  
298 nine genes (*Fvi2\_5*, *GmXyl\_7*, *GmXyl\_9*, *GmXyl\_10*, *Gmi1\_3*, *Gmi1\_5*, *GmBg\_1*, *GmBg\_2*) coding for  
299 proteins having sequence identity to known GH enzymes (Table 2), and we have proven by  
300 subcloning that four of these genes (*Fvi2\_5*, *GmXyl\_7*, *Gmi1\_3* and *GmBg\_1*) confer the predicted  
301 activity in the presence of a relevant substrate (Table S1). By using the genomes of only ten  
302 preselected cultivable polysaccharolytic isolates, we have discovered several novel GH genes,  
303 whereas glycosidases represent less than 15% of the enzymes identified by functional screening of  
304 metagenomic libraries (Ferrer et al., 2015). The genomes of cultivable macroalgal-polysaccharide-  
305 degrading bacteria thus appear particularly rich in genes involved in sugar hydrolyzation, and  
306 preselecting polysaccharolytic bacteria obviously increases the identification of novel GH genes.

## 307 **Identification of genes encoding original functional enzymes in cultivable alga-associated isolates**

308 The discovery of novel enzyme and protein families from marine organisms is interesting from the  
309 standpoint of both basic science and biotechnology. Some enzymes from marine microbes show  
310 unusually high stability or display diverse genetic and biochemical characteristics that distinguish  
311 them from their counterparts in terrestrial organisms (Zhang and Kim, 2010). Here we have identified  
312 from marine isolates five novel functional-enzyme-encoding genes, three of which (*Fvi2\_5*, *Gmi1\_3*,  
313 and *GmEst\_7*) were not previously known to confer the observed activity and were not assigned to  
314 that function in protein databases.

315 **(i)** The ***GmXyl\_7*** gene encodes a putative GH8 xylanase, GmXyl7, having high sequence identity  
316 (98%) to an enzyme that has been characterized and crystallized: the endo-1,4-beta xylanase pXyl  
317 (Q8RJN8) of *Pseudoalteromonas haloplanktis* (Collins et al., 2002; Van Petegem et al., 2003, 2002)  
318 (Table S2). Interestingly, the latter enzyme appears most active towards xylan from the red alga  
319 *Palmaria palmata*. This xylan is a linear  $\beta$ 1,3- $\beta$ 1,4 mixed-linkage seaweed xylan (Collins et al., 2002).  
320 This suggests that the main source of xylan in the natural environment of *pXyl* could be of algal  
321 origin, as it is for *GmXyl7*. Only a few xylanases have been classified in the GH8 family so far. The  
322 potential of GH8 xylanases as technological aids in baking has been clearly demonstrated, particularly  
323 with the cold-active pXyl from *P. haloplanktis* (Collins et al., 2006). Furthermore, functional analyses  
324 have shown that these enzymes have narrow substrate specificity and low affinity for smaller xylan  
325 units. This is an advantage in industrial applications, as the enzymes won't hydrolyze the released  
326 degradation products (Pollet et al., 2010). The GmXyl contig bears two other genes (*GmXyl\_9* and  
327 *GmXyl\_10*) closely related to known xylanases. Several hypotheses might be proposed to explain  
328 why *GmXyl\_9* and *GmXyl\_10* do not confer any endoxylanase activity when subcloned. It could be  
329 that our screening conditions (temperature, pH, substrate...) were not appropriate for observing the  
330 activity of the encoded proteins. Alternatively, the genes *GmXyl\_7*, *GmXyl\_9*, and *GmXyl\_10* might  
331 work in an operon regulated by a promoter in front of *GmXyl\_7*. We found no such operon, however,  
332 in operon databases such as ProOpDB (Taboada et al., 2012) and OperonDB (Perteau et al., 2009), and

333 no operon was predicted in the Softberry FGENESB software (Solovyev and Salamov, 2011).  
334 Moreover, all attempts to produce the GmXyl9 protein in *E. coli* under the control of an IPTG-  
335 inducible promoter (expression vector pET30b), and under various conditions in a bioreactor, proved  
336 unsuccessful (data not shown). On the other hand, *GmXyl\_9* and *GmXyl\_10* might be pseudogenes.  
337 Pseudogenes are sequences sharing homology with active genes but having lost their ability to  
338 function as transcriptional units. They are found in high number in bacterial genomes but are still  
339 difficult to predict (Lerat, 2005; Rouchka and Cha, 2009).

340 **(ii)** The putative beta-glucosidase encoded by *GmBg\_1* should also show interesting properties, as  
341 the few characterized beta-glucosidases isolated from marine bacteria have been found to be alkali-  
342 stable and cold-active (Chen et al., 2010; Mao et al., 2010).

343 **(iii)** Interestingly, the sequence of the potential esterase encoded by the *GmEst\_7* gene is practically  
344 identical to proteins annotated as homoserine o-acetyltransferases (HAT) (Table S2), but contains an  
345 esterase/lipase domain and hydrolyzes tributyrin. Closely related HAT-annotated proteins may thus  
346 be wrongly annotated. Another similar protein, *CgHle* of *Corynebacterium glutamicum*, also referred  
347 as a HAT in protein databases, likewise contains an esterase/lipase domain and displays esterase  
348 activity (Tölzer et al., 2009). HATs are required in methionine biosynthesis (Bourhy et al., 1997), but  
349 *CgHle* was not found to play a role in the main methionine pathway or in any alternative one, and  
350 thus appears to have been (wrongly) assigned as a HAT on the sole basis of its structure (Rückert et  
351 al., 2003; Tölzer et al., 2009). Lastly, by aligning the amino acid sequences of the *CgHle* and *GmEst\_7*  
352 proteins, we were able to retrieve the GxSxG amino acid motif typically found in lipolytic enzymes  
353 (Arpigny and Jaeger, 1999) (Figure 2), but were unable to assign these two esterases to any known  
354 esterase family. Hausmann and Jaeger (2010) note that many esterases in protein databases remain  
355 unassigned to already described esterase families. The proteins *GmEst7* and *CgHle* could thus be  
356 members of a novel family of HAT-like carboxy-esterases.

357 **(iv)** Lastly, we have identified two functional iota-carrageenase genes (*Fvi2\_5* and *Gmi1\_3*). Both of  
358 the encoded proteins belong to the GH82 family. Iotase activity was described for the first time in

359 1984 (Greer and Yaphe, 1984), but the iota-carrageenase enzymes and family (GH82) were not  
360 defined until 2000 (Barbeyron et al., 2000). Since then, only 19 proteins have been assigned to this  
361 family (CAZy Database, Lombard et al., 2014). Iota-carrageenases have been divided by Michel &  
362 Czjzek (2013) into three clades, according to their phylogeny. In the constructed phylogenetic tree,  
363 the iota-carrageenase Fvi2.5 appears to belong to clade A and Gmi2.3 to clade C (Figure 3).

