Xylanases from thermophilic archaea: A hidden treasure

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ABSTRACT

Archaea are a domain of prokaryotic organisms that often inhabit extremophilic environments. Their enzymes are therefore of enormous biotechnological interest, due to their stability and activity in harsh conditions. Although bioprospection by the metagenomics of extreme environments has yielded many novel xylanases (*endo*-xylanases and β-xylosidades), most show a highest sequence similarity to bacteria or unculturable microorganisms and not to archaea.

This graphical review focuses on the very promising but underexploited (hyper)thermophilic archaeal xylanases. There are only a few examples of xylanases isolated from culturable archaea. So far, 5 different (hyper) thermophilic archaeal strains with xylanase activity have been described, of which 4 present *endo*-xylanase activity and one shows both *endo*-xylanase and β-xylosidase activity. All of them show enzymatic activity under a high optimum temperature, neutral optimum pH (with one exception).

These facts, together with the important applications of xylanases, reveal archaeal extremophilic xylanases to be a hidden treasure of biotechnology. Bioprospection of archaeal *endo*-xylanases constitutes a relatively unexploited field, full of potential.

Contents

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1. Introduction

Microorganisms of the Archaea domain inhabit almost every environment on Earth, from terrestrial and marine environments to the rumen of ruminants and insect/termite gut (Thapa et al., 2020). Archaea are well-known for their numerous extremophilic and polyextremophilic lineages, mainly (hyper)thermophilic and/or halophilic, and also for the ecological importance of methanogenic and ammonia-oxidizing species (Naitam and Kaushik, 2021). This domain has been historically eclipsed by bacteria and fungi as a source for biotechnologically relevant enzymes. Nevertheless, their huge potential for diverse biotechnological applications is clear and becoming accessible, partially due to recent developments in genetic engineering techniques. Archaea not only are producers of active enzymes resistant to harsh industrial conditions, such as the well-known DNA polymerase from *Pyrococcus furiosus*, but they also serve as a cell factory for other bioproducts like lipids, methane, biohydrogen, polyhydroxyalcanoates, surface-layer proteins and bacteriorhodopsin, among others (Pfeifer et al., 2021).

Xylan is a natural major renewable polysaccharide after cellulose and xylanases, the crucial enzymes that convert the xylan into xylose. Most industrial operations that use xylanases demand a thermostable and alkali stable enzyme. Although efforts have been made in recent years to find xylanases that meet the requirements of demanding industrial conditions, there is still a need for a new xylanase. Despite potential, there are few studies regarding plant cell-wall degrading archaea. The termite gut has proven to be a unique reservoir of archaeal species producing cellulases and xylanases, being an environment where it is necessary to degrade the lignocellulose present in plants. The majority of gut *endo*-xylanases are acidic and thermophilic in nature due to the characteristics of these intestinal environments (Verma, 2021). *Thermoplasmatales* is an example of a thermophilic archaeon present in termites, which is also present in terrestrial and marine environments (Paul et al., 2012).

This graphical review is focused on 2 specific archaeal thermophilic enzymes of current interest: *endo*-xylanases and β-xylosidases.

2. Xylan: The substrate

A valuation of highly abundant lignocellulosic wastes is a key objective of the developing strategies of circular bioeconomy and sustainable development (Gudiña et al., 2020). Lignocellulose is formed by 40–50 % cellulose, 20–40 % hemicellulose and 20–30 % lignin, depending on its origin (Fig. 1A). These 3 polymers are tightly associated, making lignocellulose recalcitrant to microbial and enzymatic hydrolysis and therefore physicochemical pretreatments are usually required before any bioprocess (Verma, 2021). Hemicellulose is a mixture of several polysaccharides that interconnect cellulose and lignin in lignocellulose (Bhardwaj et al., 2019). Xylan, the second most abundant polysaccharide in plants after cellulose is a hemicellulolytic heteropolysaccharide formed by β-(1–4)-linked xylose main chains with acetyl, methyl-glucuronyl, and arabinosyl substituents. These side chains vary depending on their origin and may form ester bonds with ferulic and p-coumaric acids (Rohman et al., 2019). Hemicellulose and lignin are covalently cross-linked, delignification is favored for xylan hydrolysis. This aids both cellulose purification and enzyme access to cellulose in bioprocess (Sani and Krishnaraj, 2017). In addition, oligosaccharides and monosaccharides resulting from xylan hydrolysis can be converted into high value-added products.

