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Thermophilic Mould *Sporotrichum thermophile*: Biology and Potential Biotechnological Applications

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ABSTRACT

***Sporotrichum thermophile* (Syn. *Myceliophthora thermophila*) is a ubiquitous thermophilic mould that exhibits strong plant organic matter decomposing ability in the temperature range between 40 and 50°C. It has a very broad and efficient enzymatic machinery which enable the mould to thrive in different environments utilizing an array of substrates. Both genome analysis and experimental data have confirmed that the mould is capable of hydrolyzing all major polysaccharides found in plant biomass. The genome analysis and characterization of the biomass-hydrolyzing enzymes confirm that the mould efficiently degrades plant organic matter at elevated temperatures. The hydrolytic enzymes secreted by the mould have several biotechnological applications. Despite low enzyme titers, the native enzymes of the mould are more efficient than their mesophilic counterparts. Attempts are being made to mine the genome through heterologous gene cloning, expression and characterization of the recombinant enzymes. The mould is also known to synthesize various bioactive molecules, which find potential applications.**

Keywords: Thermophilic mould, *Sporotrichum thermophile*, thermostable enzymes, biomolecules, organic matter degradation

INTRODUCTION

Thermophilic moulds are a potential reservoir of thermozymes which find potential applications in various biotechnological processes (Singh *et al.*, 2016; Singh, 2016; Maheshwari *et al.*, 2000; Johri *et al.*, 1999). Enzymes from thermophilic fungi often tolerate higher temperatures than enzymes from their mesophilic counterparts and some show stability even at 70-80°C (Singh *et al.*, 2016; Singh, 2016; Johri *et al.*, 1999). It has been reported that the cellulolytic activity of some thermophilic species was several times higher than that of the most active cellulolytic mesophile, *Trichoderma reesei* (Singh *et al.*, 2016; Singh, 2016; Maheshwari *et al.*, 2000; Johri *et al.*, 1999). Furthermore, biomass-degrading enzymes from thermophilic moulds display higher hydrolysis rate than those from more conventionally used mesophiles like *Trichoderma* or *Aspergillus* (Singh *et al.*, 2016; Singh, 2016).

Sporotrichum thermophile is a thermophilic mould well known as an efficient decomposer of organic matter (Singh 2016; Maheshwari *et al.*, 2000). *Myceliophthora thermophila* is the synonym of *Sporotrichum thermophile* (Singh, 2016; Mouchacca, 2000). The mould is ubiquitous and produces a large number of thermostable enzymes and biomolecules of biotechnological potential (Singh *et al.*, 2016; Singh, 2016; Berka *et al.*, 2011; Singh and Satyanarayana, 2006a; Maheshwari *et al.*, 2000; Johri *et al.*, 1999). The mould grows well and produces various enzymes as well as biomolecules in submerged and solid state fermentations (Bala and Singh, 2016; Singh, 2016; Singh and Satyanarayana, 2011; Singh and Satyanarayana, 2006a; Kaur *et al.*, 2004; Kaur and Satyanarayana, 2004; Maheshwari *et al.*, 2000). Several extracellular hydrolytic enzymes produced by *S. thermophile* are listed in **Table 1**. Besides thermostable enzymes, *S. thermophile* is also known to produce a number of interesting bioactive molecules including thiol protease inhibitors (Yaginuma *et al.*, 1989), anti-microbial xylo-oligosaccharides (Chritakopoulos *et al.*, 2003) and fructo-oligosaccharides (Katapodis *et al.*, 2004). Heterologous expression of *Myceliophthora thermophila* enzymes has been achieved in a number of fungal and bacterial hosts (Singh 2016; Ranjan *et al.*, 2015). The genome of *Sporotrichum thermophile* is 38.74 Mb that contains seven

Table 1. List of various hydrolytic enzymes produced by *Sporotrichum thermophile*