364 Clade A contains the only iota-carrageenase whose crystal structure has been solved: CgiA\_Af of  
365 *Alteromonas fortis* (Michel et al., 2001). This enzyme folds into a right-handed  $\beta$ -helix flanked by two  
366 additional domains (domains A and B). Domain A has been found to be highly conserved in clade A  
367 iota-carrageenases and to be responsible for their processive character (Michel et al., 2003). This  
368 domain is indicated in the protein sequence of Fvi2.5, by sequence alignment with other  
369 characterized iota-carrageenases of this clade (CgiA\_Af (CGIA\_ALTFO), CgiA1\_Zg, CgiA\_C.QY3) (Figure  
370 4). Domain A is absent from the two other clades (containing only non-processive enzymes), and  
371 domain B is found in some iota-carrageenases of these clades (Rebuffet et al., 2010).

372 The enzyme Gmi1.3 is related (30% sequence identity) to the characterized clade C iota-carrageenase  
373 CgiA\_Mt of *Microbulbifer thermotolerans* (Hatada et al., 2011) (Table S2). Only two enzymes  
374 (CgiA\_Mt and PatI-0879 of *Pseudoalteromonas atlantica*) belong to this clade so far. Adding this  
375 novel iota-carrageenase will reinforce the coherence of this group (Figure 3). Gmi1.3 is very distant  
376 from the clade-A sequences (only 18% sequence identity to CgiA\_Af). This is notably due to the  
377 absence of Domain A in clade C sequences. In contrast, Gmi1.3 features several large insertions as  
378 compared to CgiA\_Af (mainly between the strands  $\beta$ 13 and  $\beta$ 14,  $\beta$ 25 and  $\beta$ 26, and  $\beta$ 27 and  $\beta$ 28,  
379 Figure 4). The absence of domain A suggests that Gmi1.3 is not a processive enzyme, but the large  
380 inserts in this new iota-carrageenase may influence its mode of action.

381 In Fvi2.5, the essential residues of the catalytic site, E245, D247, Q222, and H281 (Rebuffet et al.,  
382 2010), are strictly conserved (Figure 4). Residue E310 of domain A, involved in stabilizing the  
383 intermediate substrate-bound conformation, is also recovered in the sequence of Fvi2.5 (Michel and  
384 Czjzek, 2013; Michel et al., 2003). Gmi1.3 also features most of the essential residues of the CgiA\_Af

385 catalytic machinery (E245, Q222 and H281), but the base catalyst D247 is replaced by a glycine. This  
386 substitution is also observed in the characterized iota-carrageenase CgiA\_Mt. Thus, the identity of  
387 the base catalyst in clade C enzymes remains an open question.

## 388 **CONCLUSION**

389 The plurigenomic libraries screened in the present study were constructed with the genomic DNA of  
390 bacteria preselected for the presence of specific enzymatic activities. Yet only five activities out of  
391 the 42 identified in these natural polysaccharolytic isolates were recovered by functional screening.  
392 Expression in a heterologous host, DNA insert size, and/or the restriction enzyme used may at least  
393 partly explain this low yield. These limitations are obviously magnified in functional metagenomic  
394 analysis, as there is no preselection of specific bacterial isolates that act on the screening substrates.  
395 This explains why even lower yields are obtained by this approach. These results highlight, once  
396 again, the difficulty of identifying novel enzyme genes by functional analysis. Nevertheless, we also  
397 demonstrate that cultivable bacteria should not be left out, as with only ten bacteria we have  
398 discovered two novel iota-carrageenase genes (gaining knowledge about this poorly known enzyme  
399 family) and a putative novel HAT-like esterase family. The originality of the cultivable isolates used  
400 (low identity of their 16S rRNA genes to those of known species), of the environment from which  
401 they were isolated (few functional analyses have focused on alga-associated microbiotas), and of the  
402 method used here (construction of plurigenomic libraries from preselected original bacteria) is  
403 obviously responsible for the novelty of our discoveries.

404

## 405 **TABLES AND FIGURES**

406 **Table 1: Enzymatic activities of the *Flavobacteriia* and *Gammaproteobacteria* strains used to**  
407 **construct the plurigenomic libraries**

408 **Table 2: Gene name, accession number, ORF size, sequence identities of the diverse coding**  
409 **sequences identified on the constructed contigs from the active clones**

410 **Figure 1 : Plan of the five contigs identified in the two plurigenomic libraries.** \* : incomplete ORF; →:  
411 ORF found by subcloning to be responsible for the observed activity; → : subcloned ORFs that did not  
412 confer the observed activity

413 **Figure 2 : Multiple sequence alignment of partial amino acid sequences containing the conserved**  
414 **blocks of the HAT-like carboxy-esterases encoded by GmEst\_6 and cg0961.** The three stars indicate  
415 the GxSxG lipase active site motif.

416 **Figure 3 : Phylogenetic tree with the 19 proteins of the GH82 iota-carrageenase family and the two**  
417 **novel iota-carrageenases Fvi2.5 and Gmi1.3** (indicated with red diamonds). The characterized  
418 proteins of the GH82 family are indicated with blue squares.

419 **Figure 4: Multiple sequence alignments with the characterized iota-carrageenases of the GH82**  
420 **family and Fvi2.5 and Gmi1.3** The secondary structural elements of the crystallized iota-  
421 **carrageenase of *Alteromonas fortis* are found above the sequences.** Domain A is underlined in blue.  
422 The proton donor and the base catalyst (DE245 and D247) are indicated with red stars. The other  
423 residues important for catalysis in CgiA\_Af (Q222 and H281; E310 from domain A) are indicated with  
424 green stars.

#### 425 **SUPPLEMENTARY MATERIAL**

426 **Table S1: Summary of the subclonings performed with the ORFs putatively responsible for the**  
427 **observed activity.** ORFs in bold were found to be responsible for the observed activity

428 **Table S2 : Five best hits in the GenBank and Swissprot databases and deduced sequence**  
429 **characteristics of the functional genes discovered in this study**

430

431 **ACKNOWLEDGMENTS**

432 This project was funded by Gembloux Agro-Bio Tech (ULg), Wallonie-Bruxelles International (WBI),  
433 and the Fonds Scientifique de la Recherche (F.R.S-F.N.R.S) in the framework of the Collaboration  
434 Program Hubert Curien. GM and TB are grateful for the support of the French Government through  
435 the National Research Agency with regard to the “Blue Enzymes” ANR project (reference ANR-14-  
436 CE19-0020-01).