3. Xylanolytic enzymes

Due to its complexity and diversity, the complete hydrolysis of xylan to its monosaccharide units involves more than 20 enzymatic activities, mostly with glycosyl hydrolases and carbohydrate esterases (Juturu and Wu, 2014). These can be classified in 2 groups: xylanases and accessory enzymes. The so-called xylanases (*endo*-xylanases and β-xylosidases), cleave the β-1,4 linkages of the xylan main chain in a synergistic manner (Lagaert et al., 2014). Accessory enzymes cleave the

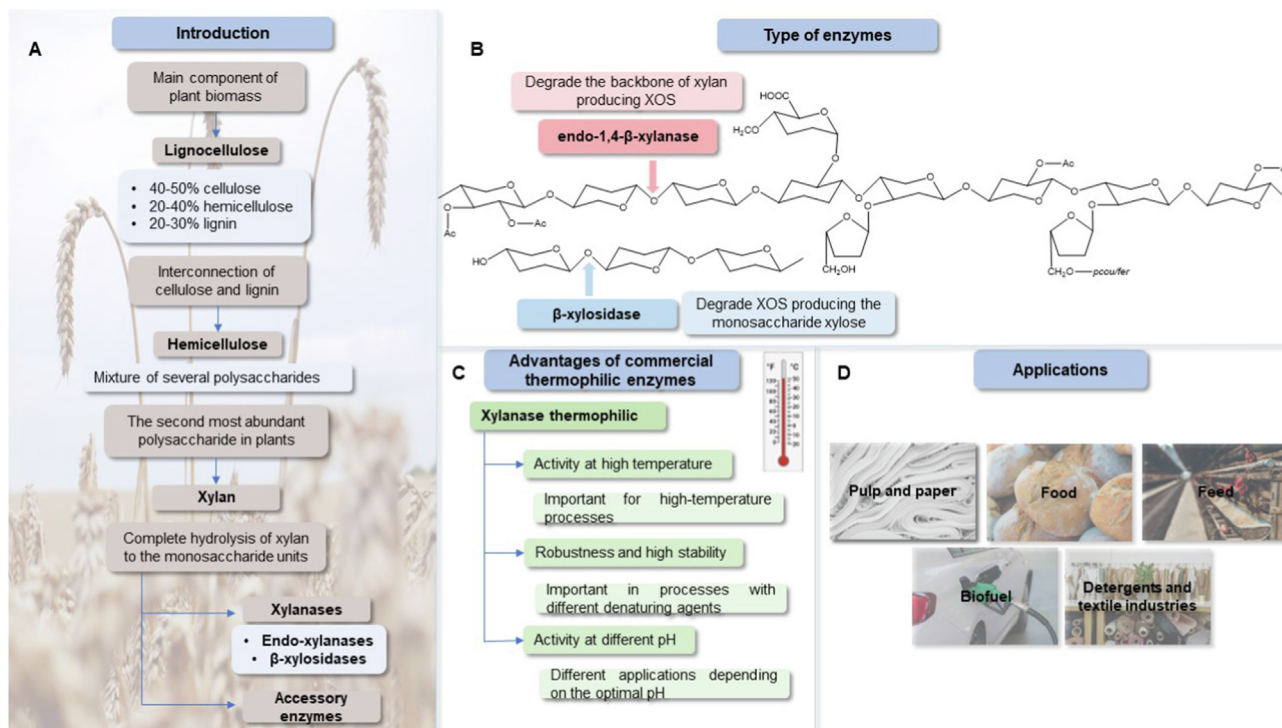
Fig. 1. Introduction to lignocellulose chemical composition and xylanolytic enzymes (**A**), types of xylanases (**B**), advantages of commercial thermophilic enzymes (**C**) and applications of xylanases (**D**).

linkages of this backbone with the side chains, and the crosslinks between xylan and lignin. endo-xylanases (EC 3.2.1.8) degrade the backbone of xylan producing xylooligosaccharides, while β-xylosidases (EC 3.2.1.37) degrade these xylooligosaccharides producing monosaccharide xylose (Juturu and Wu, 2012) (Fig. 1B). endo-xylanases are frequently secreted into the extracellular environment, as the large size of the substrate prevents penetration into the cell. It is thought that small amounts of constitutively expressed enzymes release xylooligomers that are transported into the cell for further degradation by β-xylosidases activity enzymes. These xylooligomers induce xylanase synthesis inside the cell (Verma et al., 2019).

