Review

Cellulases from Thermophiles Found by Metagenomics

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Abstract: Cellulases are a heterogeneous group of enzymes that synergistically catalyze the hydrolysis of cellulose, the major component of plant biomass. Such reaction has biotechnological applications in a broad spectrum of industries, where they can provide a more sustainable model of production. As a prerequisite for their implementation, these enzymes need to be able to operate in the conditions the industrial process requires. Thus, cellulases retrieved from extremophiles, and more specifically those of thermophiles, are likely to be more appropriate for industrial needs in which high temperatures are involved. Metagenomics, the study of genes and gene products from the whole community genomic DNA present in an environmental sample, is a powerful tool for bioprospecting in search of novel enzymes. In this review, we describe the cellulosolytic systems, we summarize their biotechnological applications, and we discuss the strategies adopted in the field of metagenomics for the discovery of new cellulases, focusing on those of thermophilic microorganisms.

Keywords: cellulases; thermophiles; metagenomics; biotechnology

1. Introduction

Cellulose is a complex polymer that can be hydrolyzed into glucose by the synergetic action of a mixture of enzymes known as cellulases. Plants fix atmospheric CO$_2$ and incorporate about half of the carbon in structural polysaccharides and lignin (lignocellulose). This structural carbon can be used as an energy source by cellulosolytic microorganisms [1]. The cellulosolytic enzymes can form an enzyme complex known as the cellulosome, in which they are anchored to a common scaffold. This structure is mostly observed in anaerobes and exclusively in bacteria. They can also act as non-complexed extracellular free cellulase systems, more often associated to aerobes and present in fungi, bacteria, and archaea [1–4]. Additionally, other auxiliary enzymes like lytic polysaccharide monoxygenases have been reported to also contribute to the degradation of cellulose by cellulases by enhancing their activity [5–7]. An enhancer effect has also been proposed for hemicellulases such as xylanases, mannanases, galactosidases, and β-1,3-1,4-glycanases, which has activity on polysaccharides present in plant biomass by allowing cellulases to better reach the substrate [8].

Microorganisms adapted to live in harsh conditions (from a human standpoint) are known as extremophiles. Their enzymes, and especially the extracellular ones, have adopted mechanisms to maintain their function in such environments and are known as extremozymes. They are interesting from a biotechnological perspective, as many industrial applications involve conditions similar to those of extreme environments, and a more sustainable production model would require biocatalysts able to operate in such conditions [9–11].
Thermophiles are extremophiles that thrive at high temperatures ranging from moderate thermophiles (capable of growth at temperatures between 50 °C and 64 °C), extreme thermophiles (between 65 °C and 79 °C), and hyperthermophiles (over 80 °C) [12]. Extreme habitats where these microorganisms can be found include deep-sea hydrothermal vents, hot springs, volcanic fields, mud pots and deserts, and human-made environments like compost, among others. Many enzymes of industrial importance have been retrieved from thermophiles, including cellulases [11].

2. Modular Structure of Cellulases and their Classification

Most cellulases have a modular design, in which two or more discrete units have cooperative functions and are connected through linker sequences. Usually, this modular design includes the catalytic domain linked to a carbohydrate-binding module (CBM), but other non-catalytic domains can also be present, and multiple catalytic domains or CBMs can exist on the same enzyme. The CBM helps in the catalytic process by increasing the concentration of the enzyme near the polysaccharides they bind [5,13,14] and by disrupting the crystalline cellulose structure, increasing substrate accessibility [15]. As previously stated, some cellulases can form the enzyme complex known as the cellulosome, where they are anchored to a protein scaffold (composed of non-catalytic proteins known as scaffoldins). These cellulases contain dockerin domains that bind to the cohesin module of the scaffoldins, although these domains have also been described in proteins not related to the cellulosome [16]. In cellulosomes, the scaffolding proteins might also contain CBM modules [17].

The classic classification of cellulases is based on the mechanism of action of their catalytic domains and on their substrate specificity. This classification allows us to distinguish three major types of cellulases: β-1,4-endoglucanases (EC 3.2.1.4), exoglucanases [non-reducing end cellobiohydrolases (EC 3.2.1.91), reducing-end cellobiohydrolases (EC 3.2.1.176) and cellohextrinases (EC 3.2.1.74)], and β-glucosidases (EC 3.2.1.21) [3,7,18]. Endoglucanases act randomly cleaving internal glycosidic bonds of cellulose chains, releasing oligosaccharides of different length (like cellobiose and cellotriose). Cellobiohydrolases act processively on the reducing and non-reducing ends of cellulose, primarily releasing cellobiose but also other short oligosaccharides. Cellohextrinases act on soluble cellooligosaccharides, also releasing cellobiose. Lastly, β-glucosidases perform the hydrolysis of cellohextrins and cellobiose into glucose, enhancing both endoglucanase and exoglucanase activities by reducing the end product inhibition [3,6,7,9]. A schematic representation of cellulases acting on cellulose is depicted in Figure 1.

Due to the enormous variety of polysaccharides that exist in nature, and the fact that cellulases are not always easy to categorize as only endo- or exo-acting enzymes [19], an alternative classification based on amino acid sequence similarity was proposed [20]. Rather than substrate specificity, this classification addresses the structure-function relationships, substrate recognition and enzymatic reaction mechanisms, and evolutionary relationships between the enzymes. The publicly available Carbohydrate-Active Enzymes Database (CAZy, http://cazy.org) contains the classification of glycoside hydrolase (GH) families in which the cellulases are included. The database at the time of writing lists 149 different GH families [21]. Endoglucanases are mainly present in 12 GH families: GH5-9, GH12, GH44, GH45, GH48, GH51, GH74, and GH124; cellobiohydrolases acting on non-reducing ends can be found in families GH5, GH6, and GH9, whereas the reducing-end acting ones are mostly present in GH7, GH9, and GH48; cellohextrinases are distributed in families GH1, GH3, GH5, and GH9; and, lastly, β-glucosidases belong in families GH1-3, GH5, GH9, GH30, GH39, and GH116 [20].
Figure 1. Overview of the two strategies (free or cell-bound cellulase systems) for degrading cellulose. In free extracellular systems, endoglucanases and exoglucanases act synergistically, with the endoglucanase cutting amorphous cellulose providing chain ends for exoglucanases to release cellobiose. Then, $\beta$-glucosidases complete the process of cellulose hydrolysis by releasing glucose. Also, cellodextrins released by endoglucanases can be further hydrolysed by cellodextrinases. The carbohydrate binding domain directs the enzymes to their specific substrates. In the cellulosome system, all cellulases are anchored to a common scaffold but are generally thought to follow the same synergic mode of action. The scaffolding is bound to the cell membrane through the surface layer homology domain, while a network of dockerin and cohesin domains amplifies the number of cellulases bound to the same scaffolding unit. Lastly, a carbohydrate binding domain is responsible for the targeting of the whole complex to the substrate.
Even if they share structural characteristics, members of the same GH family may differ widely in substrate specificity and their evolutionary history, and, due to their multidomain nature, some enzymes may contain sequences from different GH families [3,6,10]. As a further classification for GHs, some families are also grouped in clans in regard to their folding, as it is more conserved than their amino acid sequence [14]. Clans are designated by a letter, and some cellulases fall inside these groups: GH-A (with a ($\beta / \alpha$)$_8$ barrel) includes cellulases from families GH1, GH2, GH5, GH30, GH39, and GH51; GH-B (that fold in $\beta$-jelly roll) contains family GH7; GH-C (also folding with a $\beta$-jelly roll) includes family GH12; GH-M (folding with a ($\alpha / \alpha$)$_6$ barrel) comprises families GH8 and GH48; and GH-O [($\alpha / \alpha$)$_6$ barrel folding] contains family GH116.

In regard to the catalytic mechanism, GHs (including cellulases) may perform the hydrolysis of the glycosidic bond by an inverting or retaining mechanism, whether the configuration of the substrate’s anomeric carbon (C1) is changed or not after the cleavage. Retaining enzymes have a double nucleophilic displacement mechanism involving two carboxylate catalytic residues. Inverting enzymes act with a single nucleophilic displacement mechanism, also involving two carboxylate catalytic residues [1]. For cellulases, seven GH families have an inverting mechanism of catalysis (6, 8, 9, 45, 48, 74, and 124), whereas eleven act with a retaining mechanism (1-3, 5, 7, 12, 30, 39, 44, 51, and 116) [20,22].

3. Factors Influencing Thermostability of Thermophile Cellulases

As pointed out, a greater half-life of cellulases at high temperatures is a desirable trait for many industrial applications. In order to obtain more thermostable variants of cellulases, the molecular mechanisms behind thermostability have been studied. Some researchers argue that the study of smaller, single-domain enzymes would make it easier to pinpoint the mechanisms involved in a higher resistance to high temperature [23], while others have studied the effect of the number of domains and linker sequences and domain-removal on thermostability, though opposing stabilizing and destabilizing effects have been described in this regard [5].

Several stabilization factors have been proposed for the increased thermostability of thermozymes, such an increased number of ion pairs, a lower number of loops and cavities (thus making the protein more compact), a reduced ratio of protein surface area to protein volume, a higher number of proline residues in loops (limiting the conformational freedom of the protein), an increased amount of hydrophobic interactions, and a greater degree of oligomerization [24,25]. Despite that, a direct correlation between all these factors and protein thermostability cannot always be established; for example, for *Humicola insolens* exoglucanase Cel6A the addition of proline residues in the loop regions did not achieve greater stability and in some instances had the opposite effect [26]. It has been also proposed that proteins can undergo structure-based or sequence-based stabilization strategies through evolution. As thermophilic archaea emerged in already extreme environments, their enzymes would initially favour stable folding at high temperatures, whereas thermophilic bacteria would have to enhance the thermostability of their proteins by point mutations that increase the number of ion-pairs in order to colonize the new habitats. Despite this theory, it has been found that among archaea, the two different stabilization models can be adopted [24].

There are also reports on how hydrophobic and aromatic residues can play a major role in protein thermal stability, like in the endoglucanase from family GH12 from *Aspergillus niger* [27]. Other authors have described an increased percentage of the charged amino acid glutamic acid in thermophilic enzymes from family GH12 compared to mesophilic ones, which is thought to stabilize the protein’s structure through salt bridges and hydrogen bonds [23]. Moreover, some key residues for protein stability have been already identified in this protein family [1]. When comparing mesophilic and thermophilic exoglucanases from family GH7, the potential disulphide bridge formation by the presence of cysteine residues could not be linked to an increased thermostability, whereas a higher number of charged residues and lower number of polar residues was observed in the more
thermostable enzymes [28]. However, it was found that rational mutagenesis introducing disulphide bridges in an exoglucanase from this family did allow the mutant proteins to be more thermostable [29]. Lastly, eukaryotes’ post-translational modifications (including glycosylation, phosphorylation, acetylation, and methylation) have been reported to account for protein thermostability [27], and heterologous expression of the enzyme in a yeast host can be a desirable production system for industrial applications.

The yeast *Pichia pastoris*, in particular, has been extensively employed due to this property, along with its relative ease for genetic manipulation and high level of protein expression [19,30–32], coupled with inexpensive production media and relatively simple protein processing protocols [33]. Nevertheless, most studies regarding the discovery and the characterization of new thermophilic cellulases have involved the model organism *Escherichia coli* [34–38], sometimes at the expense of thermostability [39].

4. Biotechnological Applications by Thermophile Cellulases

Thermozymes have general advantages over their mesophilic counterparts in regard to their application in various industries, as they are generally more stable towards extreme temperatures and pH, as well as in the presence of chemically destabilizing agents, and function at high temperatures with higher reaction rates [35] and higher mass-transfer rates that increase the substrates’ solubility, as well as a lower risk of contamination [27]. Lastly, the process design gains flexibility (e.g., current process configurations with operations that needed pre-treatment of the substrates to lower the temperature can now be performed simultaneously without the requirement of a temperature modification between them), which in turn can reduce the cost of operation [27]. On the other hand, and as previously stated, preferred systems to produce these enzymes are not thermophilic, as thermophile production faces many technical challenges due to limited knowledge of their physiology and genetics, difficulty of growing and not being Generally Recognized As Safe [27] as defined by the US Food and Drug Administration under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act. In regard to the production process, extracellular enzymes are desirable, as they are easier to purify [27,33].

The range of industries in which degradation of cellulose by cellulases is required is considerably wide and includes biofuels (conversion of plant biomass in bioethanol), food and brewing, textiles (biostoning and biopolishing), laundry (in detergent formulations), pulp and paper (biopulping), and animal feeds [35]. Other uses include waste management, improvement of soils for agriculture [40], and extraction of compounds from plants such as olive oil, pigments, and bioactive molecules [4].

The full conversion of cellulose into glucose, which can later be converted into ethanol (named bioethanol to stress it being a biofuel, in contrast with the classic fossil fuels) has been previously stated to require the combined action of multiple cellulolytic enzymes (endo- and exoglucanases and β-glucosidases). This process has gained a lot of interest, as plant biomass poses a promising renewable substrate alternative to assess the increasing energy demands while limiting the use of fossil fuels [2,41]. In this regard, the use of non-food lignocellulosic waste from agriculture and forestry has replaced food crops to produce the substrate of choice, as the use of the latter would have the associated risk of raising basic foods prices and limiting their supply [42]. In general, biorefining (using biomass as a substrate to produce fuels, energy, or chemicals) benefits from thermostable enzymes, as heat treatment, is an important step for the pre-processing of the lignocellulosic material [43–45]. The use of thermostable cellulases for the treatment and pretreatment of the biomass reduces the energy cost of the process, improves the solubility of the substrate, reduces its viscosity, and reduces dependency on the use of environmentally harsh chemicals [39,45].

4.1. Endoglucanase-Specific Industrial Applications

Endoglucanases have been used in the textile industry for the process called biostoning. Biostoning achieves a wash-down look on denim cotton clothes, and represents an alternative to the chemical method using pumice stone. Biostoning has a number of advantages over the classical
method, such as greater yields, less labor-intensive operations, more secure workplace, shorter time
requirements, lower damage to the machinery, and a more environmentally friendly process [4].

Another textile industrial process in which endoglucanases are employed is the biopolishing of
cotton products. This process removes the microfibrils from cottons’ surfaces, enhancing the colour
brightness and making them more resistant to pilling [40], as well as softening the product [46] and
giving it a cleaner and smoother look [4]. Biopolishing is often performed after another enzymatic
process called desizing (in which amylases remove starch from the fabrics). Desizing uses temperatures
higher than 70 °C, so endoglucanases operating at such temperatures would be interesting for
combining both processes and thus reducing the required time and energy costs [46]. Other textile
processes in which endoglucanases are employed to remove cellulosic impurities, replacing chemical
treatments, include bio-carbonization of polyester-cotton blends, wool scouring, and de-fibrillation of
Lyocell [4].