437 **BIBLIOGRAPHY**

- 438 Arpigny, J.L., Jaeger, K.E., 1999. Bacterial lipolytic enzymes: classification and properties. *Biochem. J.*  
439 343 Pt 1, 177–83.
- 440 Barbeyron, T., L’Haridon, S., Corre, E., Kloareg, B., Potin, P., 2001. *Zobellia galactanovorans* gen. nov.,  
441 sp. nov., a marine species of Flavobacteriaceae isolated from a red alga, and classification of  
442 [Cytophaga] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen.  
443 nov., comb. nov. *Int. J. Syst. Evol. Microbiol.* 51, 985–997. doi:10.1099/00207713-51-3-985
- 444 Barbeyron, T., Michel, G., Potin, P., Henrissat, B., Kloareg, B., 2000. Iota-Carrageenases constitute a  
445 novel family of glycoside hydrolases, unrelated to that of kappa-carrageenases. *J. Biol. Chem.*  
446 275, 35499–505. doi:10.1074/jbc.M003404200
- 447 Biver, S., Vandebol, M., 2012. Characterization of three new carboxylic ester hydrolases isolated by  
448 functional screening of a forest soil metagenomic library. *J. Ind. Microbiol. Biotechnol.*  
449 doi:10.1007/s10295-012-1217-7
- 450 Bourhy, P., Martel, a., Margarita, D., Saint Girons, I., Belfaiza, J., 1997. Homoserine O-  
451 acetyltransferase, involved in the *Leptospira meyeri* methionine biosynthetic pathway, is not  
452 feedback inhibited. *J. Bacteriol.* 179, 4396–4398.
- 453 Chen, S., Hong, Y., Shao, Z., Liu, Z., 2010. A cold-active  $\beta$ -glucosidase (Bgl1C) from a sea bacteria  
454 *Exiguobacterium oxidotolerans* A011. *World J. Microbiol. Biotechnol.* 26, 1427–1435.  
455 doi:10.1007/s11274-010-0317-7
- 456 Cheng, H.-R., Jiang, N., 2006. Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnol.*  
457 *Lett.* 28, 55–9. doi:10.1007/s10529-005-4688-z
- 458 Collins, T., Hoyoux, A., Dutron, A., Georis, J., Genot, B., Dauvrin, T., Arnaut, F., Gerday, C., Feller, G.,  
459 2006. Use of glycoside hydrolase family 8 xylanases in baking. *J. Cereal Sci.* 43, 79–84.  
460 doi:10.1016/j.jcs.2005.08.002
- 461 Collins, T., Meuwis, M.A., Stals, I., Claeysens, M., Feller, G., Gerday, C., 2002. A novel family 8  
462 xylanase, functional and physicochemical characterization. *J. Biol. Chem.* 277, 35133–35139.  
463 doi:10.1074/jbc.M204517200
- 464 Dalmaso, G., Ferreira, D., Vermelho, A., 2015. Marine Extremophiles: A Source of Hydrolases for  
465 Biotechnological Applications. *Mar. Drugs* 13, 1925–1965. doi:10.3390/md13041925
- 466 DeAngelis, K.M., Gladden, J.M., Allgaier, M., D’haeseleer, P., Fortney, J.L., Reddy, A., Hugenholtz, P.,  
467 Singer, S.W., Vander Gheynst, J.S., Silver, W.L., Simmons, B. a., Hazen, T.C., 2010. Strategies for  
468 Enhancing the Effectiveness of Metagenomic-based Enzyme Discovery in Lignocellulolytic  
469 Microbial Communities. *BioEnergy Res.* 3, 146–158. doi:10.1007/s12155-010-9089-z

470 Deniaud-Bouët, E., Kervarec, N., Michel, G., Tonon, T., Kloareg, B., Hervé, C., 2014. Chemical and  
471 enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular  
472 matrix of brown algae. *Ann. Bot.* 114, 1203–16. doi:10.1093/aob/mcu096

473 Ekkers, D.M., Cretoiu, M.S., Kielak, A.M., Elsas, J.D. Van, 2012. The great screen anomaly--a new  
474 frontier in product discovery through functional metagenomics. *Appl. Microbiol. Biotechnol.* 93,  
475 1005–20. doi:10.1007/s00253-011-3804-3

476 Ferrer, M., Martínez-Martínez, M., Bargiela, R., Streit, W.R., Golyshina, O. V., Golyshin, P.N., 2015.  
477 Estimating the success of enzyme bioprospecting through metagenomics: current status and  
478 future trends. *Microb. Biotechnol.* n/a–n/a. doi:10.1111/1751-7915.12309

479 Finn, R.D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R.Y., Eddy, S.R., Heger, A., Hetherington,  
480 K., Holm, L., Mistry, J., Sonnhammer, E.L.L., Tate, J., Punta, M., 2014. Pfam: the protein families  
481 database. *Nucleic Acids Res.* 42, D222–30. doi:10.1093/nar/gkt1223

482 Gibson, D.M., King, B.C., Hayes, M.L., Bergstrom, G.C., 2011. Plant pathogens as a source of diverse  
483 enzymes for lignocellulose digestion. *Curr. Opin. Microbiol.* 14, 264–270.  
484 doi:10.1016/j.mib.2011.04.002

485 Goecke, F., Thiel, V., Wiese, J., Labes, A., Imhoff, J.F., 2013. Algae as an important environment for  
486 bacteria – phylogenetic relationships among new bacterial species isolated from algae.  
487 *Phycologia* 52, 14–24. doi:10.2216/12

488 Greer, C.W., Yaphe, W., 1984. Purification and properties of  $\iota$ -carrageenase from a marine bacterium.  
489 *Can. J. Microbiol.* 30, 1500–1506.

490 Handelsman, J., 2004. Metagenomics : Application of Genomics to Uncultured Microorganisms.  
491 *Microbiol. Mol. Biol. Rev.* 68, 669–685. doi:10.1128/MBR.68.4.669

492 Hatada, Y., Mizuno, M., Li, Z., Ohta, Y., 2011. Hyper-production and characterization of the  $\iota$ -  
493 carrageenase useful for  $\iota$ -carrageenan oligosaccharide production from a deep-sea bacterium,  
494 *Microbulbifer thermotolerans* JAMB-A94T, and insight into the unusual catalytic mechanism.  
495 *Mar. Biotechnol. (NY)*. 13, 411–22. doi:10.1007/s10126-010-9312-0

496 Hausmann, S., Jaeger, K.E., 2010. Lipolytic enzymes of bacteria, in: Timmis, K.N. (Ed.), *Handbook of*  
497 *Hydrocarbon and Lipid Microbiology*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1099–  
498 1126. doi:10.1007/978-3-540-77587-4

499 Hehemann, J.-H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., Michel, G., 2010. Transfer of  
500 carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464,  
501 908–12. doi:10.1038/nature08937

502 Holmström, C., Egan, S., Franks, A., McCloy, S., Kjelleberg, S., 2002. Antifouling activities expressed by  
503 marine surface associated *Pseudoalteromonas* species. *FEMS Microbiol. Ecol.* 41, 47–58.  
504 doi:10.1111/j.1574-6941.2002.tb00965.x

505 Ivanova, E.P., Alexeeva, Y. V., Flavier, S., Wright, J.P., Zhukova, N. V., Gorshkova, N.M., Mikhailov, V.  
506 V., Nicolau, D. V., Christen, R., 2004. *Formosa* algae gen. nov., sp. nov., a novel member of the  
507 family Flavobacteriaceae. *Int. J. Syst. Evol. Microbiol.* 54, 705–711. doi:10.1099/ijs.0.02763-0

508 Johansen, J.E., Nielsen, P., Sjøraholt, C., 1999. Description of *Cellulophaga baltica* gen. nov., sp. nov.  
509 and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *lytica* to  
510 *Cellulophaga lytica* gen. nov., comb. nov. *Int. J. Syst. Bacteriol.* 49, 1231–1240.