Xylanases are becoming an increasingly important group of hydrolases among industrial enzymes, a class that represents about 75 % of the market. Xylanases have applications in pulp and paper, food and feed, detergents, and textile industries, also, in the production of biofuels and xylitol by fermentation of hydrolyzed lignocellulosic wastes. Thermophilic xylanases are especially needed for processes that are performed at high temperature, and are also useful in other processes due to their robustness and high stability against different denaturing agents other than temperature (Fig. 1C-D). The interval of pH in which the enzyme is active is also an important factor to consider: e.g. alkaline xylanases are used for pulp bleaching whereas acidic xylanases are suitable for poultry feed (Chadha et al., 2019). An interesting characteristic of some β-xylosidases for biotechnological applications, is their transxylosylation (transglycosylation) activity, which consists in forming new glycosidic bonds that produce longer oligosaccharide chains, xylooligosaccharides that may have prebiotic activity (Knob et al., 2010).

Thermophilic xylanases are naturally synthesized by thermophilic microorganisms that live at optimal temperatures above 50 °C. However, the high-scale production of the enzymes from their native microorganism is difficult due to demanding culture conditions and low growth rates and yields, especially for the hyperthermophilic (existing at optimal temperatures above 80 °C). Therefore, the production of these enzymes is accomplished through heterologous host systems (Basit et al., 2018). Among the varied applications of xylanases, the main market niches for thermophilic xylanases are in bioethanol production, paper pulp bleaching and as silage additives (Chadha et al., 2019). Highscale commercial xylanases are predominantly *endo*-xylanases and β-xylosidases that are available only as xylanase cocktails, whereas production of low-scale purified xylanases is mostly restricted to research purposes. One of the main reasons for this is that β-xylosidases usually show a multimeric structure that is stabilized by disulfide bonds and glycosylation, that hinders their functional heterologous expression (Juturu and Wu, 2012). Despite significant progress made, at present the high cost, low stability, and low activity under operating conditions of the enzymes employed in biomass conversion are still considered limiting factors of the profitability of such bioprocesses. Therefore, novel and improved enzymes are actively being pursued by researchers, mainly through functional metagenomics of extremophilic environments (Kumar et al., 2018).

3.1. Bioprospecting for novel thermophilic xylanases by metagenomics

Metagenomics encompasses the study of complex microbial communities whose DNA is extracted from environmental samples without isolation nor cultivation of any microorganism. There are 2 approaches (Fig. 2): sequence-based (computational, metagenome mining) and function-based (experimental, construction and screening of metagenomic libraries) (DeCastro et al., 2016).

So far, thermophilic xylanases discovered by metagenomics and assigned to archaeal species are scarce or none. Most of these metagenomics-derived xylanases show a highest sequence identity/ similarity related to bacteria. This can be attributed to various reasons: the number of genes of hemicellulases in databases is low compared to other hydrolases (Kumar et al., 2018); a low proportion of archaea with respect to bacteria in thermophilic metagenomes (approximately 5 % compared to more than 90 %); the environments preferred by archaea (geothermal springs, deep sea, hypersaline lakes) are usually not rich in lignocellulosic materials; and importantly, the fact that many enzymes found by metagenomics are assigned to unclassified unculturable microorganisms with low sequence identity/similarity percentages to known species (DeCastro et al., 2016).