In the brewing industry, the production of malt generates high molecular weight β-glucans.
The presence of these molecules increases viscosity, lowering the efficiency and yield of the process due
to the increased difficulty for pumping and also making filtration difficult [33]. As such, the addition
of endoglucanases would alleviate those problems, allowing for the hydrolysis of β-glucans [33].
Also, endoglucanases may be used to increase the extraction of fermentable compounds both in
brewing and fermentation industries [47].

In the laundry industry, the use of endoglucanases in detergent formulations is known to improve
the colour brightness and soften cotton fabrics [4], similarly to the biopolishing in the textile industry.

In the animal feed industry, they enhance β-glucan digestibility and nutrient bioavailability [47],
and have been shown to increase weight gain and milk production of ruminants [4].

Endoglucanases have been extensively used in the pulp and paper industry for the treatment of
pulp wastes [4,47], deinking and removal of pollutants from paper without altering its brightness and
strength [4], and in the pulp process (bio-pulping), reducing the energy cost of the process and
improving the beatability of the pulp [4].

4.2. Exoglucanase-Specific Industrial Applications

As in nature, efficient degradation of cellulose from biomass in industrial applications requires
the synergic action of a mixture of cellulases [26,48]. Synergism has been described between
endoglucanases and exoglucanases, between reducing-end-acting and non-reducing-end-acting
exoglucanases, between processive endoglucanases and endo- or exoglucanases, and between
β-glucosidases and the other cellulases [48]. As such, the previously described industrial
applications benefit from the addition of exoglucanases to enzyme mixtures already containing other
cellulase classes.

4.3. β-glucosidase-Specific Industrial Applications

In addition to their application in the last step of cellulose hydrolysis to release glucose,
β-glucosidases have several additional biotechnological applications.

In the food industry, they can be used to release aromatic compounds from fruit and fermentation
products [49], like the release of terpenoids and phenylpropanoids in wine to enhance its aroma [50,51].
Other uses include juice clarification [32] and hydrolysis of bitter compounds in its extraction [52],
and, in general, improvement of quality of beverages and foods [44] including colour, aroma, flavour,
texture, and nutritional value [4].

In the pharmaceutical industry, they are used to deglycosylate ginsenosides, active compounds
with many pharmaceutical uses, as the natural glycosylated ginsenosides from ginseng root
are less active and less absorbable [50,52,53]. Similarly, they are used to convert the bioactive
isoflavonoid-glucosides from soybean and other leguminous plants into aglycones with higher
bioavailability and pharmaceutical activity [44,50,54]. Moreover, β-glucosidases can perform reverse
hydrolysis or transglycosylation catalytic pathways for the formation of new glycosidic bonds,
a property that makes them interesting for the production of functional compounds, and nutraceutical and pharmaceutical products [44]. For example, gentibiose, a product of transglycosylation by β-glucanases, can be used as a prebiotic food additive [50]. These kinds of enzymatic transformations constitute important alternatives to chemical synthesis involving the use of organic solvents [55]. In this regard, the valorization of spent coffee grounds to produce isoflavone glycosides has also been proposed [54].

5. Metagenomics for the Search of Novel Cellulases

The metabolism of thermophiles holds great potential for several industrial applications, but due to the difficulty of growing extremophiles in the laboratory, culture-independent techniques constitute instrumental methods to have access to it. The use of metagenomics, the study of whole communities’ genomes, has proven to be a useful tool for the discovery of novel cellulases, both in the functional and the sequence-based approaches [10,11]. Several studies had found cellulases in a wide variety of natural thermophilic environments, such as hydrothermal vents [56,57], continental geothermal pools and hotsprings [58,59], and man-made environments like vermicompost [60], compost [37,61,62], and biogas digesters [63]. Nevertheless, high-temperature acting enzymes have also been found by metagenomics on moderate-temperature samples like soils [40,64,65] and aquatic environments [66], and in microorganisms associated with animals like microbial communities in rabbit cecum [67], ruminants rumen [36,68,69], earthworm casts [70], and termite guts [71,72].

The main limiting factor for the discovery of new thermophile cellulases by functional metagenomics is the host organism used for the metagenomic libraries, typically the mesophilic bacterium E. coli, which may have a limited or biased expression of gene products from thermophiles [3]. One of the proposed solutions for this problem is the use of an alternative thermophilic host for the metagenomic libraries that would increase the hit detection rate for cellulases [11]. It should also be noted that bacteria hosts are not able to express fungal enzymes, as the promoter and intron regions are not recognized [3]. Lastly, the discovery of novel celllobiohydrolases through metagenomics is limited due to the lack of specific substrates other than AVICEL that can discriminate between true celllobiohydrolases and other cellulases, as AVICEL has the requirement of a synergy between an endoglucanase and an exoglucanase for detection of activity [3]. The other metagenomic approach, an analysis of the whole metagenome sequencing data, can overcome the problems that arise in the expression-based approach. Regardless, the discovery of gene products with novel characteristics is hindered due to the need of high amino acid homology with already known enzymes, and before assigning putative proteins a function, activities should be verified [11].

6. Thermophile Cellulases Characterized

Tables 1–5 list, respectively, endoglucanases, exoglucanases acting on non-reducing ends, exoglucanases acting on reducing ends, cellodextrinases and β-glucanases that can be considered thermophilic (optimum temperature at 50 °C or higher), and other key parameters for their industrial application, namely, pH optimum and temperature stability, their classification according to the CAZY database, and their source organism.

Table 1. Characterized endoglucanases (EC 3.2.1.4) from thermophiles. NM: not measured.

| Enzyme | GH Family | Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|------------|---------|---------------------|------------|----------------------|--------|-----------|
| EGPh   | 5          | >97 °C  | 5.4–6.0             | 80%; 97 °C; 3 h | Archaea (Pyrococcus horikoshii) | [46]   |
| EG1    | 5          | 83 °C   | 5.0                 | 20%; 90 °C; 2 h | Bacteria (Acidothermus cellulolyticus) | [73]   |
Table 1. Cont.

| Enzyme | GH Family | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|-----------|---------------------|------------|----------------------|--------|-----------|
| EgIII  | 5         | 50 °C               | 6.0        | NM                   | Bacteria (Bacillus amyloliquefaciens) | [74]    |
| EG     | 5         | 65 °C               | 6.0        | 72%; 55 °C; 42 h     | Bacteria (Bacillus licheniformis) | [75]    |
| CelA   | 5         | 60 °C               | 8.0        | 50%; 70 °C; 1 h      | Bacteria (Bacillus subtilis) | [76]    |
| TmCel5A| 5         | 80 °C               | 6.0        | 50%; 80 °C; 18 h     | (Thermotoga maritima) | [77]    |
| EglA   | 5         | 57 °C               | 4.0        | NM                   | Fungi (Aspergillus nidulans) | [78]    |
| EglB   | 5         | 52 °C               | 4.0        | NM                   | Fungi (Aspergillus nidulans) | [78]    |
| EBI-244| 5         | 109 °C              | 5.5        | 50%; 105 °C; 4.5 h   | Uncultured Archaea (Continental geothermal pool enrichment) | [58] |
| CelA10 | 5         | 55 °C               | 7.5        | NM                   | Uncultured organism (Aquatic community and soil sample) | [66] |
| CelA24 | 5         | 55 °C               | 7.0        | NM                   | Uncultured organism (Aquatic community and soil sample) | [66] |
| cMGL504| 5         | 50 °C               | 5.5        | NM                   | Uncultured organism (Vermicompost sample) | [60] |
| Cel5G  | 5         | 50 °C               | 4.8        | >90%; 50 °C; 30 min  | Uncultured organism (Soil metagenome) | [65] |
| En1    | 5         | 55 °C               | 5.5        | 82%; 45 °C; 16 h     | Uncultured organism (Bogas digestor metagenome) | [63] |
| RC1    | 5         | 55 °C               | 6.0–6.5    | >90%; 50 °C; 30 min  | Uncultured organism (Rabbit cecum metagenome) | [67] |
| RC3    | 5         | 50 °C               | 6.0–7.0    | NM                   | Uncultured organism (Rabbit cecum metagenome) | [67] |
| RC5    | 5         | 50 °C               | 6.5–7.0    | NM                   | Uncultured organism (Rabbit cecum metagenome) | [67] |
| CelL   | 6         | 50 °C               | 5.0        | 50%; 50 °C; 12 min   | Bacteria (Cellulosimicrobium fumiferus) | [22] |
| Cel6A  | 6         | 58 °C               | 6.5        | >80%; 56 °C; 18 h    | Bacteria (Thermobifida fusca) | [79] |
| ThCel6A| 6         | 55 °C               | 8.5        | 58%; 90 °C; 1 h      | Bacteria (Thermobifida halotolerans) | [80] |
| Cel6A  | 6         | 50–55 °C            | 5.5        | NM                   | Bacteria (Xylanimonobacter pachnodae) | [81] |
| HiCel6C| 6         | 70 °C               | 6.5        | >90%; 60 °C; 1 h     | Fungi (Humicola insolens) | [82] |
| Cel6A  | 6         | 50 °C               | 4.8        | >90%; 45 °C; 24 h    | Fungi (Orpinomyces sp.) | [83] |
| C1     | 6         | 50 °C               | 6.0        | 100%; 60 °C; 30 min  | Uncultured organism (Compost metagenome) | [61] |
| pre-LC-CelB | 6 | NM                   | NM         | NM                   | Uncultured organism (Compost metagenome) | [62] |
| pre-LC-CelI | 6 | NM                   | NM         | NM                   | Uncultured organism (Compost metagenome) | [62] |
| EGI    | 7         | 55–60 °C            | 5.0        | >80%; 60 °C; 10 min  | Fungi (Humicola grisea var. thermoides) | [84] |
| Cel7B  | 7         | 60 °C               | 4.0        | >90%; 60 °C; 1 h     | Fungi (Penicillium decumbens) | [85] |
| Cel7A  | 7         | 60 °C               | 5.0        | 100%; 60 °C; 1 h     | Fungi (Neosartorya fischeri) | [33] |
| MtEG7  | 7         | 60 °C               | 5.0        | 50%; 70 °C; 9.96 h   | (Mycelisphthora thermophila) | [31] |
### Table 1. Cont.

| Enzyme | GH Family Domains | Optimum Temperature | Optimum pH | Temperature Stability $^1$ | Source | Reference |
|--------|-------------------|---------------------|------------|----------------------------|--------|-----------|
| EGL1  | 7                 | 62 °C               | 4.8        | NM                         | Fungi (Trichoderma longibrachiatum) | [51]     |
| MaCel7A | 7               | 65–70 °C             | 6.0        | NM                         | Fungi (Melanocarpus albomyces) | [66]     |
| CelC   | 8                 | 50 °C               | 6.5        | NM                         | Bacteria (Salmonella typhimurium) | [87]     |
| CelB   | 8                 | 80 °C               | 7.0        | 50%; 90 °C; 4 h             | Bacteria (Aquifex geolicus) | [88]     |
| Egl-257 | 8            | 55 °C               | 6.5        | 100%; 50 °C; 15 min; 60 °C; 2 h | Bacteria (Bacillus circulans) | [89]     |
| CenC   | 9                 | 70 °C               | 6.0        | 60%; 70 °C; 1 h             | Bacteria (Clostridium thermocellum) | [90]     |
| CelA   | 9                 | 95 °C (endoglucanase) and 85 °C (cellulbiohydrolase) | 5.0–6.0   | >40%; 90 °C; 130 min; 73%; 100 °C; 26 min | 50%; 110 °C; 26 min | 50%; 80 °C; 1 h | Bacteria (Caldicellulosiruptor bescii) | [91] |
| Cel9A  | 9                 | 65 °C               | 6.5        | NM                         | Bacteria (Lachnolocystidium phytofermentans) | [92]     |
| CelA20 | 9                 | 55 °C               | 5.0        | NM                         | Uncultured organism (Aquatic community and soil metagenome) | [66]     |
| AcCel12B | 12          | 75 °C               | 4.5        | 50%; 60 °C; 90 h             | Bacteria (Acidothermus cellulolyticus) | [35]     |
| CelA   | 12                | 95 °C               | 6.0        | NM                         | Bacteria (Thermotoga neapolitana) | [8]      |
| CelB   | 12                | 106 °C              | 6.0–6.6   | 50%; 110 °C; 26 min; 73%; 100 °C; 4 h | 50%; 80 °C; 1 h | 73%; 100 °C; 4 h | Bacteria (Thermotoga neapolitana) | [8] |
| TMCellH2A | 12          | 90 °C               | 7.0        | 50%; 50 °C; 90 °C; 3 h         | Bacteria (Thermotoga maritima) | [93]     |
| TMCell2B | 12          | 85 °C               | 6.0        | 50%; 50 °C; 90 °C; 9 h         | Bacteria (Thermotoga maritima) | [93]     |
| CelA   | 12                | >100 °C              | 6.0–7.0   | 45%; 90 °C; 8 h               | Bacteria (Rhodothermus marinus) | [23]     |
| EglA   | 12                | 100 °C              | 6.0        | 50%; 95 °C; 40 h              | Archaea (Pyrococcus furiosus) | [94]     |
| SSO1949 | 12            | 80 °C               | 1.8        | 50%; 80 °C; 8 h               | Archaea (Sulfotobas sulfitarius) | [95]     |
| SSO1354 | 12            | 90 °C               | 4.0        | 50%; 90 °C; 180 min           | Archaea (Sulfobas sulfitarius) | [39]     |
| EglS   | 12                | 65 °C               | 6.0        | >40%; 60 °C; 30 min           | Bacteria (Streptomyces rochei) | [96]     |
| Cel12A | 12                | 50 °C               | 5.0        | NM                         | Fungi (Trichoderma reesi) | [97]     |
| EG     | 12                | 70 °C               | 3.5        | 50%; 70 °C; 3 h               | Fungi (Aspergillus niger) | [27]     |
| Pre-LC-CelA | 12         | 90 °C               | 5.0–9.0   | >100%; 90 °C; 30 min          | Uncultured organism (Compost metagenome) | [62]     |
| Pre-LC-CelD | 12        | NM                  | NM         | NM                         | Uncultured organism (Compost metagenome) | [62]     |
| Pre-LC-CelE | 12        | NM                  | NM         | NM                         | Uncultured organism (Compost metagenome) | [62]     |
| Cell2E | 12                | 92 °C               | 5.5        | >80%; 80 °C; 4.5 h            | Uncharacterized archeon (deep sea vents metagenome enrichment) | [57]     |
| GH44EG | 12                | 55 °C               | 5.0        | NM                         | Bacteria (Clostridium acetobutylicum) | [98]     |
| CelA   | 12                | 60 °C               | 5.0–8.5   | 50%; 60 °C; 70 min           | Bacteria (Paenibacillus tartus) | [99]     |
| CelJ   | 12                | 70 °C               | 6.3        | >90%; 80 °C; 10 min          | Bacteria (Ruminoclostridium thermocellum) | [100]    |
### Table 1. Cont.