511 Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence  
512 alignment based on fast Fourier transform. *Nucleic Acids Res.* 30, 3059–3066.  
513 doi:10.1093/nar/gkf436

514 Lerat, E., 2005. Recognizing the pseudogenes in bacterial genomes. *Nucleic Acids Res.* 33, 3125–  
515 3132. doi:10.1093/nar/gki631

516 Leung, H.T.C., Maas, K.R., Wilhelm, R.C., Mohn, W.W., 2015. Long-term effects of timber harvesting  
517 on hemicellulolytic microbial populations in coniferous forest soils. *ISME J.*  
518 doi:10.1038/ismej.2015.118

519 Liebl, W., Angelov, A., Juergensen, J., Chow, J., Loeschcke, A., Drepper, T., Classen, T., Pietruzka, J.,  
520 Ehrenreich, A., Streit, W.R., Jaeger, K.-E., 2014. Alternative hosts for functional (meta)genome  
521 analysis. *Appl. Microbiol. Biotechnol.* 98, 8099–109. doi:10.1007/s00253-014-5961-7

522 Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2014. The carbohydrate-  
523 active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 42, D490–D495.  
524 doi:10.1093/nar/gkt1178

525 Mao, X., Hong, Y., Shao, Z., Zhao, Y., Liu, Z., 2010. A novel cold-active and alkali-stable  $\beta$ -glucosidase  
526 gene isolated from the marine bacterium *Martellella mediterranea*. *Appl. Biochem. Biotechnol.*  
527 162, 2136–48. doi:10.1007/s12010-010-8988-y

528 Martin, M., Barbeyron, T., Martin, R., Portetelle, D., Michel, G., Vandenberg, M., 2015. The Cultivable  
529 Surface Microbiota of the Brown Alga *Ascophyllum nodosum* is Enriched in Macroalgal-  
530 Polysaccharide-Degrading Bacteria. *Front. Microbiol.* 6, 1–14. doi:10.3389/fmicb.2015.01487

531 Martin, M., Biver, S., Steels, S., Barbeyron, T., Jam, M., Portetelle, D., Michel, G., Vandenberg, M.,  
532 2014a. Identification and Characterization of a Halotolerant, Cold-Active Marine Endo-Beta-1,4-  
533 Glucanase by Using Functional Metagenomics of Seaweed-Associated Microbiota. *Appl.*  
534 *Environ. Microbiol.* 80, 4958–4967. doi:10.1128/AEM.01194-14

535 Martin, M., Portetelle, D., Michel, G., Vandenberg, M., 2014b. Microorganisms living on macroalgae:  
536 diversity, interactions, and biotechnological applications. *Appl. Microbiol. Biotechnol.* 98, 2917–  
537 2935. doi:10.1007/s00253-014-5557-2

538 Mattéotti, C., Haubruge, E., Thonart, P., Francis, F., De Pauw, E., Portetelle, D., Vandenberg, M., 2011.  
539 Characterization of a new  $\beta$ -glucosidase/ $\beta$ -xylosidase from the gut microbiota of the termite  
540 (*Reticulitermes santonensis*). *FEMS Microbiol. Lett.* 314, 147–57. doi:10.1111/j.1574-  
541 6968.2010.02161.x

542 Michel, G., Chantalat, L., Fanchon, E., Henrissat, B., Kloareg, B., Dideberg, O., 2001. The iota-  
543 carrageenase of *Alteromonas fortis*. A beta-helix fold-containing enzyme for the degradation of  
544 a highly polyanionic polysaccharide. *J. Biol. Chem.* 276, 40202–9. doi:10.1074/jbc.M100670200

545 Michel, G., Czjzek, M., 2013. Polysaccharide-degrading enzymes from marine bacteria, in: Trincone,  
546 A. (Ed.), *Marine Enzymes for Biocatalysis: sources, Biocatalytic Characteristic and Bioprocesses*  
547 *of Marine Enzymes.* Woodhead Publishing Limited, Cambridge, pp. 429–464.  
548 doi:10.1533/9781908818355.3.429

549 Michel, G., Helbert, W., Kahn, R., Dideberg, O., Kloareg, B., 2003. The Structural Bases of the  
550 Processive Degradation of  $\iota$ -Carrageenan, a Main Cell Wall Polysaccharide of Red Algae. *J. Mol.*  
551 *Biol.* 334, 421–433. doi:10.1016/j.jmb.2003.09.056

552 Park, B.H., Karpinets, T. V., Syed, M.H., Leuze, M.R., Uberbacher, E.C., 2010. CAZymes Analysis Toolkit  
553 (cat): Web service for searching and analyzing carbohydrate-active enzymes in a newly  
554 sequenced organism using CAZy database. *Glycobiology* 20, 1574–1584.  
555 doi:10.1093/glycob/cwq106

556 Pertea, M., Ayanbule, K., Smedinghoff, M., Salzberg, S.L., 2009. OperonDB: a comprehensive  
557 database of predicted operons in microbial genomes. *Nucleic Acids Res.* 37, D479–82.  
558 doi:10.1093/nar/gkn784

559 Pollet, A., Schoepe, J., Dornez, E., Strelkov, S. V., Delcour, J. a, Courtin, C.M., 2010. Functional analysis  
560 of glycoside hydrolase family 8 xylanases shows narrow but distinct substrate specificities and  
561 biotechnological potential. *Appl. Microbiol. Biotechnol.* 87, 2125–35. doi:10.1007/s00253-010-  
562 2659-3

563 Popper, Z. a, Michel, G., Hervé, C., Domozych, D.S., Willats, W.G.T., Tuohy, M.G., Kloareg, B., Stengel,  
564 D.B., 2011. Evolution and diversity of plant cell walls: from algae to flowering plants. *Annu. Rev.*  
565 *Plant Biol.* 62, 567–90. doi:10.1146/annurev-arplant-042110-103809

566 Rebuffet, E., Barbeyron, T., Jeudy, A., Jam, M., Czjzek, M., Michel, G., 2010. Identification of catalytic  
567 residues and mechanistic analysis of family GH82 iota-carrageenases. *Biochemistry* 49, 7590–9.  
568 doi:10.1021/bi1003475

569 Robert, X., Gouet, P., 2014. Deciphering key features in protein structures with the new ENDscript  
570 server. *Nucleic Acids Res.* 42, W320–W324. doi:10.1093/nar/gku316

571 Rouchka, E.C., Cha, I.E., 2009. Current Trends in Pseudogene Detection and Characterization. *Curr.*  
572 *Bioinform.* 4, 112–119. doi:10.2174/157489309788184792

573 Rückert, C., Pühler, a., Kalinowski, J., 2003. Genome-wide analysis of the L-methionine biosynthetic  
574 pathway in *Corynebacterium glutamicum* by targeted gene deletion and homologous  
575 complementation. *J. Biotechnol.* 104, 213–228. doi:10.1016/S0168-1656(03)00158-5

576 Simon, C., Daniel, R., 2011. Metagenomic analyses: Past and future trends. *Appl. Environ. Microbiol.*  
577 77, 1153–1161. doi:10.1128/AEM.02345-10

578 Solovyev, V., Salamov, A., 2011. Automatic Annotation of Microbial Genomes and Metagenomic  
579 Sequences, in: Robert W. Li (Ed.), *In Metagenomics and Its Applications in Agriculture,*  
580 *Biomedicine and Environmental Studies.* Nova Science Publishers, pp. 61–78.

