



HAL
open science

Draft Genome Sequence of the Lignocellulolytic and Thermophilic Bacterium *Thermobacillus xylanilyticus* XE

Harivony Rakotoarivonina, Valentin Loux, Christelle Doliwa, Véronique Martin, Caroline Rémond

► **To cite this version:**

Harivony Rakotoarivonina, Valentin Loux, Christelle Doliwa, Véronique Martin, Caroline Rémond. Draft Genome Sequence of the Lignocellulolytic and Thermophilic Bacterium *Thermobacillus xylanilyticus* XE. *Microbiology Resource Announcements*, 2022, 11, 10.1128/mra.00934-21 . hal-04099349

HAL Id: hal-04099349

<https://hal.science/hal-04099349>

Submitted on 16 May 2023

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.



Distributed under a Creative Commons Attribution 4.0 International License



Draft Genome Sequence of the Lignocellulolytic and Thermophilic Bacterium *Thermobacillus xylanilyticus* XE

Harivony Rakotoarivonina,^a Valentin Loux,^{b,c} Christelle Doliwa,^a Véronique Martin,^b  Caroline Rémond^a

^aUniversité de Reims Champagne Ardenne, INRAE, FARE, UMR A 614, Chaire AFERE, Reims, France

^bUniversité Paris-Saclay, INRAE, MalAGE, Jouy-en-Josas, France

^cUniversité Paris-Saclay, INRAE, BioinfOmics, MIGALE Bioinformatics Facility, Jouy-en-Josas, France

ABSTRACT *Thermobacillus xylanilyticus* is a thermophilic and hemicellulolytic bacterium able to use several lignocelluloses as its main carbon source. This draft genome sequence gives insight into the genomic potential of this bacterium and provides new resources to understand the enzymatic mechanisms used by the bacterium during lignocellulose degradation and will allow the identification of robust lignocellulolytic enzymes.

Thermophilic microorganisms may provide robust and thermostable enzymes for plant biorefining to produce biofuels and biomolecules (1, 2). *Thermobacillus xylanilyticus* XE (deposited at the Collection Nationale de Cultures Microbiennes France under no. CNCM I-1017) is a Gram-positive, thermophilic bacterium, originally isolated from farm soil situated underneath a manure heap, that is able to use nonpretreated lignocelluloses as its carbon source (3–5). This bacterium produces several hemicellulases (xylanase, arabinosidase, and feruloyl esterase) that have resistance to high temperatures (60° to 70°C) and alkaline reaction conditions (4–10) and efficiently liberate sugars and phenolic compounds from various lignocelluloses (8, 10–15).

T. xylanilyticus was grown at 50°C on basal medium complemented with 5 g/L xylan (3). The total genome was isolated from early-stationary-phase cell cultures using the Purelink genomic DNA kit (Thermo Fisher). DNA was quantified using a fluorimetry methodology (Qubit v2.0; Thermo Fisher) and by gel electrophoresis analysis. Genomic DNA was fragmented with a Covaris S220 instrument. DNA libraries were prepared with a TruSeq SBS kit v5 (Illumina) protocol and the SPRIworks fragment library system I. The genome was sequenced by using 74-nucleotide paired-end sequencing with Genome Analyzer IIx instruments (Illumina). Raw read quality was assessed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and quality filtering was performed using Sickle (<https://github.com/najoshi/sickle>). The genome sequence was assembled *de novo* using Velvet v1.1.04 (16) after quality analyses and trimming of raw reads. The annotation was performed with the AGMIAL platform (17). Default parameters were used for all software.