| Enzyme   | GH Family Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source                    | Reference |
|----------|-------------------|---------------------|------------|-----------------------|---------------------------|-----------|
| pre-LC-CelH | 44                | NM                  | NM         | NM                    | Uncultured organism (Compost metagenome) | [62]      |
| Cel45A   | 45                | 60 °C               | 5.0        | NM                    | Fungi (Trichoderma reesei) | [97]      |
| PpCel45A | 45                | 65 °C               | 4.8        | 70%; 65 °C; 48 h      | Fungi (Pichia pastoris)   | [5]       |
| STCE1    | 45                | 60 °C               | 6.0        | NM                    | (Staphylotherix coccosporum) | [101]     |
| BCC18080 | 45                | 70 °C               | 6.0        | >70%; 70 °C; 2 h      | Fungi (Syncephalastrium racemosum) | [102]     |
| BCE1     | 45                | 55 °C               | 4.5        | NM                    | Fungi (Beltraniella portoricensis) | [103]     |
| MaCel45A | 45                | 70 °C               | 6.0        | >70%; 70 °C; 2 h      | Fungi (Melanocarpus albomyces) | [86]      |
| CelB     | 51                | 80 °C               | 4.0        | 60%; 80 °C; 1 h       | (Alicyclobacillus acidocaldarius) | [104]     |
| CelA4    | 51                | 65 °C               | 2.6        | >85%; 60 °C; 1 h      | (Alicyclobacillus sp. A4) | [47]      |
| CelIVA   | 51                | 80 °C               | 3.6–4.5    | 70%; 70 °C; 2 h       | (Alicyclobacillus eucalanis) | [45]      |
| pre-LC-CelC | NM               | NM                  | NM         | NM                    | Uncultured organism (Compost metagenome) | [62]      |
| TmCel74  | 74                | 90 °C               | 6.0        | 50%; 90 °C; 5 h       | (Thermotogon maritima) Bacteria | [15]      |
| CrCel124 | 124               | NM                  | NM         | NM                    | (Ruminoclostridium thermocellum) | [105]     |

1 Temperature stability is given as a percentage of activity (residual activity) after treatment at the specified temperature and time compared to the untreated enzyme.

### Table 2. Characterized exoglucanases (1,4-β-cellobiosidase) acting on non-reducing ends from thermophiles (EC 3.2.1.91). NM: not measured.

| Enzyme       | GH Family Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source                                          | Reference |
|--------------|-------------------|---------------------|------------|-----------------------|-------------------------------------------------|-----------|
| CBHII        | 6                 | 60 °C               | 4.0        | 30%; 100 °C; 10 min   | Bacteria (Streptomyces sp. M23) Fungi            | [106]     |
| CelB         | 6                 | NM                  | 7.0–8.0    | 100%; 55 °C; 16 h     | (Thermobifida fusca) Fungi (Aspergillus nidulans) | [107]     |
| CBHII        | 6                 | 57 °C               | 5.5        | NM                    | Fungi                                            | [78]      |
| Cel6A        | 6                 | 50 °C               | 4.0        | 50%; 70 °C; 30 min    | (Clatamon thermophilum) Fungi                    | [108]     |
| CBHII (Cel6A)| 6                 | 60 °C               | 5.0–5.5    | >90%; 50 °C; 5 h      | (Chrysonobium lucknowense) Fungi                 | [109]     |
| HiCel6A      | 6                 | 60–65 °C            | NM         | 50%; 75 °C; <25 min   | (Humicola insolens) Fungi (Ileps Lactus) Fungi   | [26]      |
| Ex-4         | 6                 | 50 °C               | 5.0        | 80%; 60 °C; 60 min    | Fungi                                            | [110]     |
| PoCel6A      | 6                 | 50 °C               | 5.0        | 90%; 50 °C; 2 h       | (Penicillium oxalicum) Fungi                     | [111]     |
| PaCel6A      | 6                 | 55 °C               | 5.0–9.0    | 100%; 35 °C; 24 h     | (Paenosa anserina) Fungi                         | [112]     |
| CBHIII       | 6                 | 70 °C               | 5.0        | NM                    | Uncultured organism (Earthworm casts metagenome) | [113]     |
| G10-6        | 6                 | 55 °C               | 9.5        | NM                    | (Ruminoclostridium thermocellum) Bacteria        | [114]     |
| Chb9A        | 9                 | 60 °C               | 6.5        | NM                    | (Ruminoclostridium thermocellum) Bacteria        | [115]     |
| Csb9K        | 9                 | 65 °C               | 6.0        | 97%; 60 °C; 200 h     | (Ruminoclostridium thermocellum) Bacteria        | [116]     |

1 Temperature stability is given as a percentage of activity (residual activity) after treatment at the specified temperature and time compared to the untreated enzyme.
Table 3. Characterized exoglucanases (1,4-β-cellobiosidase) acting on reducing ends from thermophiles (EC 3.2.1.176). NM: not measured.

| Enzyme | GH Family Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|-------------------|---------------------|------------|----------------------|--------|-----------|
| CelO   | 5                 | 65 °C               | 6.6        | NM                   | Bacteria (Ruminiclostridium thermocellum) | [115]    |
| AtCel7A| 7                 | 60 °C               | 5.0        | NM                   | Fungi (Acremonium thermophilum) | [28]     |
| CBH1   | 7                 | 60 °C               | 3.0        | NM                   | Fungi (Aspergillus awamori) | [116]    |
| CBH    | 7                 | 55 °C               | NM         | NM                   | Fungi (Aspergillus fumigatus) | [117]    |
| CeCel7A| 7                 | 65 °C               | 4.0        | NM                   | Fungi (Chaetomium thermophilum) | [28]     |
| CBH3   | 7                 | 65 °C               | 5.0        | 50%; 70 °C; 1 h      | Fungi (Humicola grisea var. thermoidea) | [118]    |
| DpuCel7A| 7                | 55 °C               | 5.0        | NM                   | Metazoa (Dictyostelium purpureum) | [119]    |
| CBH1   | 7                 | 60 °C               | 5.0        | >90%; 65 °C; 10 min  | Fungi (Humicola grisea var. thermoidea) | [118]    |
| EXO1   | 7                 | 65 °C               | 5.0        | >80%; 65 °C; 10 min  | Fungi (Humicola grisea var. thermoidea) | [118]    |
| MaCel7B| 7                 | 55 °C               | NM         | NM                   | Fungi (Methanococcus albus) | [120]    |
| TeCel7A| 7                 | 65 °C               | 4.0–5.0    | 50%; 70 °C; 30 min   | Fungi (Talaromyces emersonii) | [29]     |
| Cel7A  | 7                 | 55 °C               | 5.0–6.0    | >70%; 55 °C; 2.5 h   | Fungi (Penicillium janiculus) | [122]    |
| TaCel7A| 7                 | 65 °C               | 5.0        | NM                   | Fungi (Thermoascus aurantiacus) | [28]     |
| ThCBH1 | 7                 | 50 °C               | 5.0        | NM                   | Fungi (Trichoderma harzianum) | [123]    |
| CBH1   | 7                 | 60 °C               | 5.8        | NM                   | Fungi (Trichoderma viride) | [112]    |
| CelA   | 9 (endoglucanase) and 48 (cellobiohydrolase) | 95 °C (endoglucanase) and 85 °C (cellobiohydrolase) | 5.0–6.0 40 min (endoglucanase) 4 h (cellobiohydrolase) | 50%; 95 °C; 40 min (5%); 85 °C; 4 h 100% (4%) | Bacteria (Caldicellulosiruptor bescii) | [91]     |
| CelY   | 48                | 70 °C               | 5.0–6.0    | NM                   | Bacteria (Clostridium stercorarium) | [124]    |
| CpCel48| 48                | 55 °C               | 5.0–6.0    | >70%; 55 °C; 30 min  | Bacteria (Lachnoclostridium phytafermentans) | [125]    |
| CelS   | 48                | 70 °C               | 5.5        | NM                   | Bacteria (Ruminiclostridium thermocellum) | [126]    |

1 Temperature stability is given as a percentage of activity (residual activity) after treatment at the specified temperature and time compared to the untreated enzyme.

Table 4. Characterized exoglucanases (cellodextrinases) acting on reducing ends from thermophiles (3.2.1.74).

| Enzyme | GH family Domains | Optimum Temperature | Optimum pH | Temperature Stability 1 | Source | Reference |
|--------|-------------------|---------------------|------------|-------------------------|--------|-----------|
| CcGH1  | 1                 | 60 °C               | 6.5        | 61%; 50 °C; 30 min      | Bacteria (Clostridium Cellulolyticum) | [127]    |
| GgbA   | 1                 | 95 °C               | 6.5        | 85%; 90 °C; 9 h         | Bacteria (Thermotoga neapolitana) | [128]    |

1 Temperature stability is given as a percentage of activity (residual activity) after treatment at the specified temperature and time compared to the untreated enzyme.
Table 5. Characterized β-glucanases from thermophiles (3.2.1.21). NM: not measured.

| Enzyme | GH family | Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|-----------|---------|---------------------|------------|----------------------|--------|-----------|
| CelB   | 1         |         | 101–105 °C          | 5.0        | 50%; 100 °C; 85 h    | Arquea (Pyrococcus furiosus) | [129]   |
| Tpa-glu| 1         | 90 °C   | 7.5                 | 50%; 90 °C | 6 h                  | Arquea (Thermococcus pacificus) | [130] |
| BGPh   | 1         | >100 °C | 6.0                 | 50%; 90 °C | 15 h                 | Arquea (Pyrococcus horikoshii) | [131] |
| LacS   | 1         | 90 °C   | 6.0                 | 90%; 75 °C | 80 h                 | Arquea (Sulfolobus solfataricus) | [132] |
| O08324 | 1         | 78 °C   | 5.0–6.8             | 50%; 78 °C | 860 min              | Arquea (Thermococcus sp.) | [133] |
| BglII  | 1         | 90 °C   | 6.5                 | 67%; 90 °C | 1.5 h                 | Bacteria (Anoxybacillus flavithermus) | [135] |
| GlyB   | 1         | 85 °C   | 5.5                 | 8%; 80 °C | 10 min               | Bacteria (Caldicellulosiruptor saccharolyticus) | [138] |
| Bglp   | 1         | 60 °C   | 7.0                 | 50%; 60 °C | 10 h                 | Bacteria (Clostridium cellulovorans) | [139] |
| BglA   | 1         | 55 °C   | 6.0–9.0             | 50%; 50 °C | 15 min               | Bacteria (Bacillus circulans subsp. Alkalophilus) | [136] |
| BglA   | 1         | 50 °C   | 7.0                 | 50%; 50 °C | 30 min               | Bacteria (Clostridium cellulovorans) | [138] |
| BglA   | 1         | 85 °C   | 6.25                | 50%, 70 °C | 2280 min             | Bacteria (Clostridium cellulovorans) | [138] |
| BglA   | 1         | 50 °C   | 6.0                 | NM        |                      | Bacteria (Clostridium cellulovorans) | [138] |
| DIGH   | 1         | 90 °C   | 7.0                 | 50%; 70 °C | 533 h                | Bacteria (Dictyoglomus thermophilum) | [140] |
| DturβGlu| 1         | 80 °C   | 5.4                 | 70%; 70 °C | 2 h                  | Bacteria (Dictyoglomus thermophilum) | [140] |
| FiBgl1A| 1         | 90 °C   | 6.0–7.0             | 50%; 90 °C | 25 min               | Bacteria (Sphingomonas paucimobilis) | [141] |
| BglA   | 1         | 60 °C   | 6.5                 | 91%; 60 °C | 3 h                  | Bacteria (Sphingomonas paucimobilis) | [141] |
| SdBgl1B| 1         | 50 °C   | 6.0–7.5             | NM        |                      | Bacteria (Sphingomonas paucimobilis) | [141] |
| BglA   | 1         | 50 °C   | 6.5                 | 50%; 60 °C | 15 min               | Bacteria (Sphingomonas paucimobilis) | [141] |
| BglA   | 1         | 50 °C   | 6.5                 | NM        |                      | Bacteria (Sphingomonas paucimobilis) | [141] |
| CgIT   | 1         | 75 °C   | 5.5                 | 100%; 60 °C | 24 h                 | Bacteria (Thermoaerobacter brockii) | [146] |
| TeBglA | 1         | 80 °C   | 7.0                 | 10%; 65 °C | 5 h                  | Bacteria (Thermoaerobacter ethanolicus) | [147] |
| TmBglA | 1         | 90 °C   | 6.2                 | >80%; 65 °C | 5 h                  | Bacteria (Thermotoga maritima) | [147] |
| Bgl    | 1         | 70 °C   | 6.4                 | 50%; 68 °C | 1 h                  | Bacteria (Thermoaerobacter thermosaccharolyticum) | [148] |
| BglC   | 1         | 50 °C   | 7.0                 | NM        |                      | Bacteria (Thermobifida fusca) | [149] |
| BglB   | 1         | 60 °C   | 6.2                 | 70%; 60 °C | 48 h                 | Bacteria (Thermobifida fusca) | [149] |
| BglA   | 1         | 80–90 °C| 7.0–8.0             | 100%; 70 °C | 6 h                  | Bacteria (Thermotoga petrophila) | [151] |
| TcBglA | 1         | 90 °C   | 5.5–6.5             | >40%; 80 °C | 30 min               | Bacteria (Thermus caldophilus) | [152] |
| TnGly  | 1         | 90 °C   | 5.6                 | 50%; 90 °C | 2.5 h                | Bacteria (Thermus nonproteolyticus) | [153] |
Table 5. Cont.