Hydrolytic enzymes	Reference
Cellulolytic enzymes	Coutts and Smith, 1976; Kaur and Satyanarayana 2004; Canevascini *et al.*, 1991; Bhat and Maheshwari, 1987; Bhat *et al.*, 1993; Gaikwad and Maheshwari, 1994; Canevascini and Meyer, 1979; Subramaniam *et al.*, 1999; Vafiadi *et al*., 2009; Tambor *et al.*, 2012
Xylanolytic enzymes	Gool *et al.*, 2012; Yadav and Jaitly, 2011; Katapodis *et al*. 2006; Kaur and Satyanarayana 2004; Singh and Satyanarayana, 2006a; Abdelrahim *et al.*, 2011; Christakopoulos *et al.*, 2003; Vardakou *et al.*, 2003; Katapodis *et al.*, 2003
Feruloyl esterases	Topakas *et al.*, 2004; Topakas *et al.*, 2005a;b; Vafiadi *et al.*, 2007; Topakas *et al,*. 2010
Pectinases	Kaur *et al.*, 2004; Kaur and Satyanarayana, 2004
Phytases	Mitchell *et al.*, 1997; Hassouni *et al*., 2006; Singh and Satyanarayana, 2006a;b; 2008a;b;c; 2009; 2010; Kumari *et al*., 2016; Ranjan *et al.*, 2015; Ranjan and Satyanarayana, 2016;
Laccases	Berka *et al.*, 1997; Babot *et al*., 2011; Lloret *et al.*, 2012a;b; Valls *et al*., 2013; Toledo-Núñez *et al.*, 2012
Miscellaneous enzymes	
Aldolactonase	Beeson *et al.*, 2011
	Satyanarayana *et al.*, 1985
β-Mannosidase	Dotsenko *et al.*, 2012
β-Mannanase	Dotsenko *et al.*, 2012; Klyosov *et al.*, 2012
α-Galactosidase	Dotsenko *et al.*, 2012
Amylase	Adams, 1997; Sadhukhan *et al.*, 1992
Keratinase	Liang *et al.*, 2011
Malate dehydrogenase	Wali *et al.*, 1979
Lipase	Johri *et al.*, 1990
Glutathione S-transferase	Sheehan and Casey, 1993

chromosomes having 9,110 genes (Berka *et al.,* 2011). This review focuses on the biotechnological potential of *S. thermophile*. The role of this mould in different biotechnological processes has been supported by physiological, biochemical and genomic data.

Morphology and taxonomy of the mould

Sporotrichum thermophile has frequently been isolated from the soil and from self-heating masses of composting vegetable matter (Singh and Satyanarayana, 2006a; Domsch *et al.,* 1993), where it contributes to the decomposition of organic biomass/matter (Johri *et al.,* 1999). It is proficient in degrading wood and other cellulosic substances faster than other thermophilic and mesophilic fungi (Berka *et al.,* 2011; Maheshwari *et al.,* 2000; Domsch *et al.,* 1993). This is a fast growing thermophilic mould with temperature optima at 45°C on malt extract and/or Emerson's YpSs media (Emerson, 1941). Young colonies are white cottony which turn to

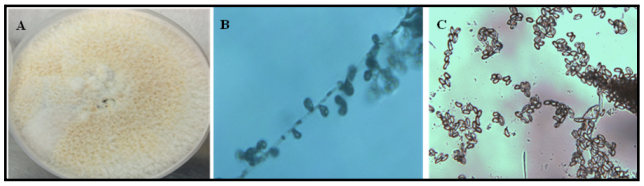

Fig.1 Morphology of *Sporotrichum thermophile* grown on YpSs agar A) Apperance of the mould on agar after 3 days B) Mycelium showing attached conidiospores under compound microscope (100x) C)Conidiospores