3.2. Molecular mechanism of xylanases

Both *endo*-xylanases and β-xylosidases catalyze the hydrolysis of glycosidic bonds. This hydrolysis can be carried out by 2 mechanisms: a general retention (Fig. 3A) or a general inversion (Fig. 3B) of the anomeric carbon configuration of the substrate. Both mechanisms of hydrolysis share the need in most cases, for 2 carboxylic acids conserved within each glycoside hydrolase family, although at different distances in function of the mechanism, and progress through transition states of the oxocarbenium ion type. The 2 carboxyl groups in inverting xylanases (side chains of glutamate and aspartate) serve as general acid and base catalysts, and are separated by an average of 10.5 Å, where the bonding occurs between substrate and a water molecule. The reaction occurs through a single displacement mechanism. One carboxylic acid, the general base, activates a water molecule to attack nucleophilically the anomeric center of the substrate. The other carboxylic acid promotes the release of the leaving group via general acid catalysis. Retention catalysis is carried out through a two-step double displacement mechanism, with each step resulting in inversion to get a final net retention of stereochemistry that involves a glycosyl-
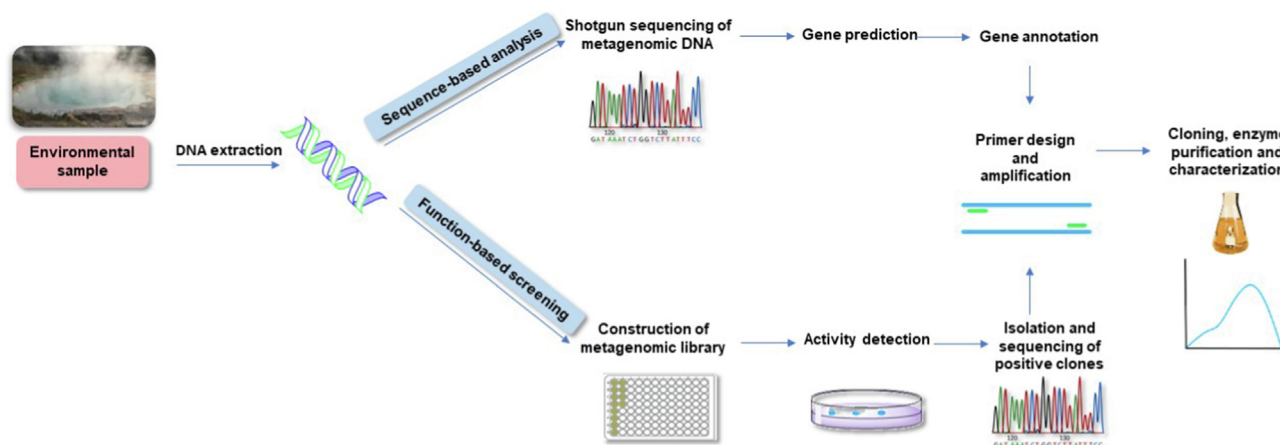

Fig. 2. The 2 main strategies used for isolation of new enzymes from environmental metagenomes.
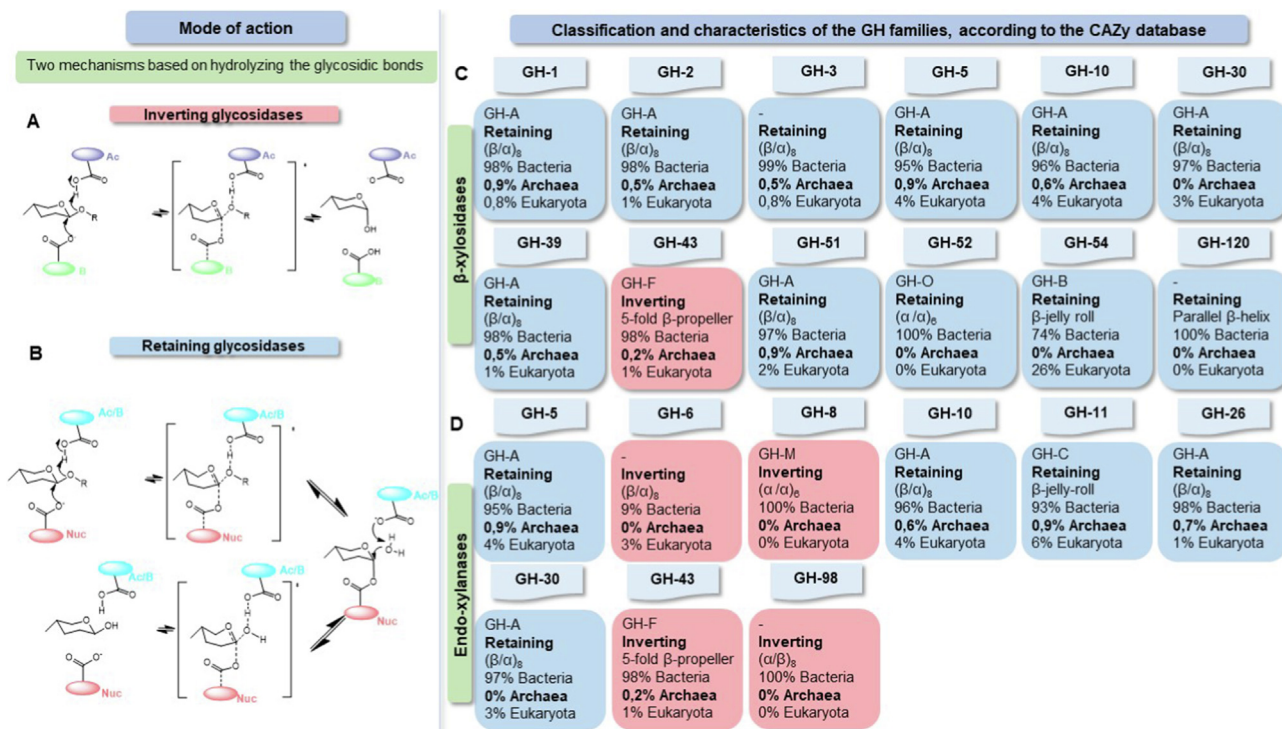
Fig. 3. Mode of action for (**A**) inverting and (**B**) retaining glycosidases. Adapted from (Collins et al., 2005). Classification and characteristics of the GH families of β-xylosidases (**C**) and *endo*-xylanases (**D**), according to the CAZy database.