| Enzyme   | GH family Domains | Optimum Temperature | Optimum pH | Temperature Stability ¹ | Source                          | Reference                      |
|----------|-------------------|---------------------|------------|--------------------------|--------------------------------|--------------------------------|
| BglA     | 1                 | 70 °C               | 5.0–6.0    | 50%; 70 °C; 38 h         | Bacteria (Thermus sp. IB-21)   | [154]                          |
|          |                   |                     |            | 50%; 80 °C; >0.4 h       |                                |                                |
|          |                   |                     |            | 50%; 90 °C; <0.3 h       |                                |                                |
|          |                   |                     |            | 50%; 70 °C; 38 h         | Bacteria (Thermus sp. IB-21)   | [154]                          |
|          |                   |                     |            | 50%; 80 °C; 2.7 h        |                                |                                |
|          |                   |                     |            | 50%; 90 °C; 24 min       |                                |                                |
| BglB     | 1                 | 80 °C               | 5.0–6.0    | 50%; 70 °C; 38 h         | Bacteria (Thermus sp. IB-21)   | [154]                          |
|          |                   |                     |            | 50%; 80 °C; 2.7 h        |                                |                                |
|          |                   |                     |            | 50%; 90 °C; 24 min       |                                |                                |
| Bgly     | 1                 | 90 °C               | 5.4        | 50%; 90 °C; 1.5 h        | Bacteria (Thermus thermophilus) | [155]                          |
|          |                   |                     |            | 50%; 95 °C; 20 min       |                                |                                |
| BglA     | 1                 | 55 °C               | 6.5        | 82%; 50 °C; 60 min       | Uncultured organism (soil metagenome) | [52] |
|          |                   |                     |            | 20%; 55 °C; 60 min       |                                |                                |
| AS-Esc10 | 1                 | 60 °C               | 8.0        | 100%; 50 °C; 1 h         | Uncultured organism (agricultural soil metagenome) | [40] |
|          |                   |                     |            | 50%; 50 °C; 15 min       |                                |                                |
| Bgl-gx1  | 1                 | 90 °C               | 6.0        | 50%; 90 °C; 5 min        | Uncultured organism (termite gut metagenome) | [71] |
|          |                   |                     |            | 50%; 85 °C; 45 min       |                                |                                |
| Bgl      | 1                 | 60 °C               | 5.0        | 50%; 60 °C; 540 min      | Fungi (Fusarium oxysporum)     | [156]                          |
|          |                   |                     |            | 50%; 80 °C; 30 min       |                                |                                |
| Bgl4     | 1                 | 55 °C               | 6.0        | 50%; 50 °C; 10 min       | Fungi (Humicola grisea var. thermodea IFO9854) | [157] |
| BglI     | 1                 | 55 °C               | 5.5–7.5    | 100%; 50 °C; 8 h         | Fungi (Orpinomyces sp. PC-2)   | [158]                          |
| Bgl1G5   | 1                 | 50 °C               | 6.0        | 50%; 50 °C; 6 h          | Fungi (Phialophora sp. G5)     | [159]                          |
| TaGH2    | 2                 | 95 °C               | 6.5        | 100%; 90 °C; 3 h         | Bacteria (Thermus antranikianii) | [160] |
| TbGH2    | 2                 | 90 °C               | 6.5        | 50%; 70 °C; 3 h          | Bacteria (Thermus brockianus)  | [160]                          |
| TbBgl    | 3                 | 90 °C               | 3.5        | 50%; 95 °C; 60 min       | Arquea (Thermofilum pendens)   | [161]                          |
| BII-G3   | 3                 | 50 °C               | 6.0        | 50%; 75 °C; 854 min      | Fungi (Bifidobacterium longum) | [162]                          |
|          |                   |                     |            | 50%; 80 °C; 854 min      |                                |                                |
|          |                   |                     |            | 50%; 85 °C; 854 min      |                                |                                |
|          |                   |                     |            | 50%; 90 °C; 854 min      |                                |                                |
| Cba2     | 3                 | 70 °C               | 4.8        | 50%; 75 °C; 854 min      | Fungi (Cellulomonas mazatensis) | [163]                         |
|          |                   |                     |            | 50%; 80 °C; 854 min      |                                |                                |
|          |                   |                     |            | 50%; 85 °C; 854 min      |                                |                                |
|          |                   |                     |            | 50%; 90 °C; 854 min      |                                |                                |
| CFlBG3   | 3                 | 55 °C               | 7.5        | 50%; 60 °C; 6 h          | Fungi (Cellulomonas fimi)      | [164]                          |
|          |                   |                     |            | 50%; 70 °C; 6 h          |                                |                                |
|          |                   |                     |            | 50%; 80 °C; 6 h          |                                |                                |
| Bgl3Z    | 3                 | 65 °C               | 5.5        | 50%; 60 °C; 5 h          | Fungi (Clostridium stercorarium) | [165]                         |
|          |                   |                     |            | 50%; 70 °C; 1575 min     |                                |                                |
| Dtur_0219| 3                 | 85 °C               | 5.0        | 50%; 75 °C; 24 h         | Fungi (Dictyoglomus turidium)  | [54]                           |
|          |                   |                     |            | 50%; 80 °C; 24 h         |                                |                                |
|          |                   |                     |            | 50%; 85 °C; 24 h         |                                |                                |
|          |                   |                     |            | 50%; 90 °C; 24 h         |                                |                                |
|          |                   |                     |            | 67.7%; 60 °C; 1 h        |                                |                                |
|          |                   |                     |            | 67.7%; 65 °C; 1 h        |                                |                                |
|          |                   |                     |            | 29.7%; 70 °C; 10 min     |                                |                                |

¹ Stability: 100% stability at 50 °C for 2 h, 90% for 3 h, 80% for 4 h, 50% for 5 h.
| Enzyme | GH family Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|-------------------|---------------------|------------|----------------------|--------|-----------|
| Bxl5   | 3                 | 75 °C               | 4.6        | 50%; 65 °C; 5 h      | Fungi  | (Chrysosporium lucknowense) [171] |
| MoCel3A| 3                 | 50 °C               | 5.0–5.5    | NM                   | Fungi  | (Magnaporthe oryzae) [41]        |
| MoCel3B| 3                 | 50 °C               | 5.0–5.5    | NM                   | Fungi  | (Magnaporthe oryzae) [41]        |
| Bgl2   | 3                 | 60 °C               | 5.4        | 50%; 40 °C; 2 h; >50%; 50 °C; 2 h; 25%; 55 °C; 1 h | Fungi  | (Neuspora crassa) [172] |
| Bgl1   | 3                 | 50 °C               | 3.5–5.0    | 100%; 45 °C; 30 min  | Fungi  | (Mucor circinelloides) [173]     |
| Bgl2   | 3                 | 55 °C               | 3.5–5.5    | 100%; 55 °C; 30 min  | Fungi  | (Mucor circinelloides) [173]     |
| NfBGL1 | 3                 | 80 °C               | 5.0        | >80%; 70 °C; 2 h     | Fungi  | (Neosartorya fischeri) [174]     |
| PtBglu3| 3                 | 65 °C               | 6.0        | >85%; 60 °C; 30 min  | Fungi  | (Pacilomyces fischeri) [32]      |
| Bgl1   | 3                 | 70 °C               | 4.8        | 50%; 65 °C; 24 h     | Fungi  | (Penicillium brasiliense) [175]   |
| pBGL1  | 3                 | 65–70 °C            | 4.5–5.50   | 96.3%; 50 °C; 12 h; 50%; 70 °C; 4 h | Fungi  | (Penicillium decumbens) [176]   |
| Bgl1   | 3                 | 70 °C               | 5.0–6.0    | 60%; 70 °C; 1.5 h    | Fungi  | (Periconia sp.) [43]             |
| Bgl2   | 3                 | 50 °C               | 5.0        | 60%; 50 °C; 30 min   | Fungi  | (Rhizomucor miehei) [50]         |
| RmBglu3B| 3                | 50 °C               | 5.0        | >70%; 50 °C; 30 min  | Fungi  | (Saccharomycopsis fibuligera) [177] |
| Bgl1   | 3                 | 70 °C               | 5.0        | >70%; 50 °C; 30 min  | Fungi  | (Saccharomycopsis fibuligera) [177] |
| Bgl2   | 3                 | 50 °C               | 5.0        | >70%; 50 °C; 30 min  | Fungi  | (Saccharomycopsis fibuligera) [177] |
| β-glucosidase | 3   | 75 °C               | 4.5        | 50%; 60 °C; 136 h; 50%; 65 °C; 55 h; 50%; 70 °C; 10 h; 50%; 75 °C; 1 h | Fungi  | (Talaromyces aculeatus) [53]      |
| Cel3a  | 3                 | 71.5 °C             | 4.02       | 50%; 65 °C; 62 min  | Fungi  | (Talaromyces emersonii) [178]     |
| Bgl3A  | 3                 | 75 °C               | 4.5        | >65%; 60 °C; 1 h     | Fungi  | (Talaromyces lepeltarius) [179]   |
| Bgl1   | 3                 | 70 °C               | 5.0        | >70%; 60 °C; 1 h     | Fungi  | (Thermoascus aurantiacus) [180]   |
| Bgl3A  | 3                 | 70 °C               | 5.0        | 50%; 60 °C; 143 min  | Fungi  | (Myceliophthora thermophila) [181] |
| RG3    | 3                 | 50–55 °C            | 5.5–6.0    | NM                   | Uncultured organism | (Rabbit cecum metagenome) [67] |
| RG14   | 3                 | 50–55 °C            | 5.5–7.0    | NM                   | Uncultured organism | (Rabbit cecum metagenome) [67] |
| BGL7   | 3                 | 50 °C               | 6.5        | NM                   | Uncultured organism | (Termite gut metagenome) [72] |
| LAB25g2| 3                 | 55 °C               | 4.5        | >82%; 50 °C; 5 d     | Uncultured organism | (Cow rumen metagenome) [68] |
| SRF2g14| 3                 | 55 °C               | 5.0        | 50%; 50 °C; 18.06 h  | Uncultured organism | (Cow rumen metagenome) [68] |
| SRF2g18| 3                 | 50 °C               | 4.0        | 50%; 50 °C; 37.5 h   | Uncultured organism | (Cow rumen metagenome) [68] |
| RuBGX1 | 3                 | 50 °C               | 6.0        | 62%; 50 °C; 10 min   | Uncultured organism | (Yak rumen metagenome) [36] |
| JMB19063| 3               | 50–55 °C            | 6.5        | NM                   | Uncultured organism | (Compost metagenome) [37] |
| GlyA1  | 3                 | 55 °C               | 6.5        | NM                   | Uncultured organism | (Cow rumen metagenome) [69] |
Table 5. Cont.

| Enzyme | GH family Domains | Optimum Temperature | Optimum pH | Temperature Stability | Source | Reference |
|--------|-------------------|---------------------|------------|-----------------------|--------|-----------|
| Bgx1   | 30                | 50 °C               | 4.0–6.0    | NM                    | Oomycota (Phytophthora infestans) | [182] |
| SSO3039| 116               | >70 °C              | 4.0        | >70%; 65 °C; 48 h     | Arquea (Sulfolobus solfataricus) | [183] |
|        |                   |                     |            | >50%; 85 °C; 8 h      | Bacteria (Thermanaerobacterium xylanolyticum) | [184] |

1 Temperature stability is given as a percentage of activity (residual activity) after treatment at the specified temperature and time compared to the untreated enzyme.

7. Conclusions

Cellulases retrieved from high-temperature environments are considered a valuable industrial resource for their vast biotechnological potential [35]. The use of culture-independent techniques such as metagenomics has allowed us to discover enzymes from unknown microorganisms thriving in extreme habitats [11]. Since the last decade, metagenomics has led to the discovery of almost half (46%) of the characterized thermophilic endoglucanases (Table 1) described in that period and a fraction (17% of each total) of the thermophilic cellobiosidases acting on the non-reducing end of cellulose (Table 2) and thermophilic β-glucosidases (Table 5). Nevertheless, metagenomics have yet to yield thermophilic cellobiosidases acting on the reducing end of cellulose (Table 3) or thermophilic celloextrinases (Table 4). The lack of enzymes found by this strategy is likely a consequence of the mechanism of action of those enzymes, as the lack of substrates specific to those activities greatly limits its positive hit ratio. While thermophilic β-glucosidases discovered in the last 5 years still account for a similar proportion of the total (15%), no more thermophilic cellobiosidases acting on non-reducing ends have been characterized by this method. On the other hand, the proportion of thermophilic endoglucanases that have been characterized and identified by metagenomics have grown to account for more than half of the total (55%) in the last 5 years. In total, almost one fifth (18%) of all the thermophilic cellulases identified and characterized so far have been found by metagenomics. Functional metagenomic bottlenecks, like the lack of substrates for specific cellulases and problems associated with heterologous expression [3], and validation of sequence-based metagenomics annotation of cellulases [11], still need to be addressed to further increase the number of cellulases identified using these strategies. Biomining for novel thermophilic cellulases through metagenomic means is thus an ongoing challenge, with great potential as a source of commercially and environmentally important byocatalysts in all sorts of biotechnological applications.

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References

1. Sandgren, M.; Ståhlberg, J.; Mitchinson, C. Structural and biochemical studies of GH family 12 cellulases: Improved thermal stability, and ligand complexes. *Prog. Biophys. Mol. Biol.* 2005, 89, 246–291. [CrossRef] [PubMed]
2. Blumer-Schuette, S.E.; Kataeva, I.; Westpheling, J.; Adams, M.W.; Kelly, R.M. Extremely thermophilic microorganisms for biomass conversion: Status and prospects. *Curr. Opin. Biotechnol.* 2008, 19, 210–217. [CrossRef] [PubMed]

3. Duan, C.-J.; Feng, J.-X. Mining metagenomes for novel cellulase genes. *Biotechnol. Lett.* 2010, 32, 1765–1775. [CrossRef] [PubMed]

4. Sharma, A.; Tewari, R.; Rana, S.S.; Soni, R.; Soni, S.K. Cellulases: Classification, Methods of Determination and Industrial Applications. *Appl. Biochem. Biotechnol.* 2016, 179, 1346–1380. [CrossRef] [PubMed]

5. Couturier, M.; Feliu, J.; Haon, M.; Navarro, D.; Lesage-Meessen, L.; Coutinho, P.M.; Berrin, J.-G. A thermostable GH45 endoglucanase from yeast: Impact of its atypical multimodularity on activity. *Microb. Cell Fact.* 2011, 10, 103. [CrossRef] [PubMed]

6. López-Mondéjar, R.; Zühlke, D.; Becher, D.; Riedel, K.; Baldrian, P. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Sci. Rep.* 2016, 6, 25279. [CrossRef] [PubMed]

7. Kaur, B.; Chadha, B.S. Approaches for Bioprospecting Cellulases. In *Extremophilic Enzymatic Processing of Lignocellulosic Feedstocks to Bioenergy*; Sani, R.K., Krishnaraj, R.N., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 53–71, ISBN 978-3-319-54683-4.