A total of 32,786,406 raw reads were obtained, representing an average coverage of 58×; 240 Mb of raw sequences was assembled into 108 scaffolds (146 contigs). The N_{50} value of the assembly was 83 kb. The largest contig size was 184,473 bp. The size of the genome was 4,109,925 nucleotides with a G+C content of 61.0%. The draft genome encoded 3,956 proteins and 55 tRNAs; 1 copy each of 23S rRNA and 16S rRNA was correctly assembled. The lignocellulolytic potential of *T. xylanilyticus* was assessed by identifying the relevant genes for lignocellulose utilization (CAZymes) by using a procedure analogous to that used for the analysis of daily releases of GenBank data by the CAZy database (www.cazy.org) (18) and involving both automated and human curation-based BLASTP (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and HMMER v3 (<http://hmmer.janelia.org/>) results against libraries of modules derived from the database. A total of 94 genes encoding glycoside hydrolases, 16 genes encoding carbohydrate esterases, 45 genes encoding glycosyl transferases,

Editor J. Cameron Thrash, University of Southern California

Copyright © 2022 Rakotoarivonina et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Harivony Rakotoarivonina, harivony.rakotoarivonina@univ-reims.fr.

The authors declare no conflict of interest.

Received 26 November 2021

Accepted 18 February 2022

Published 8 March 2022

and 2 genes encoding polysaccharide lyases were identified. Some of these genes were organized in operon structures. Except for genes encoding β -glucosidases and one putative gene encoding an exoglucanase, no other genes related to cellulose degradation were identified. Several oxidoreductases have been annotated. This genome sequence will facilitate the identification of new genes implicated in the enzymatic fractionation of lignocellulosic biomass for the production of new enzymes of interest.

Data availability. *T. xylanilyticus* raw reads and the draft genome sequence have been deposited at the European Nucleotide Archive (ENA) under the study accession number [PRJEB43105](https://ena.ebi.ac.uk/ena/record/PRJEB43105). The raw reads are available under run accession number [ERR5840465](https://ena.ebi.ac.uk/ena/record/ERR5840465). The annotated genome sequence is available under WGS sequence set [CAJRAY010000000](https://ena.ebi.ac.uk/ena/record/CAJRAY010000000). The version described in this paper is the first version.

ACKNOWLEDGMENTS

We thank Pedro M. Coutinho (Département Génie Biologique/Biological Engineering Department/AFMB, Polytech Marseille, Université Aix-Marseille) and Bernard Henrissat (Technical University of Denmark, Lyngby, Denmark) for their help in annotating the CAZymes of *T. xylanilyticus*.

We acknowledge the high-throughput sequencing facility of I2BC for its sequencing and bioinformatics expertise.

We are grateful to the University of Reims Champagne-Ardenne, the French Region Grand Est, Grand Reims, and Feder for their financial support (chair AFERE).