enzyme intermediate. The carboxyl groups in retention glycosidases (side chains of glutamates) are separated by only 5.5 Å, with one functioning as a general acid catalyst and the other as a nucleophile. In the first step, the acting general acid catalyst carries out the protonation of the glycosidic oxygen with the concomitant cleavage of the bond. The other carboxylic group acting as a nucleophile, carries out the formation of a covalent glycosyl-enzyme intermediate. In the second step, the deprotonation of the water molecule takes place, and the deprotonated water molecule attacks the anomeric center and displaces the carbohydrate (Knob et al., 2010).

3.3. Classification of archaeal xylanases

According to CAZy (The Carbohydrate-Active EnZymes database) (Elodie et al., 2022), that experimentally classifies characterized enzymes by the function of several parameters (sequence homology, structure, mechanism of action, substrate specificity, molecular mass and isoelectric point), β-xylosidases are present in families 1, 2, 3, 5, 10, 30, 39, 43, 51, 52, 54 and 120 of glycoside hydrolases (GHs). Archaeal β-xylosidases have been described for half of these families (1, 2, 3, 5, 10, 39, 43 and 51) (Fig. 3C). In the same way, *endo*-xylanases are classified under GH families 5, 6, 8, 10, 11, 26, 30, 43, and 98, and archaeal *endo*-xylanases are present in only 5 of these families, 5, 10, 11, 26 and 43 (Fig. 3D). The proportion of archaeal xylanases is less than 1 % in the GH families where they are present, with bacterial enzymes the most abundant, followed by eukaryotic ones (fungal). Except for the families GH-43 (inverting, β-propeller) and GH-11 (retaining, β-jelly roll), archaeal β-xylosidases and *endo*-xylanases show a retaining mechanism of action and a characteristic (β/α)$_8$ structural motif.

3.4. Characterized archaeal thermophilic xylanases

There are a few described examples of remarkable xylanases of archaeal origin (Table 1). The first archaeal xylanase biochemically

characterized was an *endo*-xylanase from *Thermococcus zilligii* strain AN1, a highly thermophilic and thermostable enzyme (half-life 8 min at 100 °C), with activity on several xylans but not with other polysaccharides or p-nitrophenyl substrates. It has a unique *N*-terminal sequence (Uhl and Daniel, 1999). The microorganism, with an optimal growth rate at 75 °C, was isolated from a geothermal pool in New Zealand with activity found both in the extracellular medium and in the cell pellet. A library of the *Thermococcus zilligii* strain AN1 genome was constructed, and it was concluded after hybridization experiments, that the unique *N*-terminal sequence belonged to a maltodextrin phosphorylase and not to the xylanase (Rolland et al., 2002). The secreted *endo*-xylanase of the hyperthermophilic *Pyrodictium abyssi,* isolated from marine hot abyssal sites, proved to be highly thermostable (retaining its activity for over 100 min at 105 °C) and active at 110 °C (Andrade et al., 2001). The presence of *endo*-xylanase and β-xylosidase activities was observed in the extremely halophilic *Halohrabdus utahensis* isolated from sediments of the Great Salt Lake (Utah, USA). Both enzymes showed high optimal temperatures (above 65 °C) and salt concentration tolerance (30 % NaCl). While the *endo*-xylanase was mostly extracellular, the β-xylosidase was mostly intracellular (Wainø and Ingvorsen, 2003). Another *endo*-xylanase was discovered after the isolation of 2 strains (O(alpha) and X(2)) of the hyperthermophilic *Sulfolobus solfataricus* strain MT4 based on the ability to grow on xylan. It was concluded that both strains produced the same *endo*-xylanase, which was membrane associated and highly thermostable, showing a half-life of 47 min at 100 °C, with an optimal temperature and pH of 90 °C and 7.0, respectively (Cannio et al., 2004). Finally, the extremely halophylic *Natrinema sp.* strain SSBJUP-1 was isolated from Lonar Lake (Buldhana, Maharashtra, India) This microorganism produces extracellular cellulase and xylanase with optimum activity at pH 9–10 and mostly stable up to pH 11 and 16 % of NaCl concentration. The xylanase showed maximum activity 50 °C (Patil and Bajekal, 2014). Most xylanases show optimal pH in the neutral to acid range, with alkaliphilic ones less frequent.

