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Authors

Aulitto, Martina

Fusco, Salvatore

Limauro, Danila

et al.

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REVIEW

Galactomannan degradation by thermophilic enzymes: a hot topic for biotechnological applications

Martina Aulitto[1] · Salvatore Fusco[1] · Danila Limauro[1] · Gabriella Fiorentino[1] · Simonetta Bartolucci[1] · Patrizia Contursi[1]

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Abstract

Extremophilic microorganisms are valuable sources of enzymes for various industrial applications. In fact, given their optimal catalytic activity and operational stability under harsh physical and chemical conditions, they represent a suitable alternative to their mesophilic counterparts. For instance, extremophilic enzymes are important to foster the switch from fossil-based to lignocellulose-based industrial processes. Indeed, more stable enzymes are needed, because the conversion of the lignocellulosic biomass to a wide palette of value-added products requires extreme chemo-physical pre-treatments. Galactomannans are part of the hemicellulose fraction in lignocellulosic biomass. They are heteropolymers constituted by a β-1,4-linked mannan backbone substituted with side chains of α-1,6-linked galactose residues. Therefore, the joint action of different hydrolytic enzymes (i.e. β-mannanase, β-mannosidase and α-galactosidase) is needed to accomplish their complete hydrolysis. So far, numerous galactomannan-degrading enzymes have been isolated and characterized from extremophilic microorganisms. Besides applications in biorefinery, these biocatalysts are also useful to improve the quality (i.e. digestibility and prebiotic properties) of food and feed as well as in paper industries to aid the pulp bleaching process. In this review, an overview about the structure, function and applications of galactomannans is provided. Moreover, a survey of (hyper)-thermophilic galactomannans-degrading enzymes, mainly characterized in the last decade, has been carried out. These extremozymes are described in the light of their biotechnological application in industrial processes requiring harsh conditions.

Martina Aulitto and Salvatore Fusco have contributed equally to this work.

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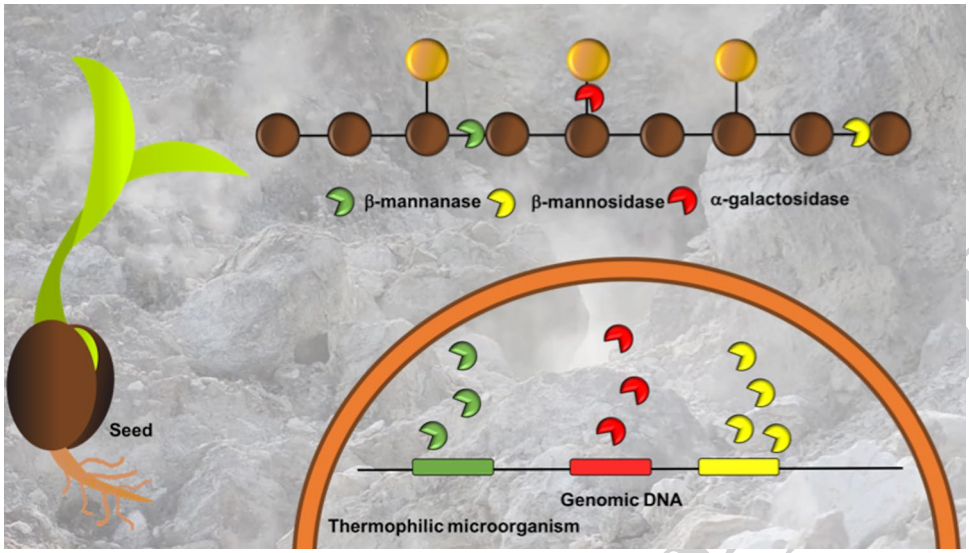
Graphical abstract



Keywords Thermophiles · Galactomannans · Galactomannan-degrading enzymes · Beta-mannanase · Beta-mannosidase · Alpha-galactosidase

Introduction

AQ1 Hemicellulose is the second most abundant biopolymer on Earth after cellulose and is a branched polysaccharide consisting of shorter chains of 500–3000 sugar units. Mannans are one of the major groups of hemicellulose present in the plant tissues and seeds, especially of *Gymnospermae*, where they exert structural, nutritional as well as signalling roles (Dhawan and Kaur 2007). Mannose-containing polysaccharides are generally classified in mannans, glucomannans, galactomannans and galactoglucomannans, based on the sugar composition (Pauly et al. 2013). In particular, galactomannans consist of a linear backbone of (1→4)-*β*-D-mannopyranosyl residues decorated with galactose units linked by *α*-1,6-glycosidic bonds. Therefore, their complete hydrolysis requires the concerted action of both main- and side-chain hydrolytic enzymes that include *β*-mannanases (EC 3.2.1.78), *β*-mannosidases (EC 3.2.1.25) and *α*-galactosidases (EC 3.2.1.22) (Moreira and Filho 2008).