cinnamon to light-brown color upon maturation (**Fig. 1**). Fungal hyphae are colourless and about 2 µm broad bearing pear shaped sessile conidiospores that are pale to dark-brown in color (http://fungalgenomics.concordia.ca/fungi/Mthe.php). The conidiospores are abundantly produced laterally and terminally from fungal mycelium (**Fig. 1**). The mould can be cultured in the temperature range between 25 and 55°C. This mould has frequently been isolated from the soil and compost samples (Singh 2016; Singh and Satyanarayana, 2006a; Maheshwari *et al.,* 2000; Domsch *et al.,*., 1993). The efficient enzymatic machinery of this mould enables it to grow on degrading wood and other cellulosic substances faster than other thermophilic and mesophilic moulds (Singh 2016; Berka *et al.,* 2011; Maheshwari *et al.,* 2000; Domsch *et al.,* 1993).

POTENTIAL BIOTECHNOLOGIES WITH THE MOULD

ENZYME INDUSTRY

Sporotrichum thermophile grows on different substrates due to secretion of a large number of hydrolytic enzymes (Singh 2016; Berka *et al.,* 2011; Singh and Satyanarayana, 2006a; Maheshwari *et al.,* 2000; Johri *et al.,* 1999). These enzymes possess unique and desired features suitable for biotechnological applications (Maheshwari *et al.,* 2000; Singh and Satyanarayana, 2011). Different hydrolytic enzymes produced by *S. thermophile* are listed in **Table 1** and **Table 2**.

Cellulolytic enzymes: Cellulolytic enzymes hydrolyze

Table 2. Different enzymes/biomolecules of *Sporotrichum thermophile* and their tested applications

Enzyme	Application potential	References
Phytase	Dephytinization of sesame oil cake	Singh and Satyanarayana, 2006a
	Dephytinization of wheat flour	Singh and Satyanarayana, 2008a
	Dephytinization of soymilk	Singh and Satyanarayana, 2008b
	Dephytinization and improved bread nutrition	Singh and Satyanarayana, 2008c
	Plant growth promotion	Singh and Satyanarayana, 2010
	Dephytinization of poultry feed	Kumari *et al*., 2016
	Dephytinization and improvement in bread, tandoori and nutrition	Ranjan *et al.,* 2015; Ranjan and Satyanarayana, 2016
Cellulase	Hydrolysis of rice straw and waste tea cup paper	Bala and Singh, 2016
Pectinase	Improvement in carrot and banana juices	Kaur *et al.,* 2004
Xylanase	Hydrolysis of rice straw and waste tea cup paper	Bala and Singh, 2016
Laccase	Degradation of water estrogens	Lloret *et al*., 2012a;b
	Increase in brightness of eucalyptus pulp	Babot *et al.,* 2011
XOS	Increase in number of cucumber regenerants and their fresh weight	Katapodis *et al*., 2003
	Inhibition of Gram positive bacteria and *H. pylori*	Christakopoulos *et al*., 2003
Feruloyl esterase	Inhibition of *Mycobacterium bovis* BCG	Vafiadi *et al.,* 2007