8. Bok, J.D.; Yernool, D.A.; Eveleigh, D.E. Purification, characterization, and molecular analysis of thermostable cellulases CelA and CelB from *Thermotoga neapolitana*. *Appl. Environ. Microbiol.* 1998, 64, 4774–4781. [PubMed]

9. Elleuche, S.; Schäfers, C.; Blank, S.; Schröder, C.; Antranikian, G. Exploration of extremophiles for high temperature biotechnological processes. *Curr. Opin. Microbiol.* 2015, 25, 113–119. [CrossRef] [PubMed]

10. Tiwari, R.; Nain, L.; Labrou, N.E.; Shukla, P. Bioprospecting of functional cellulases from metagenome for second generation biofuel production: A review. *Crit. Rev. Microbiol.* 2018, 44, 244–257. [CrossRef] [PubMed]

11. De Castro, M.-E.; Rodriguez-Belmonte, E.; González-Siso, M.-I. Metagenomics of Thermophiles with a Focus on Discovery of Novel Thermozyymes. *Front. Microbiol.* 2016, 7, 1521. [CrossRef] [PubMed]

12. Wagner, I.D.; Wiegel, J. Diversity of thermophilic anaerobes. *Ann. N. Y. Acad. Sci.* 2008, 1125, 1–43. [CrossRef] [PubMed]

13. Liu, Y.; Zhang, J.; Liu, Q.; Zhang, C.; Ma, Q. Molecular cloning of novel cellulase genes cel9A and cel12A from *Bacillus licheniformis* GXN191 and synergism of their encoded polypeptides. *Curr. Microbiol.* 2004, 49, 234–238. [CrossRef] [PubMed]

14. Crennell, S.J.; Hreggvidsson, G.O.; Nordberg Karlsson, E. The structure of *Rhodothermus marinus* Cel12A, a highly thermostable family 12 endoglucanase, at 1.8 Å resolution. *J. Mol. Biol.* 2002, 320, 883–897. [CrossRef]

15. Chhabra, S.R.; Kelly, R.M. Biochemical characterization of *Thermotoga maritima* endoglucanase Cel74 with and without a carbohydrate binding module (CBM). *FEBS Lett.* 2002, 531, 375–380. [CrossRef] [PubMed]

16. Peer, A.; Smith, S.P.; Bayer, E.A.; Lamed, R.; Borovok, I. Noncellulosomal cohesin- and dockerin-like modules in the three domains of life. *FEBS Microbiol. Lett.* 2009, 291, 1–16. [CrossRef] [PubMed]

17. Bayer, E.A.; Belaich, J.-P.; Shoham, Y.; Lamed, R. The cellulosomes: Multienzyme machines for degradation of plant cell wall polysaccharides. *Annu. Rev. Microbiol.* 2004, 58, 521–554. [CrossRef] [PubMed]

18. Sathyap, T.A.; Khan, M. Diversity of glycosyl hydrolase enzymes from metagenome and their application in food industry. *J. Food Sci.* 2014, 79, R2149–R2156. [CrossRef] [PubMed]

19. Poidevin, L.; Feliu, J.; Doan, A.; Berrin, J.-G.; Bey, M.; Coutinho, P.M.; Henrissat, B.; Record, E.; Heiss-Blanket, S. Insights into exo- and endoglucanase activities of family 6 glycoside hydrolases from *Podospora anserina*. *Appl. Environ. Microbiol.* 2013, 79, 4220–4229. [CrossRef] [PubMed]

20. Henrissat, B. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 1991, 280, 309–316. [CrossRef] [PubMed]

21. Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P.M.; Henrissat, B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 2014, 42, D490–D495. [CrossRef] [PubMed]

22. Kim, D.Y.; Lee, M.J.; Cho, H.-Y.; Lee, J.S.; Lee, M.-H.; Chung, C.W.; Shin, D.-H.; Rhee, Y.H.; Son, K.-H.; Park, H.-Y. Genetic and functional characterization of an extracellular modular GH6 endo-1,4-glucanase from an earthworm symbiont, *Cellulosimicrobium fuscii* HY-13. *Antonie Van Leeuwenhoek* 2016, 109, 1–12. [CrossRef] [PubMed]
23. Halldórsson, S.; Thórofjórdóttir, E.T.; Spiliaert, R.; Johansson, M.; Thorbjarnardóttir, S.H.; Palsdottir, A.; Hreggvidsson, G.O.; Kristjánsson, J.K.; Holst, O.; Eggertsson, G. Cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable cellulase of glycosyl hydrolase family 12. *Appl. Microbiol. Biotechnol.* 1998, 49, 277–284. [CrossRef] [PubMed]

24. Ausili, A.; Cobucci-Ponzano, B.; Di Lauro, B.; D’Avino, R.; Perugini, G.; Bertoli, E.; Scire, A.; Rossi, M.; Tafani, F.; Moracci, M. A comparative infrared spectroscopic study of glycoside hydrolases from extremophilic archaea revealed different molecular mechanisms of adaptation to high temperatures. *Proteins* 2007, 67, 991–1001. [CrossRef] [PubMed]

25. Aguilar, C.F.; Sanderson, I.; Moracci, M.; Ciaramella, M.; Nucci, R.; Rossi, M.; Pearl, L.H. Crystal structure of the β-glucosidase from the hyperthermophilic archean *Sulfolobus solfataricus*: Resilience as a key factor in thermostability. *J. Mol. Biol.* 1997, 271, 789–802. [CrossRef] [PubMed]

26. Wu, I.; Arnold, F.H. Engineered thermostable fungal Cel6A and Cel7A cellobiohydrolases hydrolyze cellulose efficiently at elevated temperatures. *Biotechnol. Bioeng.* 2013, 110, 1874–1883. [CrossRef] [PubMed]

27. Rawat, R.; Kumar, S.; Chadha, B.S.; Kumar, D.; Oberoi, H.S. An acidothermophilic functionally active novel GH12 family endoglucanase from *Aspergillus niger* HO: Purification, characterization and molecular interaction studies. *Antoni Van Leeuwenhoek* 2015, 107, 103–117. [CrossRef] [PubMed]

28. Voutilainen, S.P.; Puranen, T.; Siika-Aho, M.; Lappalainen, A.; Alapuranen, M.; Kallio, J.; Hooman, S.; Viikari, L.; Vehmaanperä, J.; Koivula, A. Cloning, expression, and characterization of novel thermostable family 7 cellobiohydrolases. *Biotechnol. Bioeng.* 2008, 101, 515–528. [CrossRef] [PubMed]

29. Voutilainen, S.P.; Murray, P.G.; Tuohy, M.G.; Koivula, A. Expression of *Talaromyces emersonii* cellobiohydrolase Cel7A in *Saccharomyces cerevisiae* and rational mutagenesis to improve its thermostability and activity. *Protein Eng. Des. Sel.* 2010, 23, 69–79. [CrossRef] [PubMed]

30. Li, Y.-L.; Li, H.; Li, A.-N.; Li, D.-C. Cloning of a gene encoding thermostable cellobiohydrolase from the thermophilic fungus *Chaetomium thermophilum* and its expression in *Pichia pastoris*. *J. Appl. Microbiol.* 2009, 106, 1867–1875. [CrossRef] [PubMed]

31. Karnaouri, A.C.; Topakas, E.; Christakopoulos, P. Cloning, expression, and characterization of a thermostable GH7 endoglucanase from *Myceliophthora thermophila* capable of high-consistency enzymatic liquefaction. *Appl. Microbiol. Biotechnol.* 2014, 98, 231–242. [CrossRef] [PubMed]

32. Yan, Q.; Hua, C.; Yang, S.; Li, Y.; Jiang, Z. High level expression of extracellular secretion of a β-glucosidase gene (PtBglu3) from *Pacilomyces thermophilus* in *Pichia pastoris*. *Protein Expr. Purif.* 2012, 84, 64–72. [CrossRef] [PubMed]

33. Liu, Y.; Dun, B.; Shi, P.; Ma, R.; Luo, H.; Bai, Y.; Xie, X.; Yao, B. A Novel GH7 Endo-β-1,4-Glucanase from *Neosartorya fischeri* P1 with Good Thermostability, Broad Substrate Specificity and Potential Application in the Brewing Industry. *PLoS ONE* 2015, 10, e0137485. [CrossRef] [PubMed]

34. Dougherty, M.J.; D’haeseleer, P.; Hazen, T.C.; Simmons, B.A.; Adams, P.D.; Hadi, M.Z. From soil to structure, a novel dimeric glucosidase/xylosidase from yak rumen metagenome promotes the enzymatic degradation of hemicellulosic xylans. *Lett. Appl. Microbiol.* 2012, 54, 79–87. [CrossRef] [PubMed]

35. McAndrew, R.P.; Park, J.I.; Heins, R.A.; Reindl, W.; Friedland, G.D.; D’haeseleer, P.; Northen, T.; Sale, K.L.; Simmons, B.A.; Adams, P.D. From soil to structure, a novel dimeric β-glucosidase belonging to glycoside hydrolase family 3 isolated from compost using metagenomic analysis. *J. Biol. Chem.* 2013, 288, 14985–14992. [CrossRef] [PubMed]

36. Lee, C.-M.; Lee, Y.-S.; Seo, S.-H.; Yoon, S.-H.; Kim, S.-J.; Hahn, B.-S.; Sim, J.-S.; Koo, B.-S. Screening and Characterization of a Novel Cellulase Gene from the Gut Microflora of *Hermetia illucens* Using Metagenomic Library. *J. Microbiol. Biotechnol.* 2014, 24, 1196–1206. [CrossRef] [PubMed]

37. Aytekin, M.; Rossi, M.; Cannio, R. Cellulose degradation by *Sulfolobus solfataricus* requires a cell-anchored endo-β-1,4-glucanase. *J. Bacteriol.* 2012, 194, 5091–5100. [CrossRef] [PubMed]
40. Biver, S.; Stroobants, A.; Portetelle, D.; Vandenbol, M. Two promising alkaline β-glucosidases isolated by functional metagenomics from agricultural soil, including one showing high tolerance towards harsh detergents, oxidants and glucose. *J. Ind. Microbiol. Biotechnol.* 2014, 41, 479–488. [CrossRef] [PubMed]

41. Takahashi, M.; Konishi, T.; Takeda, T. Biochemical characterization of *Magnaporthe oryzae* β-glucosidases for efficient β-glucan hydrolysis. *Appl. Microbiol. Biotechnol.* 2011, 91, 1073–1082. [CrossRef] [PubMed]

42. Bhalla, A.; Bansal, N.; Kumar, S.; Bischoff, K.M.; Sani, R.K. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes. *Bioresour. Technol.* 2013, 128, 751–759. [CrossRef] [PubMed]

43. Hampicharnchai, P.; Champreda, V.; Sornlake, W.; Eurwilaichitr, L. A thermostable beta-glucosidase isolated from an endophytic fungi, *Periconia sp.*, with a possible use for biomass conversion to sugars. *Protein Expr. Purif.* 2009, 67, 61–69. [CrossRef] [PubMed]

44. Fusco, F.A.; Fiorentino, G.; Pedone, E.; Contursi, P.; Bartolucci, S.; Limauro, D. Biochemical characterization of a novel thermostable β-glucosidase from *Dictyoglomus turgidum*. *Int. J. Biol. Macromol.* 2018, 113, 783–791. [CrossRef]

45. Boyce, A.; Walsh, G. Characterisation of a novel thermostable endoglucanase from *Alicyclobacillus vulcanalis* of potential application in bioethanol production. *Appl. Microbiol. Biotechnol.* 2015, 99, 7515–7525. [CrossRef] [PubMed]

46. Ando, S.; Ishida, H.; Kosugi, Y.; Ishikawa, K. Hyperthermostable Endoglucanase from *Pyrococcus horikoshii*. *Appl. Environ. Microbiol.* 2002, 68, 430–433. [CrossRef] [PubMed]

47. Bai, Y.; Wang, J.; Zhang, Z.; Shi, P.; Luo, H.; Huang, H.; Feng, Y.; Yao, B. Extremely acidic beta-1,4-glucanase, CelA4, from thermoacidophilic *Alicyclobacillus* sp. A4 with high protease resistance and potential as a pig feed additive. *J. Agric. Food Chem.* 2010, 58, 1970–1975. [CrossRef] [PubMed]

48. Vuong, T.V.; Wilson, D.B. Processivity, synergism, and substrate specificity of *Thermobifida fusca* Cel6B. *Appl. Environ. Microbiol.* 2009, 75, 6655–6661. [CrossRef] [PubMed]

49. Tang, Z.; Liu, S.; Jing, H.; Sun, R.; Liu, M.; Chen, H.; Wu, Q.; Han, X. Cloning and expression of *A. oryzae* β-glucosidase in *Pichia pastoris*. *Mol. Biol. Rep.* 2014, 41, 7567–7573. [CrossRef] [PubMed]

50. Guo, Y.; Yan, Q.; Yang, Y.; Yang, S.; Liu, Y.; Jiang, Z. Expression and characterization of a novel β-glucosidase, with transglycosylation and exo-β-1,3-glucanase activities, from *Rhizomucor miehei*. *Food Chem.* 2015, 175, 431–438. [CrossRef] [PubMed]

51. Villanueva, A.; Ramón, D.; Vallés, S.; Lluch, M.A.; MacCabe, A.P. Heterologous Expression in *Aspergillusnidulans* of a *Trichoderma longibrachiatum* Endoglucanase of Enological Relevance. *J. Agric. Food Chem.* 2000, 48, 951–957. [CrossRef] [PubMed]

52. Kim, S.-J.; Lee, C.-M.; Kim, M.-Y.; Yeo, Y.-S.; Yoon, S.-H.; Kang, H.-C.; Koo, B.-S. Screening and characterization of an enzyme with beta-glucosidase activity from environmental DNA. *J. Microbiol. Biotechnol.* 2007, 17, 905–912. [PubMed]

53. Lee, G.-W.; Yoo, M.-H.; Shin, K.-C.; Kim, K.-R.; Kim, Y.-S.; Lee, K.-W.; Oh, D.-K. β-glucosidase from *Penicillium aculeatum* hydrolyzes exo-, 3-O-, and 6-O-β-glucosides but not 20-O-β-glucoside and other glycosides of ginsenosides. *Appl. Microbiol. Biotechnol.* 2013, 97, 6315–6324. [CrossRef] [PubMed]

54. Kim, Y.-S.; Yeom, S.-J.; Oh, D.-K. Characterization of a GH3 family β-glucosidase from *Dictyoglomus turgidum* and its application to the hydrolysis of isoflavone glycosides in spent coffee grounds. *J. Agric. Food Chem.* 2011, 59, 11812–11818. [CrossRef] [PubMed]

55. Kumar, P.; Ryan, B.; Henehan, G.T.M. β-Glucosidase from *Streptomyces griseus*: Nanoparticle immobilisation and application to alkyl glycoside synthesis. *Protein Expr. Purif.* 2017, 132, 164–170. [CrossRef] [PubMed]