Table 1

Characteristics of thermostable *endo*-xylanases and β-xylosidases from different archaea (Andrade et al., 2001) (Patil and Bajekal, 2014)(Uhl and Daniel, 1999)(Cannio et al., 2004)(Wainø and Ingvorsen, 2003).

Type of enzyme	Organisms	Source	Molecular weight (kDa)	pH optimum	Temperature optimum (°C)	Key observation	Reference
Endo-xylanase	*Pyrodictium abyssi*	Alkaline hot spring in Kuirau Park (Rotorua, New Zealand)	–	–	100–110	–	Andrade et al,. 2001
Endo-xylanase	*Natrinema* sp. strain SSBJUP-1	Lonar Lake situated in Buldhana (Maharashtra, India)	–	9–10	40–60	–	Patil and Bajekal., 2014
Endo-xylanase	*Thermococcus zilligii* strain ANI	Alkaline hot spring in Kuirau Park (Rotorua, New Zealand)	95	6.0	75	Using birch wood xylan as substrate: K_m 1.58 mg ml^{-1}, and V_{max} 69.0 μmol ml^{-1} min^{-1}.	Uhl and Daniel., 1999
Endo-xylanase	*Sulfolobus solfataricus*	Originally solfatara in Pisciarelli di Agnano (Naples, Italy)	57	7.0	90	Monomeric structure.At pH 4.0, 8.0 and 9.0: retained less than 65 % activity.At 100 °C during 1 h: retained 30 % activity. The highest activity with beech wood xylan.	Cannio et al., 2004
Endo-xylanase	*Halorhabdus utahensis*	Great Salt Lake (Utah, USA)	45	7.6	55–70	At 50 °C during 8 h: retained all activity. At 55 °C during 8 h: retained 16 % activity.	Waino and Ingvorsen., 2003
β-xylosidase	*Halorhabdus utahensis*	Great Salt Lake (Utah, USA)	67	6.5	65	–	Waino and Ingvorsen., 2003

The structure of a β-glycosidase from *S. solfataricus* strain MT4 was described in 1997 (Aguilar et al., 1997). It exhibits broad substrate specificity by accepting galactose, glucose, fucose, or xylose at the non-reducing end of the substrate. It is not clear whether this β-glycosidase corresponds to the xylanase from *S. solfataricus* strain MT4 isolated and characterized a few years later (Cannio et al., 2004). However, the β-glycosidase from *S. solfataricus,* belonging to the GH-1 family, is a homotetramer with (βα)₈-barrel structure (Fig. 4). Of all the structural determinants that may contribute to thermostability, *S. solfataricus* β-glycosidase, thermostability seems to stem mainly from an increase in the number of surface ion pairs and in the number of internal water molecules. Both factors apparently provide increased resilience and resistance to denaturation.
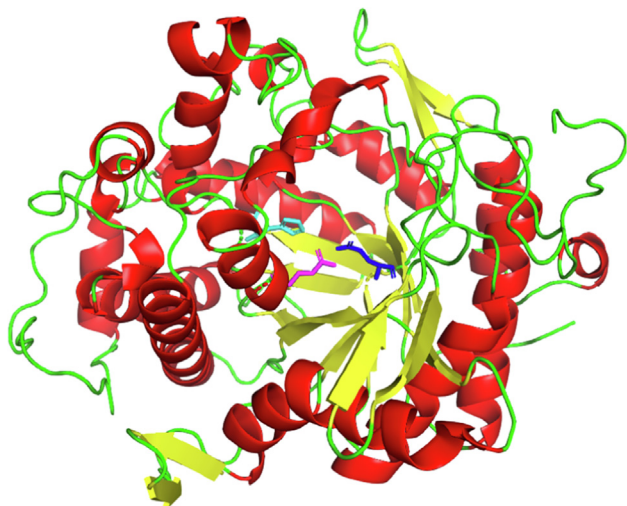