In nature, galactomannan-degrading enzymes are essential in many biological processes, such as for growth and development of plant tissues as well as for fruit ripening (Moreira and Filho 2008). Moreover, wood-decomposing microbial communities have evolved a wide arsenal of these enzymes, which are very efficient in degrading lignocellulosic material (Cragg et al. 2015). In recent years, the need to alleviate the anthropic impact on the delicate Earth ecosystem has fostered the switch from chemical-based industrial processes towards eco-friendlier bio-based setups (Kircher 2015). In this context, microbial galactomannan-degrading enzymes have found large applicability in food related processes, such as clarification of fruit juices (Vijayalaxmi et al. 2013), viscosity reduction of instant coffee and production of Konjac (Dhawan and Kaur 2007) as well as of prebiotic mannooligosaccharides (MOS) from cheap agricultural by-products (Zang et al. 2015). Moreover, these enzymes are used in the pulp/paper and detergent industries (Dhawan and Kaur 2007) and are useful tools for the sequencing of hetero-polysaccharides and carbohydrate moieties in glycoproteins (Gomes et al. 2007).

Water solubility of galactomannans is highly variable and depends upon the degree of galactose decoration (Prajapati et al. 2013). For this reason, in some of the above-mentioned industrial processes high temperature and extreme pH are applied in order to improve galactomannans solubility. Moreover, performing bioprocesses at high temperatures is advantageous to promote a better enzyme penetration into the complex polymeric substrate as well as to prevent microbial contamination in food-related processes (Nigam 2013). In this regard, (hyper)-thermophilic microorganisms represent suitable sources of robust galactomannan-degrading enzymes that can be used for the development of efficient bio-based industrial processes (Bartolucci et al. 2013; Horikoshi et al. 2010). A typical example of bioprocess is the second-generation biorefinery, in which lignocellulosic

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biomass is hydrolysed to fermentable sugars that are subsequently converted to biofuels and/or valued-added chemicals (Somerville et al. 2010). In this case, after thermochemical pretreatment of the biomass, the resulting lignocellulosic slurry must be cooled down before commercial enzymes are added for the saccharification. On the other hand, the use of thermostable galactomannan-degrading enzymes allows adding them earlier in the process to perform biomass pre-digestion, thus leading to save time and improve conversion efficiency compared to currently used enzyme cocktails (Brunecky et al. 2014). In this review, we survey the (hyper)-thermophilic galactomannan-degrading enzymes with a focus on those characterized in the last decade and discuss their biotechnological applications; for thermozymes isolated earlier the readers are referred to excellent reviews (Dhawan and Kaur 2007; Horikoshi et al. 2010).

Structure and function of galactomannans

Hemicellulose includes polymers of pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose) as well as of sugars in their acidified forms (i.e., glucuronic acid and galacturonic acid). One of the major groups of hemicellulose are mannans, which are widely distributed in the endosperm seeds and plant tissues (e.g. bulbs or tubers), where they exploit different roles, such as: (i) improving the structural resistance of the cell wall by binding the cellulose; (ii) mediating the storage of non-starch carbohydrates and (iii) functioning as important signalling molecules during the plant growth and development (Dhawan and Kaur 2007).

Galactomannans are polymers composed by a linear backbone of mannose decorated with galactose residues. Whereas mannose provides cis-OH groups that mediate hydrogen bonds formation between the polymannan chains, galactose sterically prevents polymannan chains interaction. Therefore, the galactose:mannose ratio (M:G) influences the water-solubility of galactomannans and ranges from 1:1 to 5:1. For instance, galactomannans in the *Fenugreek gum* seeds (M:G ratio is 1:1) are the most soluble ones in nature. This structural feature of galactomannans is important for their use as stabilizers in those industrial applications requiring high viscosity of the water phase (Prajapati et al. 2013). The most commonly used galactomannans in food and non-food related industries are guar gum (*C. tetragonolobo*, M:G ratio of 2:1), tara gum (*C. spinosa*, M:G ratio of 3:1) and locust bean gum (*C. siliqua*, M:G ratio of 3.5:1). For instance, they are added to ice cream preparations to improve their texture and reduce meltdown. More recently, these galactomannans are also employed in combination with other polysaccharides (i.e. xanthan gum and agar) to form gels with new properties (Dhawan and Kaur 2007; Moreira and Filho 2008; Prajapati et al. 2013).

Since in several industrial applications galactomannans have to be partially or completely hydrolysed, the isolation and characterization of galactomannans-degrading enzymes is a hot-topic. Therefore, an overview of (hyper)-thermophilic α-galactosidases, β-mannanase and β-mannosidase is provided below.

α-Galactosidases

α-galactosidases (α-D-galactoside galactohydrolases; EC 3.2.1.22), also known as melibiases, catalyse the hydrolysis of terminal non-reducing residues of α-galactose from oligosaccharides, polysaccharides, galactolipids and glycoproteins (Moreira and Filho 2008). Based on homology and catalytic features, α-galactosidases have been classified into the glycoside hydrolase (GH) families 4, 27, 36, 57, 97 and 110 in the CAZy database (http://www.cazy.org). Those belonging to the families GH27 and GH36 share a common catalytic mechanism and structural topology with family GH31 α-xylosidases and α-glucosidases; therefore, they have been pooled together in the GH-D clan (Aulitto et al. 2017b). The majority of GH27 and GH36 α-galactosidases show a conserved $(\beta/\alpha)_8$ barrel domain and two aspartate residues that are involved in the catalytic mechanism. One of these catalytic residues is embedded in a conserved consensus motif ([LIVMFY]-x(2)-[LIVMFY]-x-[LIVM]-D-[DS]-x-[WY]), which is either localized at the central region of bacterial enzymes (GH36) or at the amino-terminal region of eukaryotic variants (GH27) (Fig. 1, D327). The other aspartate residue is included in a conserved motif (RXXXD) (Fig. 1, D387), which is present only in enzymes isolated from *Thermus* sp. and *Thermotoga* sp. that constitute the sub-group GH36bt (where "bt" stands for bacterial thermophilic) (Brouns et al. 2006; Comfort et al. 2007). The hydrolysis of the substrate proceeds with the retention of stereochemistry at the anomeric centre of the substrate through a double displacement mechanism (Merceron et al. 2012), which is mediated by the two aspartate residues acting as a nucleophile (D327) and a proton donor (D387). Generally, GH27 α-galactosidases are active on both polymeric and oligomeric substrates, whereas those belonging to the family GH36 hydrolase mainly oligomeric substrates.