56. Plácido, A.; Hai, T.; Ferrer, M.; Chernikova, T.N.; Distaso, M.; Armstrong, D.; Yakunin, A.F.; Toshchakov, S.V.; Yakimov, M.M.; Kublanov, I.V.; et al. Diversity of hydrolases from hydrothermal vent sediments of the Levante Bay, Vulcano Island (Aeolian archipelago) identified by activity-based metagenomics and biochemical characterization of new esterases and an arabinopyranosidase. *Appl. Microbiol. Biotechnol.* 2015, 99, 10031–10046. [CrossRef] [PubMed]

57. Leis, B.; Heinze, S.; Angelov, A.; Pham, V.T.T.; Thürmer, A.; Jebbar, M.; Golyshin, P.N.; Streit, W.R.; Daniel, R.; Liebl, W. Functional Screening of Hydrolytic Activities Reveals an Extremely Thermostable Cellulase from a Deep-Sea Archaeon. *Front. Bioeng. Biotechnol.* 2015, 3, 95. [CrossRef] [PubMed]
58. Graham, J.E.; Clark, M.E.; Nadler, D.C.; Huffer, S.; Chokhawala, H.A.; Rowland, S.E.; Blanch, H.W.; Clark, D.S.; Robb, F.T. Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment. *Nat. Commun.* 2011, 2, 375. [CrossRef] [PubMed]

59. Schröder, C.; Elleuche, S.; Blank, S.; Antranikian, G. Characterization of a heat-active archaeal β-glucosidase from a hydrothermal spring metagenome. *Enzyme Microb. Technol.* 2014, 57, 48–54. [CrossRef] [PubMed]

60. Yasir, M.; Khan, H.; Azam, S.S.; Telke, A.; Kim, S.W.; Chung, Y.R. Cloning and functional characterization of endo-β-1,4-glucanase gene from metagenomic library of vermicompost. *J. Microbiol.* 2013, 51, 329–335. [CrossRef] [PubMed]

61. Kwon, E.J.; Jeong, Y.S.; Kim, Y.H.; Kim, S.K.; Na, H.B.; Kim, J.; Yun, H.D.; Kim, H. Construction of a Metagenomic Library from Compost and Screening of Cellulase- and Xylanase-positive Clones. *J. Korean Soc. Appl. Biol. Chem.* 2010, 53, 702–708. [CrossRef]

62. Okano, H.; Ozaki, M.; Kanaya, E.; Kim, J.-J.; Angkawidjaja, C.; Koga, Y.; Kanaya, S. Structure and stability of metagenome-derived glycoside hydrolase family 12 cellulase (LC-CelA) a homolog of Cell12A from *Rhodothermus marinus*. *FEBS Open Bio* 2014, 4, 936–946. [CrossRef] [PubMed]

63. Yan, X.; Geng, A.; Zhang, J.; Wei, Y.; Zhang, L.; Qian, C.; Wang, Q.; Wang, S.; Zhou, Z. Discovery of (hemi-)cellulase genes in a metagenomic library from a biogas digester using 454 pyrosequencing. *Appl. Microbiol. Biotechnol.* 2013, 97, 8173–8182. [CrossRef] [PubMed]

64. Alvarez, T.M.; Paiva, J.H.; Ruiz, D.M.; Cairo, J.P.L.F.; Pereira, I.O.; Ignatiev, N.; Streit, W.R. Structure and stability of (hemi-)cellulose genes in a soil derived metagenomic library. *PLoS ONE* 2013, 8, e83635. [CrossRef] [PubMed]

65. Liu, J.; Liu, W.-D.; Zhao, X.-L.; Shen, W.-J.; Cao, H.; Cui, Z.-L. Cloning and functional characterization of a novel endo-β-1,4-glucanase gene from a soil-derived metagenomic library. *Appl. Microbiol. Biotechnol.* 2011, 89, 1083–1092. [CrossRef] [PubMed]

66. Pottkämper, J.; Barthen, P.; Ilmberger, N.; Schwaneberg, U.; Schulte, M.; Ignatiev, N.; Streit, W.R. Microbial metagenomics for the identification of bacterial cellulases that are stable in ionic liquids. *Green Chem.* 2011, 13, 957. [CrossRef]

67. Feng, Y.; Duan, C.-J.; Pang, H.; Mo, X.-C.; Wu, C.-F.; Yu, Y.; Hu, Y.-L.; Wei, J.; Tang, J.-L.; Feng, J.-X. Cloning and identification of novel cellulase genes from uncultured microorganisms in rabbit cecum and characterization of the expressed cellulases. *Appl. Microbiol. Biotechnol.* 2007, 75, 319–328. [CrossRef] [PubMed]

68. Del Pozo, M.V.; Fernández-Arrojo, L.; Gil-Martínez, J.; Montesinos, A.; Chernikova, T.N.; Nechitaylo, T.Y.; Waliszek, A.; Tortajada, M.; Rojas, A.; Huws, S.A.; et al. Microbial β-glucosidases from cow rumen metagenome enhance the saccharification of lignocellulose in combination with commercial cellulase cocktail. *Enzyme Microb. Technol.* 2012, 5, 73. [CrossRef] [PubMed]

69. Ramírez-Escudero, M.; Del Pozo, M.V.; Marin-Navarro, J.; González, B.; Golyshin, P.N.; Polaina, J.; Ferrer, M.; Sanz-Aparicio, J. Structural and Functional Characterization of a Ruminal β-Glycosidase Defines a Novel Subfamily of Glycoside Hydrolase Family 3 with Permuted Domain Topology. *J. Biol. Chem.* 2016, 291, 24200–24214. [CrossRef] [PubMed]

70. Beloqui, A.; Nechitaylo, T.Y.; López-Cortés, N.; Ghazi, A.; Guazzaroni, M.-E.; Polaina, J.; Strittmatter, A.W.; Reva, O.; Waliczek, A.; Yakimov, M.M.; et al. Diversity of glycosyl hydrolases from cellulose-depleting communities enriched from casts of two earthworm species. *Appl. Environ. Microbiol.* 2010, 76, 5934–5946. [CrossRef] [PubMed]

71. Wang, Q.; Qian, C.; Zhang, X.-Z.; Liu, N.; Liu, N.; Yan, X.; Zhou, Z. Characterization of a novel thermostable β-glucosidase from a metagenomic library of termite gut. *Enzyme Microb. Technol.* 2012, 51, 319–324. [CrossRef] [PubMed]

72. Zhang, M.; Liu, N.; Qian, C.; Wang, Q.; Wang, Q.; Long, Y.; Huang, Y.; Zhou, Z.; Yan, X. Phylogenetic and functional analysis of gut microbiota of a fungus-growing higher termite: Bacteroidetes from higher termites are a rich source of β-glucosidase genes. *Microb. Ecol.* 2014, 68, 416–425. [CrossRef] [PubMed]

73. Himmel, M.E.; Adney, W.S.; Tucker, M.P.; Grohmann, K. Thermostable Purified Endoglucanases from *Acidothermus cellulolyticus* ATCC 43068. U.S. Patent 5,275,944, 4 January 1994.

74. Nurachman, Z.; Kurniasih, S.D.; Puspitawati, F.; Hadi, S.; Radjasa, O.K.; Natalia, D. Cloning of the Endoglucanase Gene from a *Bacillus amyloquefaciens* PSM 3.1 in *Escherichia coli* Revealed Catalytic Triad Residues Thr-His-Glu. *Am. J. Biochem. Biotechnol.* 2010, 6, 268–274. [CrossRef]
75. Bischoff, K.M.; Rooney, A.P.; Li, X.-L.; Liu, S.; Hughes, S.R. Purification and characterization of a family of 5 endoglucanase from a moderately thermophilic strain of Bacillus licheniformis. Biotechnol. Lett. 2006, 28, 1761–1765. [CrossRef] [PubMed]

76. Jung, Y.-J.; Lee, Y.-S.; Park, I.-H.; Chandra, M.S.; Kim, K.-K.; Choi, Y.-L. Molecular cloning, purification and characterization of thermostable beta-1,3,1,4 glucanase from Bacillus subtilis A8-8. Indian J. Biochem. Biophys. 2010, 47, 203–210. [PubMed]

77. Chhabra, S.R.; Shockley, K.R.; Ward, D.E.; Kelly, R.M. Regulation of endo-acting glycosyl hydrolases in the hyperthermophilic bacterium Thermotoga maritima grown on glucan- and mannan-based polysaccharides. Appl. Environ. Microbiol. 2002, 68, 545–554. [CrossRef] [PubMed]

78. Bauer, S.; Vasu, P.; Persson, S.; Mort, A.J.; Somerville, C.R. Development and application of a suite of polysaccharide-degrading enzymes for analyzing plant cell walls. Proc. Natl. Acad. Sci. USA 2006, 103, 11417–11422. [CrossRef] [PubMed]

79. Calza, R.E.; Irwin, D.C.; Wilson, D.B. Purification and characterization of two alkaline endoglucanases from Thermomonospora fusca. Biochemistry 1985, 24, 7797–7804. [CrossRef]

80. Yin, Y.-R.; Zhang, F.; Hu, Q.-W.; Xian, W.-D.; Hozzein, W.N.; Zhou, E.-M.; Nie, G.-X.; Li, W.-J. DNA fragment encoding two similar thermostable cellulases, CelA and CelB, and characterization of the cellulase genes of Humicola grisea var. thermoidea with Potential for Applications in Various Industries. PLoS ONE 2015, 10, e0124925. [CrossRef] [PubMed]

81. Cazemier, A.E.; Verdoes, J.C.; Op den Camp, H.J.M.; Hackstein, J.H.P.; van Ooyen, A.J. A beta-1,4-endoglucanase-encoding gene from Cellulomonas pachnodae. Appl. Microbiol. Biotechnol. 1999, 52, 232–239. [CrossRef] [PubMed]

82. Xu, X.; Li, J.; Zhang, W.; Huang, H.; Shi, P.; Luo, H.; Liu, B.; Zhang, Y.; Zhang, Z.; Fan, Y.; et al. A Neutral Thermostable β,1-4-Glucanase from Humicola insolens'Y1 with Potential for Applications in Various Industries. PLoS ONE 2015, 10, e0124925. [CrossRef] [PubMed]

83. Li, X.L.; Chen, H.; Ljungdahl, L.G. Two cellulases, CelA and CelC, from the polycentric anaerobic fungus Orpinomyces strain PC-2 contain N-terminal docking domains for a cellulase-hemicellulase complex. Appl. Environ. Microbiol. 1997, 63, 4721–4728. [PubMed]

84. Takashima, S.; Nakamura, A.; Hidaka, M.; Masaki, H.; Uozumi, T. Cloning, sequencing, and expression of the cellulase genes of Humicola grisea var. thermosta. J. Biotechnol. 1996, 50, 137–147. [CrossRef]

85. Wei, X.-M.; Yin, Y.; Qu, Y. Molecular Cloning and Characterization of Two Major Endoglucanases from Penicillium decumbens. J. Microbiol. Biotechnol. 2010, 20, 265–270. [CrossRef] [PubMed]

86. Miettinen-Oinonen, A.; Londesborough, J.; Joutsjoki, V.; Lantto, R.; Vehmaanperä, J. Three cellulases from Melanocarpus albomyces for textile treatment at neutral pH. Enzyme Microb. Technol. 2004, 34, 332–341. [CrossRef]

87. Yoo, J.-S.; Jung, Y.-J; Chung, S.-Y.; Lee, Y.-C.; Choi, Y.-L. Molecular cloning and characterization of CMCase gene (celC) from Salmonella typhimurium UR. J. Microbiol. 2004, 42, 205–210. [PubMed]

88. Kim, J.O.; Park, S.R.; Lim, W.J.; Ryu, S.K.; Kim, M.K.; An, C.L.; Cho, S.J.; Park, Y.W.; Kim, J.H.; Yun, H.D. Cloning and characterization of thermostable endoglucanase (CelY) from the hyperthermophilic Aquifex aeolicus VFS. Biochem. Biophys. Res. Commun. 2000, 279, 420–426. [CrossRef] [PubMed]

89. Hakamada, Y.; Endo, K.; Takizawa, S.; Kobayashi, T.; Shirai, T.; Yamane, T.; Ito, S. Enzymatic properties, crystallization, and deduced amino acid sequence of an alkaline endoglucanase from Bacillus circulans. Biochim. Biophys. Acta 2002, 1570, 174–180. [CrossRef]

90. Ul Haq, I.; Akram, F.; Khan, M.A.; Hussain, Z.; Nawaz, A.; Iqbal, K.; Shah, A.J. CelC, a multidomain thermostable GH9 processive endoglucanase from Clostridium thermocellum: Cloning, characterization and saccharification studies. World J. Microbiol. Biotechnol. 2015, 31, 1699–1710. [CrossRef] [PubMed]

91. Zverlov, V.; Mahr, S.; Riedel, K.; Bronnenmeier, K. Properties and gene structure of a bifunctional cellulolytic enzyme (CelA) from the extreme thermophile “Anaerocellum thermophilum” with separate glycosyl hydrolase family 9 and 48 catalytic domains. Microbiology 1998, 144, 457–465. [CrossRef] [PubMed]

92. Zhang, X.-Z.; Sathtitsukasano, N.; Zhang, Y.-H.P. Glycoside hydrolase family 9 processive endoglucanase from Clostridium phytofermentans: Heterologous expression, characterization, and synergy with family 48 cellulbiohydrolase. Biosour. Technol. 2010, 101, 5534–5538. [CrossRef] [PubMed]