Fig. 4. Tertiary structure cartoon of the monomer of *S. solfataricus* β-glycosidase (PDB code: 1GOW). The α-helices, β-strands and loops are colored red, yellow and green, respectively. Residues implicated in catalysis are shown: the conserved nucleophile Glu387 in blue, and the Glu206 and His150 in magenta and cyan, respectively. Figure produced using PyMOL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

One striking observation of this study is that very few thermophilic *endo*-xylanases and β-xylosidades have been isolated and characterized at present from microorganisms of the Archaea domain, and notably not one in the 5 years. Even more striking is the absence of reports on their heterologous expression. In comparison, those from bacteria are much more abundant. This fact contrasts with the extremely advantageous characteristics of the archaeal xylanases for industrial use, with their very high optimal activity temperatures, thermostability and tolerance to salinity and pH. It also contrasts with the occurrence of archaea in the extremophilic environments that are currently being investigated by metagenomics bioprospection.

4. Perspectives

Archaeal-derived xylanases are promising biocatalysts for the industry, and a future research aim is the discovery of novel thermophilic xylanases (*endo*-xylanases and β-xylosidases) produced by archaea and to express and characterize them in view of their biotechnological applications. To fulfill this objective, it is necessary to improve the metagenomic methods used to bioprospect enzymes to facilitate the access to the genomes of rare species in microbial communities. There is a wide field of research in this hidden treasure for biotechnology.

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CRediT authorship contribution statement

Almudena Saavedra-Bouza: Investigation, Writing – original draft, Visualization. **Juan-José Escuder Rodríguez:** Writing – review & editing. **María-Eugenia deCastro:** Writing – review & editing. **Manuel Becerra:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **María-Isabel**

González-Siso: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aguilar, C.F., Sanderson, I., Moracci, M., Ciaramella, M., Nucci, R., Rossi, M., Pearl, L.H., 1997. Crystal structure of the β-glycosidase from the hyperthermophilic archeon *Sulfolobus solfataricus*: resilience as a key factor in thermostability. J Mol Biol 271 (5), 789–802.

Andrade, C.M.M.C., Aguiar, W.B., Antranikian, G., 2001. Physiological aspects involved in production of xylanolytic enzymes by deep-sea hyperthermophilic archaeon *Pyrodictium abyssi*. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology 91–93, 655–669. https://doi.org/10.1385/ABAB:91-93:1-9:655.

Basit, A., Liu, J., Rahim, K., Jiang, W., Lou, H., 2018. Thermophilic xylanases: from bench to bottle. Crit Rev Biotechnol 38, 989–1002. https://doi.org/10.1080/07388551.2018.1425662.

Bhardwaj, N., Kumar, B., Verma, P., 2019. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. Bioresour Bioprocess 6, 40. https://doi.org/10.1186/s40643-019-0276-2.

Cannio, R., di Prizito, N., Rossi, M., Morana, A., 2004. A xylan-degrading strain of *Sulfolobus solfataricus*: Isolation and characterization of the xylanase activity. Extremophiles 8, 117–124.

Chadha, B.S., Kaur, B., Basotra, N., Tsang, A., Pandey, A., 2019. Thermostable xylanases from thermophilic fungi and bacteria: Current perspective. Bioresour Technol 277, 195–203. https://doi.org/10.1016/j.biortech.2019.01.044.

DeCastro, M.E., Rodríguez-Belmonte, E., González-Siso, M.I., 2016. Metagenomics of thermophiles with a focus on discovery of novel thermozymes. Front Microbiol 7, 1–21. https://doi.org/10.3389/fmicb.2016.01521.

Elodie Drula, Marie-Line Garron, Suzan Dogan, Vincent Lombard, Bernard Henrissat, Nicolas Terrapon (2022) The carbohydrate-active enzyme database: functions and literature Nucleic Acids Res 50: D571–D577.Full publication. doi: 10.1093/nar/gkab1045.

Gudiña, E., Amorim, C., Braga, A., Costa, A., Rodrigues, J., Silverio, S., et al. (2020). "Biotech Green Approaches to Unravel the Potential of Residues into Valuable Products," in *Sustainable Green Chemical Processes and their Allied Applications, Nanotechnology in the Life Sciences*, ed. A. A. Inamudin (Springer Nature Switzerland AG2020), 97–150. doi: 10.1007/978-3-030-42284-4_5.