Thermophilic α-galactosidases from bacteria and fungi are attractive candidates due to their efficient catalytic activity and high stability under harsh conditions (Sarmiento et al. 2015). Recently, several thermophilic enzymes have been discovered and characterized, such as the bacterial α-galactosidases from *Neosarotrya fischeri* P1 (Wang et al. 2014), *Bacillus megaterium VHM1* (Patil et al. 2010), *Bacillus coagulans* (Zhao et al. 2018) as well as the fungal α-galactosidases from *Lenzites elegans* (Sampietro et al. 2012), *Talaromyces leycettanus* JCM12802 (Wang et al.

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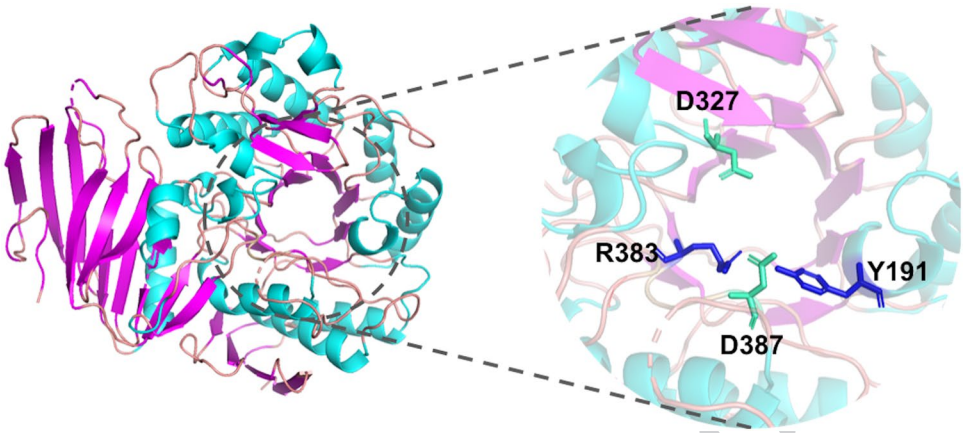

Fig. 1 Structure of α-galactosidase *Tm*GalA from *Thermotoga maritima* strain MSB8 (PBD code 1ZY9). α-Helices and β-strands are reported in cyan and magenta, respectively. The central region of the conserved $(\beta/\alpha)_8$ barrel domain is zoomed out (on the right) to show the two catalytic aspartic residues (D327 and D387) as well as two substrate-interacting residues in light green and dark blue (Y191 and R383), respectively

2016), *Pseudobalsamia microspore* (Yang et al. 2015a) and *Rhizopus* sp. F78 (Cao et al. 2007) (Table 1). Depending on their origin, α-galactosidases differ with respect to their pH optima, thermostability and thermoresistance (Lee et al. 2017; Schroder et al. 2017; Zhao et al. 2008). Generally, fungal and bacterial α-galactosidases perform better at acidic (from 3.5 to 5.0) and neutral pH values (from 6.0 to 7.5), respectively. So far, the most thermoactive α-galactosidase has been isolated from *Thermotoga neapolitana* 5068 and shows an optimal temperature of 100–103 °C (Duffaud et al. 1997), whereas the most thermostable one (*Tt*GalA) has been found in *Thermus thermophilus* (half-life of 30 h at 70 °C) (Table 1). *Tt*GalA is a hexamer in solution as other α-galactosidases belonging to GH36 (Aulitto et al. 2017b). Interestingly, the multimeric structure of these enzymes has been linked to their better thermostability if compared to monomeric and dimeric α-galactosidases (Gote et al. 2006).

Thermostable α-galactosidases are useful for several biotechnological applications, among which protease-resistant ones are particularly suitable to improve the nutritional values of food and feed; in particular, they can be supplemented to animal feed, together with proteases, to eliminate indigestible oligosaccharides (Ghazi et al. 2003). So far, only two thermophilic protease-resistant α-galactosidases have been isolated, in particular: (i) one produced by the thermophilic fungus *Rhizomucor miehei* (*Rm*Gal36) that was reported to be resistant and even slightly activated in the presence of proteases (Katrolia et al. 2012) and (ii) the enzyme Aga-BC7050, from the bacterium *Bacillus coagulans*, that exhibited excellent protease tolerance and negligible product-inhibition by low-molecular weight sugars (Zhao et al. 2018) (Table 1).