581 Taboada, B., Ciria, R., Martinez-Guerrero, C.E., Merino, E., 2012. ProOpDB: Prokaryotic Operon  
582 DataBase. *Nucleic Acids Res.* 40, D627–31. doi:10.1093/nar/gkr1020

583 Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary  
584 Genetics Analysis Version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi:10.1093/molbev/mst197

585 Tölzer, C., Pal, S., Watzlawick, H., Altenbuchner, J., Niefind, K., 2009. Crystallization and preliminary  
586 crystallographic analysis of cgHle, a homoserine acetyltransferase homologue, from  
587 *Corynebacterium glutamicum*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 65, 34–38.  
588 doi:10.1107/S1744309108039146

589 Uchiyama, T., Miyazaki, K., 2009. Functional metagenomics for enzyme discovery: challenges to  
590 efficient screening. *Curr. Opin. Biotechnol.* 20, 616–622. doi:10.1016/j.copbio.2009.09.010

591 Van Petegem, F., Collins, T., Meuwis, M. a, Gerday, C., Feller, G., Van Beeumen, J., 2002.  
592 Crystallization and preliminary X-ray analysis of a xylanase from the psychrophile  
593 *Pseudoalteromonas haloplanktis*. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 58, 1494–1496.

594 Van Petegem, F., Collins, T., Meuwis, M., Gerday, C., Feller, G., Beeumen, J. Van, 2003. The Structure  
595 of a Cold-adapted Family 8 Xylanase at 1 . 3 Å Resolution. *J. Biol. Chem.* 278, 7531–7539.  
596 doi:10.1074/jbc.M206862200

597 Zhang, C., Kim, S.-K., 2010. Research and application of marine microbial enzymes: status and  
598 prospects. *Mar. Drugs* 8, 1920–34. doi:10.3390/md8061920

599

Table 1: Enzymatic activities of the *Flavobacteriia* and *Gammaproteobacteria* strains used to construct the plurigenomic libraries

Strains	16S rRNA accession numbers <sup>1</sup>	Closest bacterial strain (EZTaxon)	Enzymatic activities detected with our screening tests							
			Ag	ι-carr	κ-carr	Amyl	Cell	β-gluc	Est	Xyl
<b>Bacterial isolates of the <i>Flavobacteriia</i> plurigenomic library <i>Fv</i></b>										
<i>Cellulophaga sp.</i> An8	LN881186	97.9% with <i>Cellulophaga geojensis</i> M-M6	++	+	+	-	++	-	-	+
<i>Cellulophaga sp.</i> An9	LN881202	98.3% with <i>Cellulophaga baltica</i> NN015840	++	+	+	-	++	-	+	+
<i>Cellulophaga sp.</i> An20	LN881252	97.5% with <i>Cellulophaga baltica</i> NN015840	++	+	++	-	++	-	+	+
<i>Maribacter sp.</i> An21	LN881276	95.3% with <i>Maribacter aestuarii</i> GY20	+	++	++	-	+	-	+	++
<i>Zobellia sp.</i> An14	LN881227	97.6% with <i>Zobellia laminariae</i> KMM3676	-	+	-	-	-	-	-	-
<b>Bacterial isolates of the <i>Gammaproteobacteria</i> plurigenomic library <i>Gm</i></b>										
<i>Cowellia sp.</i> An23	LN881284	96.2% with <i>Colwellia meonggei</i> MA1-3	+	++	++	-	+	-	++	+
<i>Paraglaciecola sp.</i> An27	LN881305	96.5% with <i>Paraglaciecola mesophila</i> KMM241	+	+	+	-	+	+	+	++
<i>Pseudoalteromonas sp.</i> An33	LN881360	99.4% with <i>Pseudoalteromonas espeijana</i> NCIMB 2127	-	+	+	-	++	+	++	++
<i>Shewanella sp.</i> An4	LN881152	97.6% with <i>Shewanella japonica</i> KMM 3299	-	+	-	-	-	-	+	-
<i>Shewanella sp.</i> An36	LN881379	99.0% with <i>Shewanella dovemarinesis</i> MAR441	-	+	+	-	-	-	+	-

<sup>1</sup>Published in Martin et al., 2015; ++ activity detected within 24 hours; + activity detected after more than 24 hours; - no activity observed

**Table 2 : Gene name, accession number, ORF size, sequence identities of the diverse coding sequences identified on the constructed contigs from the active clones**

ORFs that were subcloned are indicated in bold. ORFs indicated in red were found by subcloning to be responsible for the observed activity