93. Liebl, W.; Ruile, P.; Bronnenmeier, K.; Riedel, K.; Lottspeich, F.; Greif, I. Analysis of a Thermotoga maritima DNA fragment encoding two similar thermostable cellulases, CelA and CelB, and characterization of the recombinant enzymes. Microbiology 1996, 142, 2533–2542. [CrossRef] [PubMed]
96. Bauer, M.W.; Driskill, L.E.; Callen, W.; Snead, M.A.; Mathur, E.J.; Kelly, R.M. An Endoglucanase, EglA, from the Hyperthermophilic Archaean Pyrococcus Furiosus Hydrolyzes β,1-4 Bonds in Mixed-Linkage (1→3),(1→4)-β-D-Glucans and Cellulose. *J. Bacteriol.* 1999, 181, 284–290. [PubMed]
97. Huang, Y.; Krauss, G.; Cottaz, S.; Driguez, H.; Lipps, G. A highly acid-stable and thermostable endo-beta-glucanase from the thermoacidophilic archaeon Sulfolobus solfataricus. *Biochem. J.* 2005, 385, 581–588. [CrossRef] [PubMed]
98. Warner, C.D.; Hoy, J.A.; Shilling, T.C.; Linnen, M.J.; Ginder, N.D.; Ford, C.F.; Honzatko, R.B.; Reilly, P.J. Characterization of a novel endoglucanase from *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 2010, 76, 338–346. [CrossRef] [PubMed]
99. Hansen, C.K.; Didierichsen, B.; Jørgensen, P.L. celA from *Bacillus latus* PL236 encodes a novel cellulose-binding endo-beta-1,4-glucanase. *J. Bacteriol.* 1992, 174, 3522–3531. [CrossRef] [PubMed]
100. Ahsan, M.M.; Matsumoto, M.; Karita, S.; Kimura, T.; Sakka, K.; Ohmiya, K. Purification and Characterization of the Family J Catalytic Domain Derived from the *Clostridium thermocellum* Endoglucanase CelJ. *Biosci. Biotechnol. Biochem.* 1997, 61, 427–431. [CrossRef] [PubMed]
101. Koga, J.; Baba, Y.; Shimonaka, A.; Nishimura, T.; Hanamura, S.; Kono, T. Purification and characterization of a new family 45 endoglucanase, STCEI, from *Staphylotherium cocoisporum* and its overproduction in *Humicola insolens*. *Appl. Environ. Microbiol.* 2008, 74, 4210–4217. [CrossRef] [PubMed]
102. Wonganu, B.; Pootanakit, K.; Boonyapakron, K.; Champreda, V.; Tanaponpipat, S.; Eurwilaichitr, L. Cloning, expression and characterization of a thermostolerant endoglucanase from *Syncopehalastrum racemosum* (BCC18080) in *Pichia pastoris*. *Protein Expr. Purif.* 2008, 58, 78–86. [CrossRef] [PubMed]
103. Baba, Y.; Shimonaka, A.; Koga, J.; Murashima, K.; Kubota, H.; Kono, T. Purification and characterization of a new endo-1,4-beta-D-glucanase from *Beltraniella portoricensis*. *Biosci. Biotechnol. Biochem.* 2005, 69, 1198–1201. [CrossRef] [PubMed]
104. Eckert, K.; Schneider, E. A thermoacidophilic endoglucanase (CelB) from *Alicyclobacillus acidocaldarius* displays high sequence similarity to arabinofuranosidases belonging to family 51 of glycoside hydrolases. *Eur. J. Biochem.* 2003, 270, 3593–3602. [CrossRef] [PubMed]
105. Břás, J.L.A.; Cartmell, A.; Carvalho, A.L.M.; Verzé, G.; Bayer, E.A.; Vazana, Y.; Correia, M.A.S.; Prates, J.A.M.; Ratnaparkhe, S.; Boraston, A.B.; et al. Structural insights into a unique cellulase fold and mechanism of cellulase hydrolysis. *Proc. Natl. Acad. Sci. USA* 2011, 108, 5237–5242. [CrossRef] [PubMed]
106. Park, C.-S.; Kawaguchi, T.; Sumitani, J.-I.; Takada, G.; Izumori, K.; Arii, M. Cloning and sequencing of an exoglucanase gene from *Streptomyces* sp. M 23, and its expression in *Streptomyces lividans* TK-24. *J. Biosci. Bioeng.* 2005, 99, 434–436. [CrossRef] [PubMed]
107. Zhang, S.; Lao, G.; Wilson, D.B. Characterization of a *Thermomonospora fusca* exocellulase. *Biochemistry* 1995, 34, 3386–3395. [CrossRef]
108. Liu, S.-A.; Li, D.-C.; Er, S.-J.; Zhang, Y. Cloning and expressing of cellulase gene (cbh2) from thermophilic fungi *Chaetomium thermophilum* CT2. *Sheng Wu Gong Cheng Xue Bao* 2005, 21, 892–899. [PubMed]
109. Bukhtojarov, F.E.; Ustinov, B.B.; Salanovich, T.N.; Antonov, A.I.; Gusakov, A.V.; Okunev, O.N.; Sinitsyn, A.P. Cellulase complex of the fungus *Trichoderma viride* MC-2 and its expression in *Pichia pastoris*. *Appl. Environ. Microbiol.* 2008, 74, 3386–3395. [CrossRef] [PubMed]
110. Toda, H.; Nagahata, N.; Amano, Y.; Nozaki, K.; Kanda, T.; Okazaki, M.; Shimosaka, M. Gene cloning and overproduction of an exoglucanase gene from *Trichoderma viride* JU-A10 for bioethanol production. *Bioreour. Technol.* 2011, 102, 8339–8342. [CrossRef] [PubMed]
111. Song, J.; Liu, B.; Liu, Z.; Yang, Q. Cloning of two cellbiohydrolase genes from *Trichoderma viride* and heterogenous expression in yeast *Saccharomyces cerevisiae*. *Mol. Biol. Rep.* 2010, 37, 2135–2140. [CrossRef] [PubMed]
113. Singh, R.N.; Akinenko, V.K. Isolation of a Cellobiohydrolase of Clostridium thermocellum Capable of Degrading Natural Crystalline Substrates. Biochem. Biophys. Res. Commun. 1993, 192, 1123–1130. [CrossRef] [PubMed]

114. Kataeva, I.; Li, X.-L.; Chen, H.; Choi, S.-K.; Ljungdahl, L.G. Cloning and sequence analysis of a new cellulase gene encoding CelK, a major cellulosome component of Clostridium thermocellum: Evidence for gene duplication and recombination. J. Bacteriol. 1999, 181, 5288–5295. [PubMed]

115. Zverlov, V.V.; Velikodvorskaya, G.A.; Schwarz, W.H. A newly described cellulosomal cellobiohydrolase, CelO, from Clostridium thermocellum: Investigation of the exo-mode of hydrolysis, and binding capacity to crystalline cellulose. Microbiology 2002, 148, 247–255. [CrossRef] [PubMed]

116. Takada, G.; Kawaguchi, T.; Sumitani, J.; Arai, M. Expression of Aspergillus aculeatus No. F-50 cellobiohydrolase I (cbhI) and beta-glucosidase 1 (bgl1) genes by Saccharomyces cerevisiae. Biosci. Biotechnol. Biochem. 1998, 62, 1615–1618. [CrossRef] [PubMed]

117. Moroz, O.V.; Maranta, M.; Shaghasi, T.; Harris, P.V.; Wilson, K.S.; Davies, G.J. The three-dimensional structure of the cellobiohydrolase Cel7A from Aspergillus fumigatus at 1.5 Å resolution. Acta Crystallogr. Sect. F Struct. Biol. Commun. 2015, 71, 114–120. [CrossRef] [PubMed]

118. Li, Y.; Li, D.; Teng, F. Purification and characterization of a cellobiohydrolase from the thermophilic fungus Chaetomium thermophilus CT2. Wei Sheng Wu Xue Bao 2006, 46, 143–146. [PubMed]

119. Hobdery, S.E.; Knott, B.C.; Haddad Momeni, M.; Taylor, L.E.; Borisova, A.S.; Podkaminer, K.K.; VanderWall, T.A.; Himmel, M.E.; Decker, S.R.; Beckham, G.T.; et al. Biochemical and Structural Characterizations of Two Dictyostelium Cellulbiohydrolases from the Amoebobazo Kingdom Reveal a High Level of Conservation between Distant Phylogenetic Trees of Life. Appl. Environ. Microbiol. 2016, 82, 3395–3409. [CrossRef] [PubMed]

120. Takashima, S.; Ikura, H.; Nakamura, A.; Hidaka, M.; Masaki, H.; Uozumi, T. Isolation of the gene and characterization of the enzymatic properties of a major exoglucanase of Humicola grisea without a cellulose-binding domain. J. Biochem. 1998, 124, 717–725. [CrossRef] [PubMed]

121. Voutilainen, S.P.; Boer, H.; Linder, M.B.; Puranen, T.; Rouvinen, J.; Vehmaanperä, J.; Koivula, A. Heterologous expression of Melanocarpus albomyces cellobiohydrolase Cel7B, and random mutagenesis to improve its thermostability. Enzyme Microb. Technol. 2007, 41, 234–243. [CrossRef]

122. Texier, H.; Dumon, C.; Neugnot-Roux, V.; Maestracci, M.; O’Donohue, M.J. Redefining XynA from Penicillium funiculosum IMI 378536 as a GH7 cellobiohydrolase. J. Ind. Microbiol. Biotechnol. 2012, 39, 1569–1576. [CrossRef] [PubMed]

123. Colussi, F.; Serpa, V.; Delabona, P.D.S.; Manzine, L.R.; Voltatodio, M.L.; Alves, R.; Mello, B.L.; Pereira, N.; Farinas, C.S.; Golubev, A.M.; et al. Purification, and biochemical and biophysical characterization of cellobiohydrolase I from Trichoderma harzianum IOC 3844. J. Microbiol. Biotechnol. 2011, 21, 808–817. [CrossRef] [PubMed]

124. Bronnenmeier, K.; Rücknagel, K.P.; Staudenbauer, W.L. Purification and properties of a novel type of exo-1,4-beta-glucanase (avicelase II) from the cellulolytic thermophile Clostridium stercorarium. Eur. J. Biochem. 1991, 200, 379–385. [CrossRef] [PubMed]

125. Zhang, X.-Z.; Zhang, Z.; Zhu, Z.; Sathitsuksanoh, N.; Yang, Y.; Zhang, Y.-H.P. The noncellulosomal family 48 cellobiohydrolase from Clostridium phytofermentans ISDg: Heterologous expression, characterization, and processivity. Appl. Microbiol. Biotechnol. 2010, 86, 525–533. [CrossRef] [PubMed]

126. Kruus, K.; Wang, W.K.; Ching, J.; Wu, J.H.D. Exoglucanase activities of the recombinant Clostridium thermocellum CelS, a major cellulosome component. J. Bacteriol. 1995, 177, 1641–1644. [CrossRef] [PubMed]

127. Liu, W.; Bevan, D.R.; Zhang, Y.-H.P. The family 1 glycoside hydrolase from Clostridium cellulolyticum H10 is a cellobextrin glucohydrolase. Appl. Biochem. Biotechnol. 2010, 161, 264–273. [CrossRef] [PubMed]

128. Vernooi, D.A.; McCarthy, J.K.; Eveleigh, D.E.; Bok, J.D. Cloning and characterization of the glucooligosaccharide catabolic pathway β-glucan glucohydrolase and cellobiose phosphorylase in the marine hyperthermophile Thermotoga neapolitana. J. Bacteriol. 2000, 182, 5172–5179. [CrossRef] [PubMed]

129. Kengen, S.W.M.; Luysink, E.J.; Stams, A.J.M.; Zehnder, A.J.B. Purification and characterization of an extremely thermostable β-glucosidase from the hyperthermophilic archaeon Pyrococcus furiosus. Eur. J. Biochem. 1993, 213, 305–312. [CrossRef] [PubMed]
130. Kim, Y.J.; Lee, J.E.; Lee, H.S.; Kwon, K.K.; Kang, S.G.; Lee, J. Novel substrate specificity of a thermostable β-glucosidase from the hyperthermophilic archaeon, *Thermococcus pacificus* P-4. *Korean J. Microbiol*. **2015**, *51*, 68–74. [CrossRef]

131. Matsui, I.; Sakai, Y.; Matsui, E.; Kikuchi, H.; Kawarabayasi, Y.; Honda, K. Novel substrate specificity of a membrane-bound beta-glycosidase from the hyperthermophilic archaeon *Pyrococcus horikoshii*. *FEBS Lett*. **2000**, *467*, 195–200. [CrossRef]

132. Wu, Y.; Yuan, S.; Chen, S.; Wu, D.; Chen, J.; Wu, J. Enhancing the production of galacto-oligosaccharides by mutagenesis of *Sulfolobus solfataricus* β-galactosidase. *Food Chem*. **2013**, *138*, 1588–1595. [CrossRef] [PubMed]

133. Sinha, S.K.; Datta, S. β-Glucosidase from the hyperthermophilic archaeon *Thermococcus* sp. is a salt-tolerant enzyme that is stabilized by its reaction product glucose. *Appl. Microbiol. Biotechnol*. **2016**, *100*, 8399–8409. [CrossRef] [PubMed]

134. Di Lauro, B.; Rossi, M.; Moracci, M. Characterization of a beta-glycosidase from the thermophilic bacterium *Alicyclacillus acidocaldarius*. *Extremophiles* **2006**, *10*, 301–310. [CrossRef] [PubMed]

135. Liu, Y.; Li, R.; Wang, J.; Zhang, X.; Jia, R.; Gao, Y.; Peng, H. Increased enzymatic hydrolysis of sugarcane bagasse by a novel glucose- and xylose-stimulated β-glucosidase from *Anoxybacillus flavithermus* subsp. *yunnanensis* E13T. *BMC Biochem*. **2017**, *18*, 4. [CrossRef] [PubMed]

136. Paavilainen, S.; Hellman, J.; Korpela, T. Purification, characterization, gene cloning, and sequencing of a new β-glucosidase from *Bacillus circulans* subsp. *alkalophilus*. *Appl. Environ. Microbiol*. **1993**, *59*, 927–932. [PubMed]

137. Xu, H.; Xiong, A.-S.; Zhao, W.; Tian, Y.-S.; Peng, R.-H.; Chen, J.-M.; Yao, Q.-H. Characterization of a glucose-, xylose-, sucrose-, and D-galactose-stimulated β-glucosidase from the alkalophilic bacterium *Bacillus halodurans* C-125. *Curr. Microbiol*. **2011**, *62*, 833–839. [CrossRef] [PubMed]

138. Love, D.R.; Fisher, R.; Bergquist, P.L. Sequence structure and expression of a cloned β-glucosidase from the extreme thermophile. *MGG Mol. Genet*. **1988**, *213*, 84–92. [CrossRef] [PubMed]

139. Kosugi, A.; Arai, T.; Doi, R.H. Degradation of cellulose-produced cello-oligosaccharides by an extracellular non-cellulosomal beta-glucan glucohydrolase, BglA, from *Clostridium cellulovorans*. *Biochem. Biophys. Res. Commun.* **2006**, *349*, 20–23. [CrossRef] [PubMed]

140. Zou, Z.-Z.; Yu, H.-L.; Li, C.-X.; Zhou, X.-W.; Hayashi, C.; Sun, J.; Liu, B.-H.; Imanaka, T.; Xu, J.-H. A new thermostable β-glucosidase mined from *Dictyoglomus thermophilum*: Properties and performance in octyl glucoside synthesis at high temperatures. *Bioresour. Technol*. **2012**, *118*, 425–430. [CrossRef] [PubMed]

141. Jabbour, D.; Klippel, B.; Antraniank, G. A novel thermostable and glucose-tolerant β-glucosidase from *Fervidobacterium islandicum*. *Appl. Microbiol. Biotechnol*. **2012**, *93*, 1947–1956. [CrossRef] [PubMed]