Besides their hydrolytic activity, α-galactosidases are also powerful tools for the synthesis of oligosaccharides

Table 1 Overview of the recent characterized thermophilic α-galactosidases

Source organism	Enzyme	GH family	T_{opt} (°C)	pH_{opt}	Thermostability (half-life)	MW (kDa)	Reference
Bacillus coagulans	*Aga-BC7050*	36	55	6.0	60 °C for 30 min	85	Zhao et al. (2018)
Bacillus megaterium VHM1	N.R.	36	55	7.0	55 °C for 120 min	N.R.	Patil et al. (2010)
Bifidobacterium breve 203	Aga2	36	50	5.5	N.R.	80.5	Zhao et al. (2008)
Caldicellulosiruptor bescii	*CbAga36*	36b	70	5.0	15 h at 70°C 10h at 80 °C	84	Lee et al. (2017)
Dictyoglomus thermophilum	*Agal1*	36b	80	6.5	1 h at 70 °C	84.5	Schroder et al. (2017)
Lenzites elegans	N.R.	36	60–80	4.5	60 °C for 2 h	158	Sampietro et al. (2012)
Meiothermus ruber	*Agal2*	36bt	60	6.5	4 h at 70 °C	N.R.	Schroder et al. (2017)
Neosartorya fischeri P1	Gal27A	27	60–70	4.5	N.R.	49.2	Wang et al. (2014)
Pseudobalsamia microspora	PMG	27	55	5.0	N.R.	62	Yang et al. (2015a, b)
Rhizopus sp. F78	Aga-F78	36	50	4.8	N.R.	82	Cao et al. (2007)
Rhizomucor miehei	RmGal36	36	60	4.5	55 °C for 30 min	85	Katrolia et al. (2012)
Talaromyces leycettanus JCM12802	rAga27A	27	70	4.0	70 °C/65 °C for 1 h	55	Wang et al. (2016)
Thermus thermophilus HB27	*Tt*GalA	36bt	90	6.0	30 h at 70 °C	55	Aulitto et al. (2017b)

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via transglycosylation, i.e. the ability to transfer the galactosyl moiety to an acceptor molecule and to form α-1,6 or α-1,3 linkages. Examples of thermophilic enzymes are the α-galactosidases from *Bacillus strearothermophilus* and *Thermus brockianus* (Horikoshi et al. 2010). Although the transglycosylation properties of α-galactosidases have been well studied, the chemical structure of the synthetized products remains largely unexplored.

β-Mannanases

β-Mannanases (1,4-β-D-mannan mannanohydrolase; EC 3.2.1.78), also referred to as mannan endo-1,4-beta-mannosidases, are enzymes that carry out the random hydrolysis of β-1,4-mannosidic linkages in mannans, glucomannans and galactomannans. This reaction consists of an acid–base-assisted catalysis via a retaining double displacement mechanism, which involves a covalent glycosyl-enzyme intermediate (Merceron et al. 2012). Interestingly, this kind of reaction mechanism allows some β-mannanases to catalyse also transglycosylation reactions, which can be useful for the synthesis of MOS (Ghosh et al. 2013). Despite these oligosaccharides are indigestible for the human gut, many studies have proved their potential role as dietary fibres and prebiotics, which foster the proliferation of intestinal beneficial bugs (Zang et al. 2015).

So far, the majority of the characterized β-mannanases are grouped into GH families 5, 26 and 113 on the basis of amino acid sequences and structural similarities among their catalytic domains (Cheng et al. 2016; Songsiriritthigul et al. 2010; Summpunn et al. 2011). Enzymes belonging to these GH families share a common $(\beta/\alpha)_8$ barrel-shaped protein architecture (Fig. 2). Catalysis is mediated by glutamate residues located on β-strand 4 (Fig. 2, D134, the nucleophile) and on β-strand 7 (Fig. 2, D273, the acid/base proton donor) (Kumagai et al. 2011). Whereas bacterial β-mannanases mainly belong to the GH5 and GH113 families (Fusco et al. 2018; Zang et al. 2015), the more acid-tolerant and catalytic efficient fungal β-mannanases are grouped into the GH5 and GH26 families (Do et al. 2009; Hakamada et al. 2014; Harnpicharnchai et al. 2016; Katsimpouras et al. 2016; Liao et al. 2014; Naganagouda et al. 2009; Wang et al. 2015; Yu et al. 2015) (Table 2). More recently, the identification of Man134A, produced by the filamentous fungus *Aspergillus nidulans*, led to the establishment of the new family of GH134 in the CAZy database (Shimizu et al. 2015).

β-Mannanases are useful for many industrial applications, including: (i) the reduction of the antinutritional effect of mannan polymers found in corn and soy beans used for poultry feed (Ghazi et al. 2003), (ii) the clarification of fruit juices and wines (Vijayalaxmi et al. 2013) or viscosity reduction of instant coffee (Luo et al. 2012) as well as (iii) in the pulp/paper and detergent industries (Katrolia et al. 2013). In these two latter cases, thermostable and broad pH-tolerant enzymes are of particularly interest, given the high temperature and alkaline pH conditions applied. In particular, this has fostered the isolation and characterization of alkali-tolerant thermostable enzymes, like the cases of *Rm*Man5A from *Rhizomucor miehei* (Katrolia et al. 2013), Man5A from *Humicola insolens* Y1 (Luo et al. 2012) and Mn428 from *Streptomyces sp*. CS428 (Pradeep et al. 2016)