Juturu, V., Wu, J., 2012. Research review paper Microbial xylanases: Engineering, production and industrial applications. Biotechnol Adv 30, 1219–1227. https://doi.org/10.1016/j.biotechadv.2011.11.006.

Juturu, V., Wu, J.C.H., 2014. Microbial exo-xylanases: a mini review. Appl Biochem Biotechnol 174, 81–92. https://doi.org/10.1007/s12010-014-1042-8.

Knob, A., Terrasan, C.R.F., Carmona, E.C., 2010. β-Xylosidases from filamentous fungi: An overview. World J Microbiol Biotechnol 26, 389–407. https://doi.org/10.1007/s11274-009-0190-4.

Kumar, V., Dangi, A.K., Shukla, P., 2018. Engineering Thermostable Microbial Xylanases Toward its Industrial Applications. Mol Biotechnol 60, 226–235. https://doi.org/10.1007/s12033-018-0059-6.

Lagaert, S., Pollet, A., Courtin, C.M., Volckaert, G., 2014. β-Xylosidases and α-L-arabinofuranosidases: Accessory enzymes for arabinoxylan degradation. Biotechnol Adv 32, 316–332. https://doi.org/10.1016/j.biotechadv.2013.11.005.

Naitam, M.G., Kaushik, R., 2021. Archaea: An Agro-Ecological Perspective. Curr Microbiol 78, 2510–2521. https://doi.org/10.1007/s00284-021-02537-2.

Patil, J.U., Bajekal, S.S., 2014. Characterization of xylanase and cellulase from extremely haloalkaliphilic archaeon *Natrinema sp.* SSBJUP-1 isolated from Lonar Lake. Int J Pharma Bio Sci 5.

Paul, K., Nonoh, J.O., Mikulski, L., Brune, A., 2012. "Methanoplasmatales", thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. Appl Environ Microbiol 78, 8245–8253. https://doi.org/10.1128/AEM.02193-12.

Pfeifer, K., Ergal, İ., Koller, M., Basen, M., Schuster, B., Rittmann, S.K.M.R., 2021. Archaea Biotechnology. Biotechnol Adv 47,. https://doi.org/10.1016/J.BIOTECHADV.2020.107668 107668.

Rohman, A., Dijkstra, B.W., Puspaningsih, N.N.T., 2019. β-Xylosidases: Structural Diversity, Catalytic Mechanism, and Inhibition by Monosaccharides. Int J Mol Sci 20, 5524. https://doi.org/10.3390/ijms20225524.

Rolland, J.-L., Gueguen, Y., Flament, D., Pouliquen, Y., Street, P.F.S., Dietrich, J., 2002. Comment on "The first description of an archaeal hemicellulase: The xylanase from *Thermococcus zilligii* AN1": Evidence that the unique N-terminal sequence proposed comes from a maltodextrin phosphorylase. Extremophiles 6, 349–350. https://doi.org/10.1007/s00792-001-0258-z.

Sani, R.K., Krishnaraj, R.N. (Eds.), 2017. Extremophilic Enzymatic Processing of Lignocellulosic Feedstocks to Bioenergy. Springer International Publishing, Cham.

Thapa, S., Mishra, J., Arora, N., Mishra, P., Li, H., O'Hair, J., Bhatti, S., Zhou, S., 2020. Microbial cellulolytic enzymes: diversity and biotechnology with reference to lignocellulosic biomass degradation. Rev Environ Sci Biotechnol 19 (3), 621–648.

Uhl, A.M., Daniel, R.M., 1999. The first description of an archaeal hemicellulase: The xylanase from *Thermococcus zilligii* strain AN1. Extremophiles 3, 263–267. https://doi.org/10.1007/s007920050126.

Verma, D., 2021. Extremophilic Prokaryotic Endoxylanases : Diversity, Applicability, and Molecular Insights. Front Microbiol 12, 1–21. https://doi.org/10.3389/fmicb.2021.728475.

Verma, R., Kumar, A., Kumar, S., 2019. Synthesis and characterization of cross-linked enzyme aggregates (CLEAs) of thermostable xylanase from *Geobacillus thermodenitrificans* X1. Process Biochemistry 80, 72–79. https://doi.org/10.1016/j.procbio.2019.01.019.

Wainø, M., Ingvorsen, K., 2003. Production of β-xylanase and β-xylosidase by the extremely halophilic archaeon *Halorhabdus utahensis*. Extremophiles 7, 87–93. https://doi.org/10.1007/s00792-002-0299-y.