142. Gfen, G.; Anbar, M.; Morag, E.; Lamed, R.; Bayer, E.A. Enhanced cellulose degradation by targeted integration of a cohesin-fused β-glucosidase into the *Clostridium thermocellum* cellulosome. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10298–10303. [CrossRef] [PubMed]

143. Brognaro, H.; Almeida, V.M.; de Araujo, E.A.; Piyadov, V.; Santos, M.A.M.; Marana, S.R.; Polikarpov, I. Biochemical Characterization and Low-Resolution SAXS Molecular Envelope of GH1 β-Glycosidase from *Saccharophagus degradans*. *Mol. Biotechnol*. **2016**, *58*, 777–788. [CrossRef] [PubMed]

144. Marques, A.R.; Coutinho, P.M.; Videira, P.; Fialho, A.M.; Sá-Correia, I. *Sphingomonas paucimobilis* β-glucosidase BglI: A member of a new bacterial subfamily in glycoside hydrolase family 1. *Biochem. J.* **2003**, *370*, 793–804. [CrossRef] [PubMed]

145. Perez-Pons, J.A.; Cayetano, A.; Reborndoa, X.; Llereras, J.; Guasch, A.; Querol, E. A beta-glucosidase gene (bgl3) from *Streptomycetes* sp. strain QM-B814. Molecular cloning, nucleotide sequence, purification and characterization of the encoded enzyme, a new member of family 1 glycosyl hydrolases. *Eur. J. Biochem*. **1994**, *223*, 557–565. [CrossRef] [PubMed]

146. Breves, R.; Bronnenmeier, K.; Wild, N.; Lottspeich, F.; Staudenbauer, W.L.; Hofemeister, J. Genes encoding two different β-glucosidases of *Thermoanaerobacter brockii* are clustered in a common operon. *Appl. Environ. Microbiol*. **1997**, *63*, 3902–3910. [PubMed]

147. Song, X.; Xue, Y.; Wang, Q.; Wu, X. Comparison of three thermostable β-glucosidases for application in the hydrolysis of soybean isoflavone glycosides. *J. Agric. Food Chem*. **2011**, *59*, 1954–1961. [CrossRef] [PubMed]

148. Pei, J.; Song, Y.; Zhan, L.; Fan, S.; Shi, H. *Thermoanaerobacterium thermosaccharolyticum* β-glucosidase: A glucose-tolerant enzyme with high specific activity for cellubiose. *Biotechnol. Biofuels* **2012**, *5*, 31. [CrossRef] [PubMed]
149. Spiridonov, N.A.; Wilson, D.B. Cloning and biochemical characterization of BglC, a beta-glucosidase from the cellulolytic actinomycete Thermobifida fusca. Curr. Microbiol. 2001, 42, 295–301. [CrossRef] [PubMed]

150. Wright, R.M.; Yablonsky, M.D.; Shalita, Z.P.; Goyal, A.K.; Eveleigh, D.E. Cloning, characterization, and nucleotide sequence of a gene encoding Microbispora bispora BglB, a thermostable beta-glucosidase expressed in Escherichia coli. Appl. Environ. Microbiol. 1992, 58, 3455–3465. [PubMed]

151. Haq, I.U.; Khan, M.A.; Muneer, B.; Hussain, Z.; Azfal, S.; Majeed, S.; Rashid, N.; Javed, M.M.; Ahmad, I. Cloning, characterization and molecular docking of a highly thermostable β-1,4-glucosidase from Thermotoga petrophila. Biotechnol. Lett. 2012, 34, 1703–1709. [CrossRef] [PubMed]

152. Oh, E.-J.; Lee, Y.-J.; Chol, J.J.; Seo, M.S.; Lee, M.S.; Kim, G.A.; Kwon, S.-T. Mutational analysis of thermostable β-glucosidase from Thermus thermophilus KNOUC202: Gene and biochemical properties of the enzyme expressed in Escherichia coli. Appl. Biochem. Microbiol. 2010, 46, 515–524. [CrossRef]

153. Xiangyuan, H.; Shuzheng, Z.; Shoujun, Y. Cloning and expression of thermostable beta-glycosidase gene from Thermus nonprototylcyitus HG102 and characterization of recombinant enzyme. Appl. Biochem. Biotechnol. 2001, 94, 243–255. [CrossRef]

154. Kang, S.K.; Cho, K.K.; Ahn, J.K.; Bok, J.D.; Kang, S.H.; Woo, J.H.; Lee, H.G.; You, S.K.; Choi, Y.J. Three forms of thermostable lactose-hydrolase from Thermus sp. IB-21: Cloning, expression, and enzyme characterization. J. Biotechnol. 2005, 116, 337–346. [CrossRef] [PubMed]

155. Oh, E.; Lee, J.-A. Cloning and expression of Humicola grisea and application of an acidophilic and thermostable β-glucosidase involved in saponin metabolism from intestinal bacteria. J. Biochem. 1999, 125, 728–736. [CrossRef] [PubMed]

156. Zhao, Z.; Ramachandran, P.; Kim, T.-S.; Chen, Z.; Jeya, M.; Lee, J.-K. Characterization of an acid-tolerant β-1,4-glucosidase from Fusarium oxysporum and its potential as an animal feed additive. Microbiol. Biotechnol. 2013, 97, 10003–10011. [CrossRef] [PubMed]

157. Takashima, S.; Nakamura, A.; Hidaka, M.; Masaki, H.; Uozumi, T. Molecular Cloning and Expression of the Novel Fungal -Glucosidase Genes from Humicola grisea and Trichoderma reesei. J. Biochem. 2019, 125, 728–736. [CrossRef] [PubMed]

158. Li, X.-L.; Ljungdahl, L.G.; Ximenes, E.A.; Chen, H.; Felix, C.R.; Cotta, M.A.; Dien, B.S. Properties of a recombinant beta-glucosidase from polycentric anaerobic fungus Orpinomyces sp. PC-2 and its application for cellulose hydrolysis. Appl. Biochem. Biotechnol. 2004, 113, 233–250. [CrossRef]

159. Li, X.; Zhao, J.; Shi, P.; Yang, P.; Wang, Y.; Luo, H.; Yao, B. Molecular cloning and expression of a novel β-glucosidase gene from Philalopora sp. G5. Appl. Biochem. Biotechnol. 2013, 169, 941–949. [CrossRef] [PubMed]

160. Schröder, C.; Blank, S.; Antranikian, G. First Glycoside Hydrolase Family 2 Enzymes from Thermus antranikianii and Thermus brockianus with β-Glucosidase Activity. Front. Bioeng. Biotechnol. 2015, 3, 76. [CrossRef] [PubMed]

161. Li, D.; Li, X.; Dang, W.; Tran, P.L.; Park, S.-H.; Oh, B.-C.; Hong, W.-S.; Lee, J.-S.; Park, K.-H. Characterization and application of an acidophilic and thermostable β-glucosidase from Thermophilus pendens. J. Biosci. Bioeng. 2013, 115, 490–496. [CrossRef] [PubMed]

162. Yan, S.; Wei, P.; Chen, Q.; Chen, X.; Wang, S.; Li, J.; Gao, C. Functional and structural characterization of a β-glucosidase involved in saponin metabolism from intestinal bacteria. Biochem. Biophys. Res. Commun. 2018, 496, 1349–1356. [CrossRef] [PubMed]

163. Lau, A.T.Y.; Wong, W.K.R. Purification and characterization of a major secretory cellulobiase, Cba2, from Cellulomonas biazotica. Protein Expr. Purif. 2001, 23, 159–166. [CrossRef] [PubMed]

164. Gao, J.; Wakarchuk, W. Characterization of five β-glycoside hydrolases from Cellulomonas fimi ATCC 484. J. Bacteriol. 2014, 196, 4103–4110. [CrossRef] [PubMed]

165. Bronnenmeier, K.; Staudenbauer, W.L. Purification and properties of an extracellular β-glucosidase from the cellulolytic thermophile Clostridium stercorarium. Appl. Microbiol. Biotechnol. 1988, 28, 380–386. [CrossRef]

166. Li, Y.-K.; Lee, J.-A. Cloning and expression of β-glucosidase from Flavobacterium meningosepticum: A new member of family B β-glucosidases. Enzyme Microb. Technol. 1999, 24, 144–150. [CrossRef]

167. Xie, J.; Zhao, D.; Zhao, L.; Pei, J.; Xiao, W.; Ding, G.; Wang, Z. Overexpression and characterization of a Ca²⁺ activated thermostable β-glucosidase with high ginsenoside Rb1 to ginsenoside 20(S)-Rg3 bioconversion productivity. J. Ind. Microbiol. Biotechnol. 2015, 42, 839–850. [CrossRef] [PubMed]
168. Colabardini, A.C.; Valkonen, M.; Huuskonen, A.; Siika-aho, M.; Koivula, A.; Goldman, G.H.; Saloheimo, M. Expression of Two Novel β-Glucosidases from Chaetomium atrobrunneum in Trichoderma reesei and Characterization of the Heterologous Protein Products. Mol. Biotechnol. 2016, 58, 821–831. [CrossRef] [PubMed]

169. Liu, D.; Zhang, R.; Yang, X.; Zhang, Z.; Song, S.; Miao, Y.; Shen, Q. Characterization of a thermostable β-glucosidase from Aspergillus fumigatus ZS and its functional expression in Pichia pastoris X33. Microb. Cell Fact. 2012, 11, 25. [CrossRef] [PubMed]

170. Xu, R.; Teng, F.; Zhang, C.; Li, D. Cloning of a Gene Encoding β-Glucosidase from Chaetomium thermophilum CT2 and Its Expression in Pichia pastoris. J. Mol. Microbiol. Biotechnol. 2012, 11, 25. [CrossRef] [PubMed]

171. Dotsenko, G.S.; Sinitsyna, O.A.; Hinz, S.W.A.; Wery, J.; Sinitsyn, A.P. Characterization of a GH family 3 β-glycoside hydrolase from Chrysosporium lucknowense and its application to the hydrolysis of β-glucan and xylan. Bioresour. Technol. 2012, 112, 345–349. [CrossRef] [PubMed]

172. Pei, X.; Zhao, J.; Cai, P.; Sun, W.; Ren, J.; Wu, Q.; Zhang, S.; Tian, C. Heterologous expression of a GH3 β-glucosidase from Neurospora crassa in Pichia pastoris with high purity and its application in the hydrolysis of soybean isoflavone glycosides. Protein Expr. Purif. 2016, 119, 75–84. [CrossRef] [PubMed]

173. Kato, Y.; Nomura, T.; Ogita, S.; Takano, M.; Hoshino, K. Two new β-glucosidases from ethanol-fermenting fungus Mucor circinelloides NBRC 4572: Enzyme purification, functional characterization, and molecular cloning of the gene. Appl. Microbiol. Biotechnol. 2013, 97, 10045–10056. [CrossRef] [PubMed]

174. Yang, X.; Ma, R.; Shi, P.; Huang, H.; Bai, Y.; Wang, Y.; Yang, P.; Fan, Y.; Yao, B. Molecular characterization of a highly-active thermophilic β-glucosidase from Neosartorya fischeri P1 and its application in the hydrolysis of soybean isoflavone glycosides. PLoS ONE 2014, 9, e106785. [CrossRef] [PubMed]

175. Krogh, K.B.R.M.; Harris, P.V.; Olsen, C.L.; Johansen, K.S.; Hojer-Pedersen, J.; Borjesson, J.; Olsson, L. Characterization and kinetic analysis of a thermostable GH3 β-glucosidase from Penicillium brasilianum. Appl. Microbiol. Biotechnol. 2010, 86, 143–154. [CrossRef] [PubMed]

176. Chen, M.; Qin, Y.; Liu, Z.; Liu, K.; Wang, F.; Qu, Y. Isolation and characterization of a β-glucosidase from Penicillium decumbens and improving hydrolysis of corncob residue by using it as cellulase supplementation. Enzyme Microb. Technol. 2010, 46, 444–449. [CrossRef] [PubMed]

177. Machida, M.; Ohtsuki, I.; Fukui, S.; Yamashita, I. Nucleotide sequences of Saccharomycopsis fibuligera genes for extracellular β-glucosidases as expressed in Saccharomyces cerevisiae. Appl. Environ. Microbiol. 1988, 54, 3147–3155. [PubMed]

178. Murray, P.; Aro, N.; Collins, C.; Grassick, A.; Penttilä, M.; Saloheimo, M.; Tuohy, M. Expression in Trichoderma reesei and characterisation of a thermostable family 3 beta-glucosidase from the moderately thermophilic fungus Talaromyces emersonii. Protein Expr. Purif. 2004, 38, 248–257. [CrossRef] [PubMed]

179. Xia, W.; Xu, X.; Qian, L.; Shi, P.; Bai, Y.; Luo, H.; Ma, R.; Yao, B. Engineering a highly active thermophilic β-glucosidase to enhance its pH stability and saccharification performance. Biotechnol. Biofuels 2016, 9, 147. [CrossRef] [PubMed]

180. Hong, J.; Tamaki, H.; Kumagai, H. Cloning and functional expression of thermostable beta-glucosidase gene from Thermus aurantius. Appl. Microbiol. Biotechnol. 2007, 73, 1331–1339. [CrossRef] [PubMed]

181. Karnaouri, A.; Topakas, E.; Paschos, T.; Taouki, I.; Christakopoulos, P. Cloning, expression and characterization of an ethanol tolerant GH3 β-glucosidase from Myceliophthora thermophila. PeerJ 2013, 1, e46. [CrossRef] [PubMed]

182. Brunner, F.; Wirtz, W.; Rose, J.K.C.; Darvill, A.G.; Govers, F.; Scheel, D.; Nürnberger, T. A β-glucosidase/xylanase from the phytopathogenic oomycete, Phytophthora infestans. Phytochemistry 2002, 59, 689–696. [CrossRef]
183. Ferrara, M.C.; Cobucci-Ponzano, B.; Carpentieri, A.; Henrissat, B.; Rossi, M.; Amoresano, A.; Moracci, M. The identification and molecular characterization of the first archaeal bifunctional exo-β-glucosidase/N-acetyl-β-glucosaminidase demonstrate that family GH116 is made of three functionally distinct subfamilies. *Biochim. Biophys. Acta* **2014**, *1840*, 367–377. [CrossRef] [PubMed]

184. Sansenya, S.; Mutoh, R.; Charoenwattanasatien, R.; Kurisu, G.; Ketudat Cairns, J.R. Expression and crystallization of a bacterial glycoside hydrolase family 116 β-glucosidase from *Thermoanaerobacterium xylanolyticum*. *Acta Crystallogr. Sect. F Struct. Biol. Commun.* **2015**, *71*, 41–44. [CrossRef] [PubMed]

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