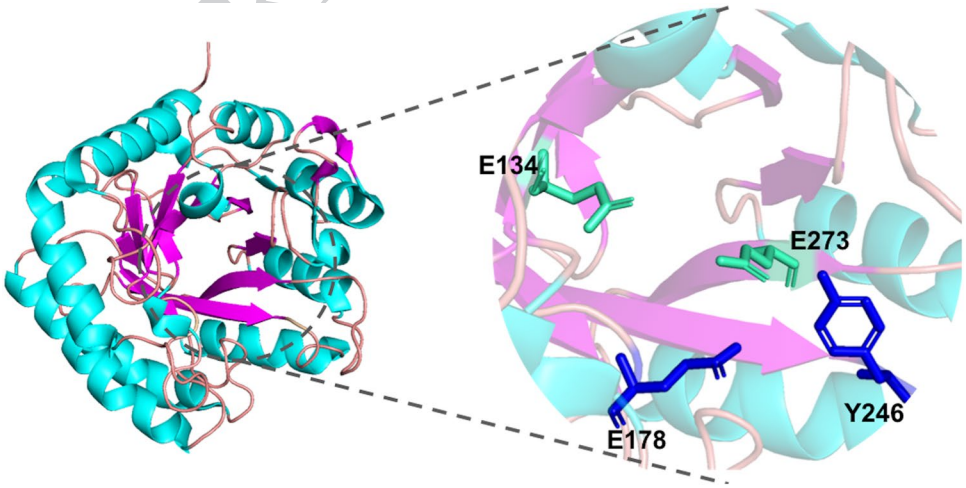

Fig. 2 Monomer structure of the dimeric β-mannanase *St*Man from *Streptomyces thermolilacinus* strain NBRC14274 (PDB code 2QHA). α-Helices and β-strands are reported in cyan and magenta, respectively. The active site of the enzyme is zoomed out (on the right) to show the two catalytic glutamic residues E134 (the nucleophile) and E273 (the proton donor), which both belong to the central β-barrel. Moreover, two substrate-interacting residues are reported in blue (E178 and Y246). These amino acids are localized at an edge of the β-barrel and modulate the substrate accessibility to the active site

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Table 2 Overview of the recent characterized thermophilic β-mannanase

Source organism	Enzyme name	GH family	T_{opt}	pH_{opt}	Thermostability (half-life)	MW (kDa)	Reference
Aspergillus niger BCC4525	MANF3	5	70 °C	5.5	N.R.	40	Harnpicharnchai et al. (2016)
Aspergillus niger BK01	B6V876_ASPNG[a]	5	80 °C	4.5	56 h at 70 °C 15 min at 80 °C 2.5 min at 90 °C	53	BC et al. (2009)
Aspergillus niger CBS 513.88	MAN-P	5	80 °C	4.5	15 min at 85 °C	45	Yu et al. (2015)
Aspergillus niger gr	N.R.	N.R.	55 °C	5.5	6 h at 55 °C	66	Naganagouda et al. (2009)
Bacillus halodurans PPKS-2	N.R.	N.R.	70 °C	11	N.R.	22	Vijayalaxmi et al. (2013)
Bacillus licheniformis	ManB	26	50 °C	6.0	80 h at 50 °C 3 min at 60 °C	41	Songsiriritthigul et al. (2010)
Bacillus pumilus GBSW19	*Bp*Man5	5	65 °C	6.5	12 h at 60 °C	45	Zang et al. (2015)
Bacillus subtilis BCC41051	ManA	N.R.	70 °C	7.0	N.R.	38	Summpunn et al. (2011)
Bacillus subtilis BE-91	N.R.	N.R.	65 °C	6.0	30 min at 70/75 °C	28	Lifeng Cheng et al. (2016)
Bacillus subtilis CSB39	*Mn*CSB39	N.R.	70 °C	7.5	30 min at 90 °C	30	Sudip Regmi et al. (2016)
Bacillus subtilis TBS2	ReTMan26	26	60 °C	6.0	6 min at 60 °C 4.2 min at 70 °C 2 min at 80 °C 20 min at 90 °C 12 min at 100 °C	42	Zhangcai Luo et al. (2017)
Clostridium thermocellum ATCC27405	*Ct*Man	26	60 °C	6.9	N.R.	53	Ghosh et al. (2013)
Dictyoglomus thermophilum CGMCC 7283	*Dt*ManB	N.R.	80 °C	6.0	46 h at 80 °C	54	Ke Hu et al. (2014)
Dictyoglomus turgidum	*Dtur*CelB	5	70 °C	5.4	2 h at 70 °C	40	Fusco et al. (2018)
Humicola insolens Y1	Man5A	5	70 °C	5.5	15 min at 60 °C	47	Huiying Luo et al. (2012)
Myceliophthora thermophila	*Mt*Man26A	26	60 °C	6.0	14.4 h at 60 °C	60	Katsimpouras et al. (2016)
Neosartorya fischeri P1	Man5P1	5	80 °C	4.0	10 min at 70 °C	40	Yang et al. (2015)
Penicillium oxalicum GZ-2	*Po*Man5A	5	80 °C	4.0	58 h at 60 °C	62	Hanpeng Liao et al. (2014)
Reinekea sp. KIT-YO10	Rman	N.R.	70 °C	8.0	N.R.	44	Hakamada et al. (2014)
Rhizomucor miehei	*Rm*Man5A	5	55 °C	7.0	30 min at 70 °C	43	Priti Katrolia et al. (2013)
Streptomyces sp. CS428	Mn428	N.R.	60 °C	12.5	1 h at 80 °C	35	Pradeep et al. (2016)
Streptomyces thermolilacinus NBRC14274	*St*Man	5	55 °C	6.0	30 min at 61 °C (Ca^{2+}) 30 min at 46 °C (EDTA)	37	Kumagai et al. (2011)
Talaromyces leycettanus JCM12802	Man5A1	5	90 °C	4.5	30 min at 80 °C	72	Wang et al. (2015)
Talaromyces leycettanus JCM12802	Man5A2	5	85–90 °C	4.0	1 h at 70 °C	60	Wang et al. (2015)
Thermobifida fusca BCRC19214	N.R.	N.R.	80 °C	8.0	N.R.	49	Cheng et al. (2016)
Thermobifida fusca NBRC14071	*Tf*Man	5	75 °C	6.0	30 min at 78 °C (Ca^{2+}) 30 min at 72 °C (EDTA)	37	Kumagai et al. (2011)