REFERENCES

- Bhalla A, Bansal N, Kumar S, Bischoff KM, Sani RK. 2013. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes. *Bioresour Technol* 128:751–759. <https://doi.org/10.1016/j.biortech.2012.10.145>.
- Viikari L, Alapuranen M, Puranen T, Vehmaanperae J, Siika-Aho M. 2007. Thermostable enzymes in lignocellulose hydrolysis. *Adv Biochem Eng Biotechnol* 108:121–145. https://doi.org/10.1007/10_2007_065.
- Touzel J-P, O'Donohue M, Debeire P, Samain E, Breton C. 2000. *Thermobacillus xylanilyticus* gen. nov., sp. nov., a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil. *Int J Syst Evol Microbiol* 50: 315–320. <https://doi.org/10.1099/00207713-50-1-315>.
- Rakotoarivonina H, Hermant B, Monthe N, Remond C. 2012. The hemicellulolytic enzyme arsenal of *Thermobacillus xylanilyticus* depends on the composition of biomass used for growth. *Microb Cell Fact* 11:159. <https://doi.org/10.1186/1475-2859-11-159>.
- Harris GW, Pickersgill RW, Connerton I, Debeire P, Touzel J-P, Breton C, Perez S. 1997. Structural basis of the properties of an industrially relevant thermophilic xylanase. *Proteins* 29:77–86. [https://doi.org/10.1002/\(SICI\)1097-0134\(199709\)29:1%3C77::AID-PROT6%3E3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0134(199709)29:1%3C77::AID-PROT6%3E3.0.CO;2-C).
- Connerton I, Cummings N, Harris GW, Debeire P, Breton C. 1999. A single domain thermophilic xylanase can bind insoluble xylan: evidence for surface aromatic clusters. *Biochim Biophys Acta* 1433:110–121. [https://doi.org/10.1016/S0167-4838\(99\)00151-X](https://doi.org/10.1016/S0167-4838(99)00151-X).
- Debeche T, Cummings N, Connerton I, Debeire P, O'Donohue MJ. 2000. Genetic and biochemical characterization of a highly thermostable alpha-L-arabinofuranosidase from *Thermobacillus xylanilyticus*. *Appl Environ Microbiol* 66:1734–1736. <https://doi.org/10.1128/AEM.66.4.1734-1736.2000>.
- Rakotoarivonina H, Hermant B, Chabbert B, Touzel J-P, Remond C. 2011. A thermostable feruloyl-esterase from the hemicellulolytic bacterium *Thermobacillus xylanilyticus* releases phenolic acids from non-pretreated plant cell walls. *Appl Microbiol Biotechnol* 90:541–552. <https://doi.org/10.1007/s00253-011-3103-z>.
- Samain E, Debeire P, Touzel JP. 1997. High level production of a cellulase-free xylanase in glucose-limited fed batch cultures of a thermophilic *Bacillus* strain. *J Biotechnol* 58:71–78. [https://doi.org/10.1016/S0168-1656\(97\)00140-5](https://doi.org/10.1016/S0168-1656(97)00140-5).
- Rakotoarivonina H, Revol PV, Aubry N, Remond C. 2016. The use of thermostable bacterial hemicellulases improves the conversion of lignocellulosic biomass to valuable molecules. *Appl Microbiol Biotechnol* 100:7577–7590. <https://doi.org/10.1007/s00253-016-7562-0>.
- Remond C, Aubry N, Cronier D, Noel S, Martel F, Roge B, Rakotoarivonina H, Debeire P, Chabbert B. 2010. Combination of ammonia and xylanase pretreatments: impact on enzymatic xylan and cellulose recovery from wheat straw. *Bioresour Technol* 101:6712–6717. <https://doi.org/10.1016/j.biortech.2010.03.115>.
- Rémond C, Boukari I, Chambat G, O'Donohue M. 2008. Action of a GH 51 alpha-L-arabinofuranosidase on wheat-derived arabinoxylans and arabinoxylooligosaccharides. *Carbohydrate Polymers* 72:424–430. <https://doi.org/10.1016/j.carbpol.2007.09.008>.
- Rakotoarivonina H, Hermant B, Aubry N, Rabenoelina F, Baillieux F, Remond C. 2014. Dynamic study of how the bacterial breakdown of plant cell walls allows the reconstitution of efficient hemicellulase cocktails. *Bioresour Technol* 170: 331–341. <https://doi.org/10.1016/j.biortech.2014.07.097>.
- Dupoiron S, Lameloise ML, Pomet M, Bennaceur O, Lewandowski R, Allais F, Teixeira ARS, Remond C, Rakotoarivonina H. 2017. A novel and integrative process: from enzymatic fractionation of wheat bran with a hemicellulase cocktail to the recovery of ferulic acid by weak anion exchange resin. *Ind Crops Prod* 105:148–155. <https://doi.org/10.1016/j.indcrop.2017.05.004>.
- Husson E, Auxenfans T, Herbaut M, Baralle M, Lambertyn V, Rakotoarivonina H, Remond C, Sarazin C. 2018. Sequential and simultaneous strategies for biorefining of wheat straw using room temperature ionic liquids, xylanases and cellulases. *Bioresour Technol* 251:280–287. <https://doi.org/10.1016/j.biortech.2017.12.047>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Bryson K, Loux V, Bossy R, Nicolas P, Chaillou S, van de Guchte M, Penaud S, Maguin E, Hoebeke M, Bessières P, Gibrat J-F. 2006. AGMIAL: implementing an annotation strategy for prokaryote genomes as a distributed system. *Nucleic Acids Res* 34:3533–3545. <https://doi.org/10.1093/nar/gkl471>.
- Drula E, Garron ML, Dogan S, Lombard V, Henrissat B, Terrapon N. 2022. The carbohydrate-active enzyme database: functions and literature. *Nucleic Acids Res* 50:D571–D577. <https://doi.org/10.1093/nar/gkab1045>.