Clone name Activity Size	Gene name	Accession number	ORF size (aa)	Identity percentage with best hit against GenBank (Source organisms), accession number
Fvi2 lota-carrageenase 6.2kb	<i>Fvi2_1</i>	LN913026	159*	87% 2OG-Fe(II) oxygenase ( <i>Maribacter forsetii</i> ) WP_036154505.1
	<i>Fvi2_2</i>	LN913027	261	74% Short-chain dehydrogenase ( <i>Maribacter forsetii</i> ) WP_034666723.1
	<i>Fvi2_3</i>	LN913028	197	80% NAD(P)H oxidoreductase ( <i>Zobellia uliginosa</i> ) WP_038232878.1
	<i>Fvi2_4</i>	LN913029	626	91% Potassium transporter ( <i>Cellulophaga geojensis</i> ) EWH14754
	<b><i>Fvi2_5</i></b>	<b>LN913030</b>	<b>491</b>	<b>97% Hypothetical protein (<i>Cellulophaga lytica</i>) WP_013622437.1</b>
	<i>Fvi2_6</i>	LN913031	108	63% Competence protein TfoX ( <i>Zobellia galactanivorans</i> )WP_013995580.1
GmXyl Xylanase 12.4kb	<i>GmXyl_1</i>	LN913032	451*	99% Ton B-dependent receptor ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599008.1
	<i>GmXyl_2</i>	LN913033	247	99% Multidrug transporter ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599009.1
	<i>GmXyl_3</i>	LN913034	336	97% Cupin ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599009.1
	<i>GmXyl_4</i>	LN913035	494	98% Tryptophan halogenase ( <i>Pseudoalteromonas citrea</i> ) WP_033028792.1
	<i>GmXyl_5</i>	LN913036	236	97% Hypothetical protein ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599012.1
	<i>GmXyl_6</i>	LN913037	273	98% Crp/Fnr transcriptional regulator ( <i>Pseudoalteromonas</i> sp. BSi20429) WP_007585178.1
	<b><i>GmXyl_7</i></b>	<b>LN913038</b>	<b>426</b>	<b>99% endo-1,4-beta-xylanase (<i>Pseudoalteromonas arctica</i>) CBY88881.1</b>
	<i>GmXyl_8</i>	LN913039	324	96% Gluconolactonase ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599014.1
	<b><i>GmXyl_9</i></b>	<b>LN913040</b>	<b>377</b>	<b>99% endo-1,4-beta-xylanase (<i>Pseudoalteromonas haloplanktis</i>) WP_024599015.1</b>
	<b><i>GmXyl_10</i></b>	<b>LN913041</b>	<b>528*</b>	<b>98% endo-1,4-beta-xylanase (<i>Pseudoalteromonas</i> sp. Bsw20308) WP_007375178.1</b>
Gmi1 lota-carrageenase 13.5kb	<i>Gmi1_1</i>	LN913042	1236	99% TonB-dependent receptor ( <i>Pseudoalteromonas</i> sp. BSW 20308) WP_007375140.1
	<i>Gmi1_2</i>	LN913043	595	91% Hypothetical protein ( <i>Pseudoalteromonas</i> sp. BSW 20308) WP_007375139.1
	<b><i>Gmi1_3</i></b>	<b>LN913044</b>	<b>545</b>	<b>100% Hypothetical protein (<i>Pseudoalteromonas</i> sp. P1-11) WP_055255568.1</b>
	<b><i>Gmi1_4</i></b>	<b>LN913045</b>	<b>333</b>	<b>90% Glycoside Hydrolase (<i>Pseudoalteromonas</i> sp. S3431) ; WP_033028792.1</b>
	<b><i>Gmi1_5</i></b>	<b>LN913046</b>	<b>642</b>	<b>94% Hypothetical protein (<i>Pseudoalteromonas haloplanktis</i>) ; WP_024594190.1</b>
	<i>Gmi1_6</i>	LN913047	793*	98% Multispecies TonB-dependant receptor ( <i>Pseudoalteromonas</i> ). ; WP_007378531.1
GmBg Beta-glucosidase 10.4kb	<b><i>GmBg_1</i></b>	<b>LN913056</b>	<b>838</b>	<b>86% Beta-glucosidase (<i>Pseudoalteromonas</i> sp. ECSMB14103) WP_039037212.1</b>
	<b><i>GmBg_2</i></b>	<b>LN913057</b>	<b>571</b>	<b>73% Glycoside hydrolase family 9 (<i>Pseudoalteromonas marina</i>) WP_024599009.1</b>
	<i>GmBg_3</i>	LN913058	354	70% Hypothetical protein ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024594157.1
	<i>GmBg_4</i>	LN913059	456	93% MATE family efflux transporter ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024603968.
	<i>GmBg_5</i>	LN913060	208	90% Riboflavin synthase subunit $\alpha$ ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024599030.1
	<i>GmBg_6</i>	LN913061	287	88% Hypothetical protein ( <i>Pseudoalteromonas agarivorans</i> ) WP_004586762.1
	<i>GmBg_7</i>	LN913062	205*	93% Multispecies hypothetical protein ( <i>Pseudoalteromonas</i> ) WP_024603971.1
GmEst Carboxy-esterase 9.3 kb	<i>GmEst_1</i>	LN913063	126*	95% Prepilin cleavage protein ( <i>Pseudoalteromonas Atlantica</i> ) ABG40490.1
	<i>GmEst_2</i>	LN913064	395	94% Serine/threonine protein kinase ( <i>Paraglaciecola mesophila</i> ) GAC24755.1
	<i>GmEst_3</i>	LN913065	241	43% 2OG-Fe(II) oxygenase ( <i>Brevundimonas</i> sp. KM4) KJV41957.1
	<i>GmEst_4</i>	LN913066	754	99% Peroxidase ( <i>Pseudoalteromonas</i> sp. PLSV) WP_033185651.1
	<i>GmEst_5</i>	LN913067	261	99% D-beta-hydroxybutyrate dehydrogenase ( <i>Paraglaciecola mesophila</i> ) WP_006992904.1
	<b><i>GmEst_6</i></b>	<b>LN913068</b>	<b>368</b>	<b>98% Polyhydroxybutyrate depolymerase (<i>Pseudoalteromonas</i> sp.) WP_033185653.1</b>
	<b><i>GmEst_7</i></b>	<b>LN913069</b>	<b>399</b>	<b>99% Homoserine o-acetyltransferase (<i>Paraglaciecola mesophila</i>) GAC24751</b>
	<i>GmEst_8</i>	LN913070	161*	100% Multispecies H <sup>+</sup> gluconate transporter ( <i>alteromonadales</i> ) WP_006992901

\* incomplete ORF

## SUPPLEMENTARY MATERIAL

**Table S1 : Summary of the subclonings performed with the ORFs putatively responsible for the observed activity.**  
ORFs in bold were found to be responsible for the observed activity

Positive clones per activity	Contig name	Contig size	Origin of the contig	Putative genes for the observed activity	Subcloned gene	Subcloned gene size (aa)	Subclone activity
<b><i>Flavobacteriia</i> library Fv</b>							
<b>1 iota-carrageenase</b>	Fvi2	6.2 kb	<i>Cellulophaga sp.</i> An8	<b>Hypothetical protein</b>	<b><i>Fvi2_5</i></b>	<b>491</b>	<b>+</b>
<b><i>Gammaproteobacteria</i> library Gm</b>							
<b>19 xylanase</b>	CjXyl	12.4 kb	<i>Pseudoalteromonas sp.</i> An33	<b>GH8 endo-1,4-<math>\beta</math>-xylanase</b>	<b><i>GmXyl_7</i></b>	<b>426</b>	<b>+</b>
				GH10 endo-1,4- $\beta$ -xylanase	<i>GmXyl_9</i>	377	-
				GH10 endo-1,4- $\beta$ -xylanase	<i>GmXyl_10</i>	741	-
<b>10 iota-carrageenases</b>	Gmi1	13.5 kb	<i>Pseudoalteromonas sp.</i> An33	<b>Hypothetical protein</b>	<b><i>Gmi1_3</i></b>	<b>545</b>	<b>+</b>
				Glycoside hydrolase	<i>Gmi1_4</i>	333	-
				Hypothetical protein	<i>Gmi1_5</i>	642	-
<b>1 beta-glucosidase</b>	CjBg	10.4 kb	<i>Pseudoalteromonas sp.</i> An33	<b>Beta-glucosidase</b>	<b><i>GmBg_1</i></b>	<b>838</b>	<b>+</b>
				Glycosidase hydrolase GH9	<i>GmBg_2</i>	571	-
<b>1 esterase</b>	GmEst	9.4 kb	<i>Paraglaciecola sp.</i> An27	Polyhydroxybutyrate depolymerase	<i>GmEst_5</i>	368	-
				<b>Homoserin o-acetyltransferase</b>	<b><i>GmEst_6</i></b>	<b>399</b>	<b>+</b>

**Table S2 : Five best hits in the GenBank and Swissprot databases and deduced sequence characteristics of the functional genes discovered in this study**