N.R. not reported

[a]UniProt code

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(Table 2). For instance, *Rm*Man5A is particularly suited for applications in the detergent industry because it is remarkably tolerant towards sodium dodecyl sulfate (SDS), which has been shown to inhibit the activity of many other β-mannanases (Jiang et al. 2006; Luo et al. 2012, 2009). Moreover, for some industrial applications (e.g. in the kraft/pulp industry), another important feature is the resistance of the enzymes to neutral and alkaline proteases. One example is Man5A that has been shown to retain more than 97% of its catalytic activity after 60 or 30 min of proteolytic treatment with trypsin, α-chymotrypsin, collagenase, subtilisin A, and proteinase K (Luo et al. 2012). Worth mentioning are also the multi-stress tolerant enzymes isolated from *Neosartorya fischeri* P1 (Man5P1) and *Bacillus subtilis* CSB39 (*Mn*CSB39), which are resistant to the presence of SDS, Ag^+ ions, surfactants, NaCl and urea, as well as to the action of proteases (Regmi et al. 2016; Yang et al. 2015b). Altogether, the above-mentioned features make these enzymes very interesting candidates for various industrial applications.

β-Mannosidases

β-Mannosidases (β-D-mannopyranoside hydrolases, EC 3.2.1.25) are exo-acting enzymes that attack the non-reducing end of β-linked MOS or mannobiose to release mannose units (Malgas et al. 2015); therefore, they are essential to complete the hydrolysis of mannans to monomeric sugars. In the CAZy database, the majority of β-mannosidases are classified as GH2 or GH5, with the exception of the mannosidase produced by *Pyrococcus furiosus* (Pfuβm) which belongs to the GH1 family (Bauer et al. 1996). Being more conserved at structural than sequence level, β-mannosidases are grouped into the GH-A clan according to their three-dimensional structure (Chauhan and Gupta 2017). These enzymes share a modular architecture with five conserved distinct domains among which those structured in a β-sheet fold (domains 1, 2 and 4) are reminiscent of carbohydrate binding modules (Fig. 3). Domain 5 plays a role in the orientation of active site and in the interaction between different monomers of the multimeric enzyme (Chauhan and Gupta 2017; Tailford et al. 2007), whereas domain 3 contains the catalytic centre with a typical $(\beta/\alpha)_8$ catalytic barrel fold (Fig. 3). Moreover, two carboxylic acid residues, one of which functions as the acid-base (Fig. 3, E462 on β-strand 4) and the other as the nucleophile (Fig. 3, E555 on β-strand 7), represent a common feature among all the β-mannosidases (Blanchard and Withers 2001). The fact that mannosidases can display affinity for smaller or longer oligosaccharides, resides in the structural differences (i.e. length and shape) of lid loops that modify the accessibility of the longer substrates to the active site (Dias et al. 2004). Moreover, different quaternary architectures have been observed, ranging from monomeric to octameric (Chauhan and Gupta 2017). Although β-mannosidases have been reported to occur in many bacteria, yeasts, fungi, marine algae, germinating seeds, invertebrates and vertebrates (Dhawan and Kaur 2007) there are only few reports about the purification and characterization of microbial β-mannosidases. This is due to their low representation in the secretome of hemicellulolytic microorganisms, which makes their purification rather difficult (Béki et al. 2003). This problem could be solved by cloning and heterologous expression of mannosidase-encoding genes.

Most of the moderate thermophilic mannosidases characterized so far have been isolated from aerobic lignocellulose-degrading eubacteria, actinomycetes and fungi (Chauhan and Gupta 2017; Horikoshi et al. 2010). Instead, hyperthermophilic mannosidases have been isolated only from

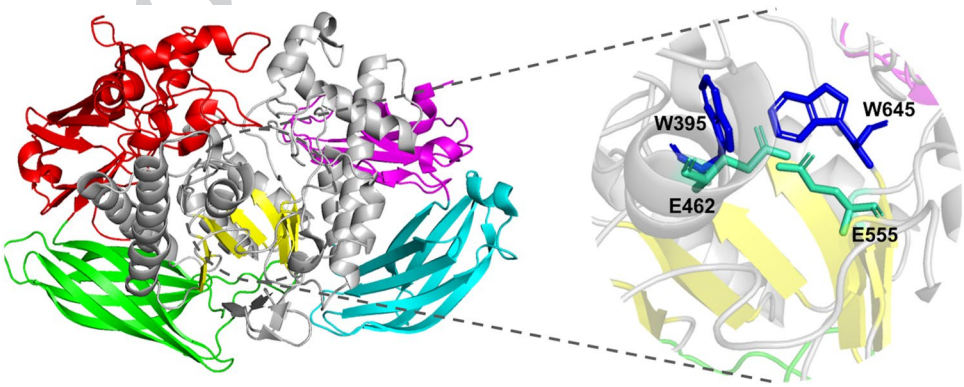

Fig. 3 Structure of the β-mannosidase *Bt*Man2A from *Bacteroides thetaiotaomicron* (PDB code 2JE8). The domain 1, 2, 3, 4 and 5 are reported in red, green, grey, cyan and magenta, respectively. The β-barrel of the central domain 3 is highlighted in yellow and is zoomed out (on the right) to show the two catalytic glutamic residues E462 (the proton donor) and E555 (the nucleophile). Moreover, two substrate-interacting tryptophan residues are reported in blue (W395 and W645). W395 is predicted to hydrogen bond to O-3 of the substrate and contribute to the topology of the active site, whereas W645 presumably binds the substrate via hydrophobic contacts with the mannosyl residue at the −1 subsite in respect to the cleavage site

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bacterial and archaeal microorganisms (Fliedrová et al. 2012; Shi et al. 2013, 2011; Zhang et al. 2009). The most thermophilic and thermostable enzyme has been isolated from the archaeon *Pyrococcus furiosus* and is optimally active at 105 °C with a half-life of 77 min at 110 °C, thus expanding considerably the upper temperature limit of the catalytic activity of β-mannosidases (Bauer et al. 1996). Other hyperthermophilic enzymes are those produced by *Thermotoga maritima* (T_{opt} 95 °C) (Zhang et al. 2009), *Thermotoga neapolitana* (T_{opt} 90 °C) (Parker et al. 2001) and *Pyrococcus horikoshii* (T_{opt} 90 °C) (Bauer et al. 1996). Within thermophilic and hyperthermophilic β-mannosidases pH optima vary from acidic (pH 4.0) to neutral/mild alkaline values (pH 7.4) and interestingly most of them are stable over a wide pH range (Horikoshi et al. 2010). As for other galactomannan-degrading enzymes, the physio-chemical features of thermophilic β-mannosidases make these enzymes particularly attractive for all the above-mentioned biotechnological applications (Table 3).

Enabling the production and use of thermophilic galactomannan-degrading enzymes at industrial level

The isolation and characterization of new thermophilic enzymes is important to better understand the physio-chemical principles behind their intrinsic stability. However, this is not enough to enable their effective use at industrial level; indeed, sustainable production strategies have to be established to reach this goal. Suitable production models of galactomannan-degrading enzyme are either single bacteria or microbial consortia endowed with a set of enzymes for the complete hydrolysis of galactomannans. One of the earlier reports is represented by *Thermotoga neapolitana* 5068, which was found to produce an extracellular β-mannanase as well as intracellular β-mannosidase and α-galactosidase when cultivated in a medium supplemented with guar gum (Duffaud et al. 1997). The intracellular localization of some enzymes is a typical strategy of bacteria to prevent monosaccharide uptake by competing microbes, whereas fungi rely on extracellular mannosidases, as the strong antagonistic activity exerted by these organisms precludes the growth of other microbes in their microenvironment (Béki et al. 2003). The *T. neapolitana* enzymes are very thermoactive and thermostable, indeed, they show optimal temperatures from 90 °C to 103 °C and retain their catalytic activity for several hours at 80 °C (Duffaud et al. 1997). However, production scale-up as well as the use of renewable carbon sources have not been attempted using this bacterium.

Besides being able to react to the presence of the polymeric substrate by expressing hydrolytic enzymes, a suitable production microorganism should be culturable using cheap carbon sources. This is the case of two thermotolerant fungal strains identified as *Aspergillus niger gr* and *Aspergillus flavus gr*, which were isolated from garden soil and compost samples in India (Naganagouda et al. 2009). With the aim of establishing optimal conditions for the maximum production of galactomannan-degrading enzymes, these authors have studied the effect of different carbon sources on the ability of these fungi to produce enzymes. In particular, they have tested simple sugars (glucose, sucrose, galactose, and xylose), commercial mannans (i.e. locust bean and guar gums) as well as untreated or defatted copra meal, i.e. a well-dried coconut kernel, which is a by-product of coconut water and oil extraction (Naganagouda et al. 2009). Both fungal strains showed the maximum production of extracellular mannanases when cultivated with defatted copra meal (about 26 and 24 U/ml) if compared to other commercial mannans (about 4–5 U/ml) or simple sugars (from 0.001 to 0.021 U/ml). Worth of note is also that, even though these fungi can be grown at 37 °C, their enzymes are active at high temperature (around 60–65 °C) and have good thermostability, retaining 50% of residual activity for 6–8 h at 60 °C. Therefore, these strains offer an attractive source of robust enzymes for the food and feed processing industries (Naganagouda et al. 2009).