Gene	Five first best hits		pi/Mw	Family	SP
Activity	GenBank	Swissprot			
<b>Fvi1_5</b> lota-carrageenase	97% Hypothetical protein Celly28_77 ( <i>Cellulophaga lytica</i> DSM7489) ADY30694.1 90% Iota-carrageenase ( <i>Cellulophaga lytica</i> ) AIM61676.1 90% Iota-carrageenase ( <i>Cellulophaga sp.</i> KL-A) AIA09360.1 88% Iota-carrageenase ( <i>Cellulophaga baltica</i> NN016038) AIY14611.1 78% Iota-carrageenase ( <i>Cellulophaga sp.</i> QY3) AEV89930.1	97% Uncharacterized protein ( <i>Cellulophaga lytica</i> ) FORBP4 90% Uncharacterized protein ( <i>Cellulophaga geojensis</i> KL-A) W7R1L4 90% Iota-carrageenase ( <i>Cellulophaga sp.</i> KL-A) A0A059WND7 78% Iota-carrageenase ( <i>Cellulophaga sp.</i> QY3) M1EU02 78% Uncharacterized protein ( <i>Cellulophaga algicola</i> ) E6XD22	9.33 54 kDA	GH82	NO
<b>Gmi1_3</b> lota-carrageenase	100% Hypothetical protein ( <i>Pseudoalteromonas sp.</i> P1-11) WP_055255568.1 96% Hypothetical protein ( <i>Pseudoalteromonas sp.</i> P1-25) WP_055017468.1 90% Hypothetical protein ( <i>Pseudoalteromonas sp.</i> S3431) KDC54733.1 85% Hypothetical protein ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024594191.1 37% Hypothetical protein ( <i>Shewanella sp.</i> P1-14-1) WP_055024040.1	33% Uncharacterized protein ( <i>Gammproteobacteria bacterium</i> Q1) A0A0J8GTJ1 30% Iota-carrageenase ( <i>Microbulbifer thermotolerans</i> ) E3W9G3 25% Uncharacterized protein ( <i>Catenovulum agarivorans</i> ) W7QNT2 27% Uncharacterized protein ( <i>Gammproteobacteria bacterium</i> Q1) A0A0J8GRK4 25% Uncharacterized protein ( <i>Gammproteobacteria bacterium</i> Q1) A0A0J8GR29	9.46 59 kDA	GH82	YES
<b>GmXyl_7</b> Xylanase	99% family 8 endo-1,4-beta-xylanase ( <i>Pseudoalteromonas arctica</i> ) CBY88881.1 98% hypothetical protein ( <i>Pseudoalteromonas sp.</i> BSi20429) GAA68809.1 98% hypothetical protein ( <i>Pseudoalteromonas haloplanktis</i> ) WP_024593845.1 98% endo-1,4-beta-xylanase ( <i>Pseudoalteromonas haloplanktis</i> ) CAD20872.1 97% Hypohtetical protein ( <i>Pseudoalteromonas multispecies</i> ) WP_007375181.1	100% Uncharacterized protein (marine sediment metagenome) A0A0F9TRL3 99% Family 8 endo-1,4-beta-xylanase ( <i>Pseudoalteromonas arctica</i> ) F2QKE3 98% Uncharacterized protein ( <i>Pseudoalteromonas sp.</i> BSi20429) G7F6N6 98% Endo-1,4-beta-xylanase ( <i>Pseudoalteromonas haloplanktis</i> ) Q8RJN8 97% Uncharacterized protein ( <i>Pseudoalteromonas citrea</i> ) A0A063KSA3	8.18 48 kDA	GH8	YES
<b>GmBg_1</b> Beta-glucosidase	86% beta-glucosidase ( <i>Pseudoalteromonas sp.</i> ECSMB14103) WP_039037212.1 86% beta-glucosidase ( <i>Pseudoalteromonas sp.</i> BSi20480) GAA76322.1 86% glucan 1,4-beta-glucosidase ( <i>Pseudoalteromonas marina</i> ) ERG25892.1 54% beta-glucosidase ( <i>Microbulbifer sp.</i> HZ11) WP_043317086.1 54% 1,4-beta-D-glucan glucohydrolase ( <i>Microbulbifer hydrolyticus</i> ) AAT81216.1	86% Glucan 1,4-beta-glucosidase ( <i>Pseudoalteromonas marina</i> ) U1K041 54% 1,4-beta-D-glucan glucohydrolase ( <i>Microbulbifer hydrolyticus</i> ) Q693B4 52% Beta-glucosidase ( <i>Alteromonadales bacterium</i> TW-7) A0XZU5 52% Beta-glucosidase ( <i>Pseudoalteromonas sp.</i> BSi20429) G7F3X4 51% Periplasmic beta-glucosidase ( <i>Pseudoalteromonas haloplanktis</i> ) F3BMX0	6.18 92 kDA	GH3	YES
<b>GmEst_6</b> Carboxy-esterase	99% Homoserine O-acetyltransferase ( <i>Paraglaciecola mesophila</i> ) GAC24751.1 99% Homoserine O-acetyltransferase ( <i>Pseudoalteromonas sp.</i> ) WP_033185654.1 93% Homoserine O-acetyltransferase ( <i>Pseudoalteromonas atlantica</i> ) ABG40496.1 81% Homoserine O-acetyltransferase ( <i>Paraglaciecola agarilytica</i> ) GAC07247.1 80% Homoserine O-acetyltransferase ( <i>Paraglaciecola chathamensis</i> ) GAC12013.1	99 % Homoserine O-acetyltransferase ( <i>Paraglaciecola mesophila</i> ) K6YL61 93% Homoserine O-acetyltransferase ( <i>Pseudoalteromonas atlantica</i> ) Q15UE2 81% Homoserine O-acetyltransferase ( <i>Paraglaciecola agarilytica</i> ) K6XME2 80% Homoserine O-acetyltransferase ( <i>Glaciecola sp.</i> ) F4AS30 75% Homoserine O-acetyltransferase ( <i>Paraglaciecola polaris</i> ) K6Z9C2	6.6 44 kDA	ABH 1	YES

ABH : alpha/beta-Hydrolase; GH : Glycoside hydrolase; pi/Mw : Isoelectric point/molecular weight; SP: signal peptide

Figure 1  
[Click here to download high resolution image](#)

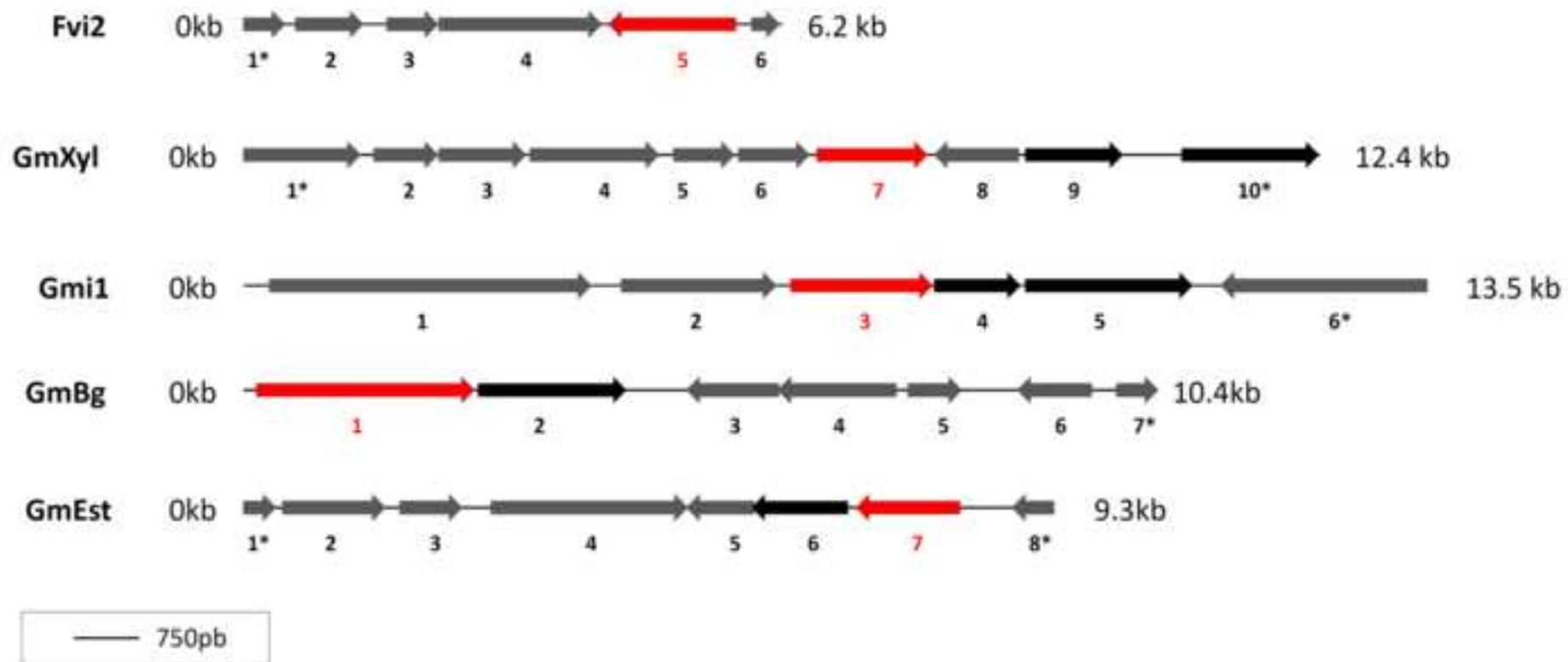


Figure 2  
[Click here to download high resolution image](#)