Although the main components of lignocellulose are ubiquitous regardless of the origin of the feedstock, there are some structural and chemical differences in the composition of hemicellulose and lignin that influence the degradability of the materials. Therefore, fungal and/or bacterial enzyme mixtures cannot efficiently hydrolyse all kinds of raw materials. For this reason, they need to be customized by adding

Table 3 Overview of the recent characterized thermophilic β−mannosidase

Source organism	Enzyme	GH family	T_{opt} (°C)	pH_{opt}	Thermostability (half-life)	MW (kDa)	Reference
Thermotoga maritima	N.D.	–	95	7.0	828 min at 80 °C	93.2–96.8	(Zhang et al. 2009)
Streptomyces sp. S27	*Man2S27*	2	50	7.0	95.4% activity after 60 min at 40 °C	92.6	(Pengjun Shi et al. 2011)
Thermotoga thermarum	*Tth Man5*	5	85	5.5	120 min at 90 °C	70	(Hao Shi et al. 2013)
Aspergillus niger CCIM K2	N.D.	5	65	3.5	N.D.	158	(Fliedrová et al. 2012)

N.D. not defined

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complementary enzymes that can aid the complete hydrolysis of different plants biomass (Aulitto et al. 2018; Karnaouri et al. 2016). To do so, it is also important to optimize the recombinant production of single hydrolytic enzymes. In this regard, even though *Escherichia coli* represents a valuable production microorganism of recombinant thermophilic proteins (Contursi et al. 2014a; Fiorentino et al. 2011; Fusco et al. 2013; Limauro et al. 2014; Pedone et al. 2014; Prato et al. 2008), it has also been shown to be inadequate for the overproduction of thermophilic enzymes (Aulitto et al. 2017b). In recent years, the thermophilic bacterium *Thermus thermophilus* has emerged has a suitable workhorse for the recombinant production of thermophilic enzymes. For instance, the strain HB27 has been recently used for the homologous expression of the α-galactosidase *Tt*GalA (Aulitto et al. 2017b), in particular, using the pMKE2 vector system that drivers the recombinant expression via the combined action of nitrate and anoxia (Moreno et al. 2005). This system is an example of the importance of thermophilic expression systems, indeed, it led to a significant overproduction of the enzyme *Tt*GalA (5 mg/l) if compared to the mesophilic counterpart (0.5 mg/l). However, even though several expression systems in thermophiles have been designed, their development is still at research level (Antonucci et al. 2018; Prato et al. 2006). Indeed, additional research efforts will be necessary before considering their exploitation at industrial scale (Turner et al. 2007).

Future perspectives

The isolation and characterization of extremophilic microorganisms has become a research hot topic in the last two decades (Horikoshi et al. 2010; Turner et al. 2007). For instance, (hyper)-thermophilic microorganisms have gained attention because they represent valuable sources of robust enzymes (Aulitto et al. 2017a; Duffaud et al. 1997; Naganagouda et al. 2009) as well as of novel bioactive molecules (Gaglione et al. 2017; Notomista et al. 2015) that can be exploited for several biomedical and industrial applications (Elleuche et al. 2015; Sarmiento et al. 2015). As shown in this review, the growing concerns about the environmental impact of using chemical processes at industrial level has led to an increased interest in the development and/or optimisation of sustainable alternatives, which are based on the use of galactomannan-degrading enzymes as catalysts. However, commercial viability of industrial bioprocesses greatly depends on the cost load of the enzymes production, which is even more evident when thermophiles or hyperthermophiles are used as workhorses. As discussed above, the setup of novel expression systems as well as the optimization of the available ones will be crucial to pave the way to the cost-effective use of thermophiles at industrial level (Turner et al. 2007). Additionally, industrial application of galactomannan-degrading enzymes is also hindered by difficulties in recovering and reusing these biocatalysts (i.e. recirculation). A solution to overcome these drawbacks is represented by enzyme immobilization, which can also improve their stability under both storage and operational conditions. Whereas classical strategies introduce additional costs related to the production of the immobilization carriers (e.g. polysaccharides and mesoporous silica) (Sheldon and van Pelt 2013), the use of virus particles might allow coupling the production of both the enzymes and the immobilization supports (Carette et al. 2007). Since conjugation procedures as well as industrial applications of immobilized enzymes often require prolonged incubations at extreme chemical and physical conditions (Steinmetz et al. 2008), thermophilic viruses represent suitable nanocarriers. Indeed, they are naturally adapted to cope with detrimental conditions, such as extreme temperature, acidity/alkalinity, pressure and salinity (Contursi et al. 2010, 2014b; Fusco et al. 2015a; Prangishvili 2013). One example is the UV-inducible fusellovirus *Sulfolobus* spindle-shaped virus 1 (SSV1), which has been extensively characterized in relation to its interactions with the host cells (Ceballos et al. 2012; Fusco et al. 2015b, c). Moreover, this virus was more recently shown to produce very robust virus particles (Quemin et al. 2015). For this reason, we are currently characterizing the SSV1 virus particles in order to use them as nanocarriers for the immobilisation of galactomannan-degrading enzymes.

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Affiliations

Martina Aulitto[1] · Salvatore Fusco[1] · Danila Limauro[1] · Gabriella Fiorentino[1] · Simonetta Bartolucci[1] · Patrizia Contursi[1]

✉ Patrizia Contursi
contursi@unina.it

[1] Department of Biology, University of Naples Federico II, via Cinthia, 80126 Naples, Italy

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