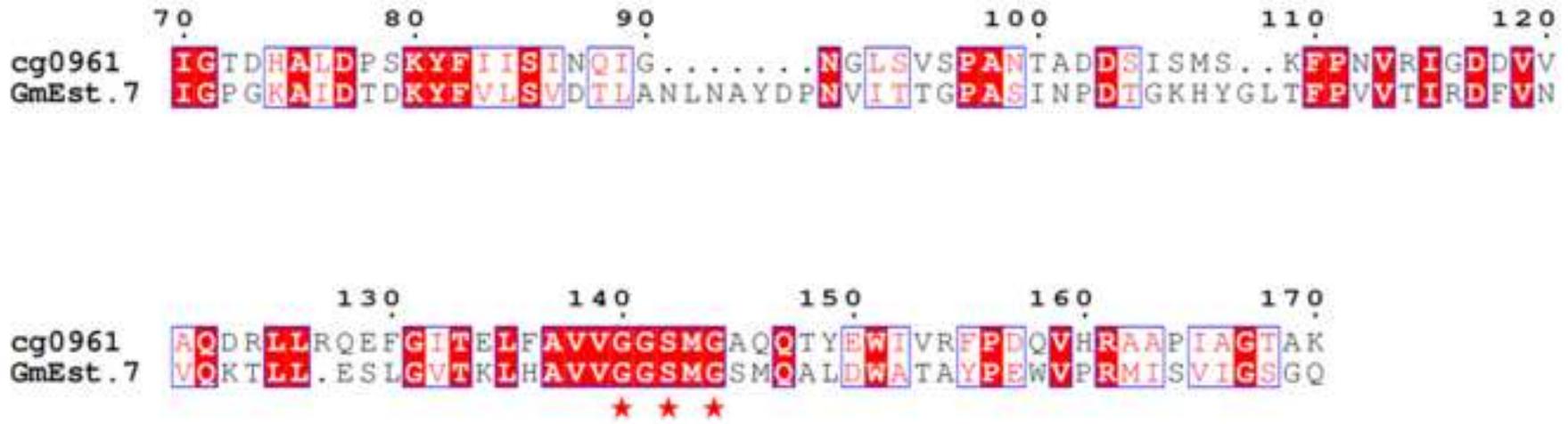


Figure 3  
[Click here to download high resolution image](#)

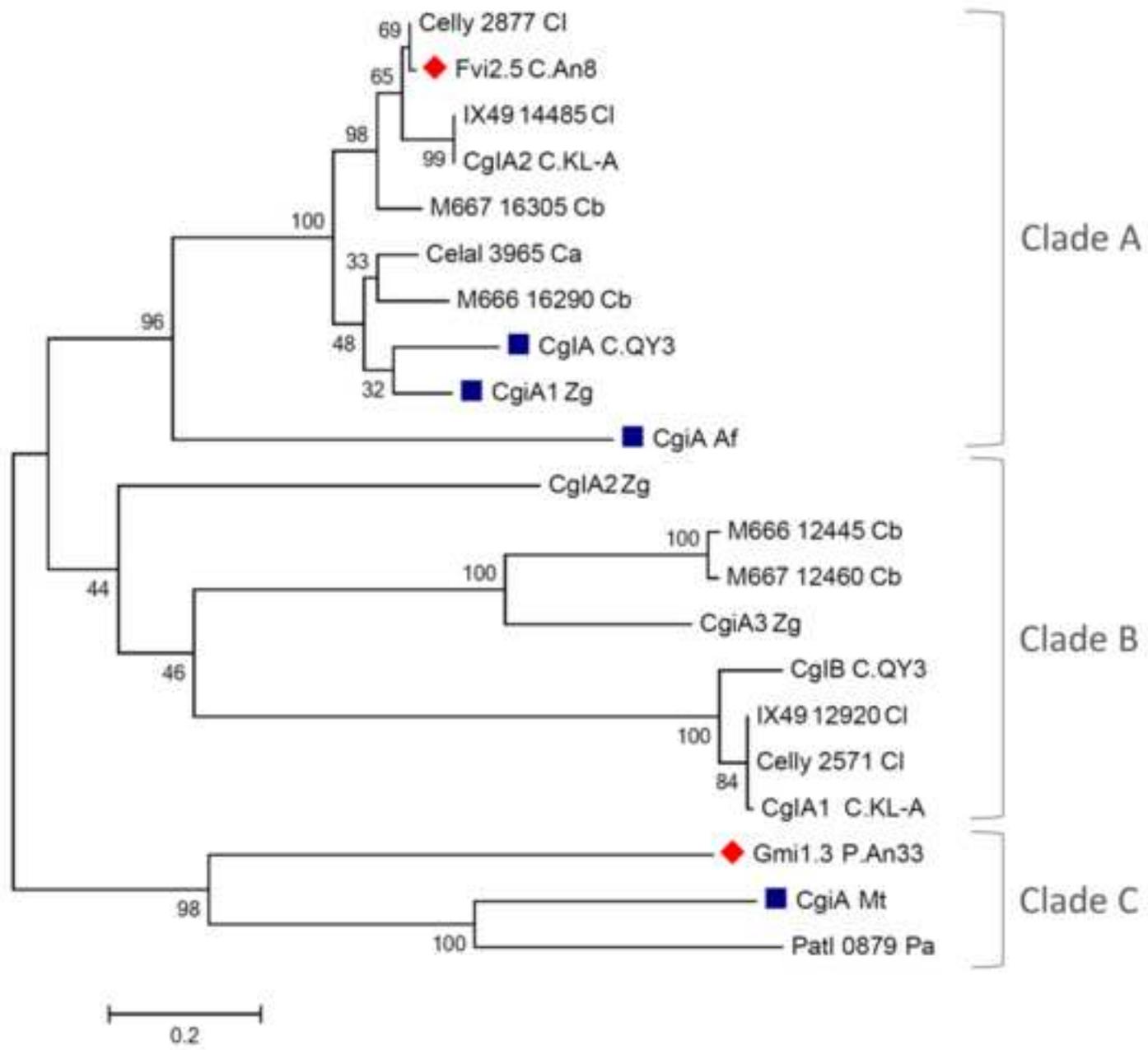


Figure 4

[Click here to download high resolution image](#)