cellulose, a linear polysaccharide of glucose residues with β-1, 4-glycosidic linkages, for producing many industrially important products (Singh, 2016; Kaur and Satyanarayana, 2004; Maheshwari *et al.,* 2000). The mould produced cellulase in a mineral salts medium containing yeast extract and cellulose (Coutts and Smith, 1976) at 45°C after 4 days similar to that of *Trichoderma viride*. Bhat and Maheshwari (1987) reported the production of cellulolytic enzymes by *Sporotrichum thermophile* strains and observed faster degradation of cellulose than *Trichoderma reesei*. *Sporotrichum thermophile* produced multiple forms of $\beta-$ glucosidase in cellulose medium (Bhat *et al.,* 1993). The mould produced high titers of cellulase on wheat bran and citrus pectin in a ratio of 1:1, inoculated with 6 $x10^8$ conidiospores at pH 7.0 and 45°C after 96 h in SSF (Kaur and Satyanarayana, 2004). Rapid growth and secretion of cellulases was observed in a medium containing cellulose as the carbon source by Gaikwad and Maheshwari (1994). The mould produced an intracellular β-glucosidase in a medium containing cellulose, cellobiose, laminaribiose, and arbutin (Canevascini and Meyer, 1979). A thermostable cellobiose dehydrogenase (CDH) from *S. thermophile* was purified, cloned, and characterized by Subramaniam *et al.*(1999). The enzyme was optimally active at 60°C with activation energy of 26.3 kJ/mol. Two cellobiose dehydrogenases (I and II) of *S. thermophile* ATCC42464 were purified to homogeneity by different chromatographic techniques (Canevascini *et al.,* 1991). Both enzymes are slightly glycosylated with molecular masses of 91 kDa and 192 kDa for enzymes I and II, respectively. A glucuronoyl esterase of *S. thermophile* was expressed in *Pichia pastoris* (Topakas *et al.,* 2010). Recombinant enzyme was optimally active at pH 7.0 and 55°C. The purified glucuronoyl esterase of *S. thermophile* had a molecular mass of 58 kDa and pI 6.7 (Vafiadi *et al.,* 2009). The enzyme was optimally active at 60°C and pH 6.0. An endoglucanse of *S. thermophile* was expressed in *A. niger* at relatively high levels (Tambor *et al.,* 2012). Recombinant enzyme hydrolyzed carboxymethylcellulose two times faster

than cellulase of *Trichoderma reesei*. *Sporotrichum thermophile* BJAMDU5 produced high cellulases in cost effective cane molasses medium supplemented with yeast extract at 45°C after 72 h (Bala and Singh 2016).

Xylanolytic enzymes: Xylanolytic enzymes hydrolyze xylan, the major component of hemicellulose consisting of a β -1,4-linked D-xylosyl residues backbone with substituent pentoses, hexoses and uronic acids in the side chains (Katapodis *et al.,* 2006). Xylanases are useful in the hydrolysis of lignocellulosic biomass to fermentable sugars, in bread making and clarification of beer and juices (Katapodis *et al.,* 2006). Xylanase production by *S. thermophile* was studied in xylan containing medium at pH 6.0 (Yadav and Jaitly, 2011). Corn cob and ammonium phosphate were identified as significant factors by central composite design affecting xylanase production by *S. thermophile* (Katapodis *et al.,* 2006). High titres of xylanase (1900 U/g DMB) were produced by *S. thermophile* after 96 h in SSF using wheat bran and citrus pectin at pH 7.0 and 45°C (Kaur and Satyanarayana, 2004). High xylanase production by *S. thermophile was* observed when kallar grass was supplemented with 0.5 % xylan at pH 6.0 (Abdelrahim *et al.,* 2011). Xylanase exhibited its optimal activity at pH 6.0 and 70°C. *Sporotrichum thermophile* BJAMDU5 produced high titres of xylanase in cost effective cane molasses medium supplemented with yeast extract at 45°C after 72 h (Bala and Singh, 2016).

Feruloyl esterases: *Sporotrichum thermophile* produced feruloyl esterase that exhibited a native molecular mass of 57.0±1.5 kDa, with a mass of 33±1 kDa on SDS-PAGE (Topakas *et al.,* 2004). The enzyme was optimally active at pH 6.0 and 55-60 °C with a pI value of 3.1. Ferulic acid was released 47-fold higher from destarched wheat bran after xylanase and esterase treatment (Topakas *et al.,* 2004). A homodimer feruloyl esterase of *S. thermophile* was optimally active at pH 6.0 and 55 °C (Topakas *et al.,* 2005a). The esterase was highly thermostable and pH stable. Despite a lower catalytic efficiency than its mesophilic counterpart, *S. thermophile* type-B esterase released more ferulic acid from plant cell wall (Topakas *et al.,* 2005b). Ferulic acid esterase of 39 kDa from *S. thermophile* was functionally expressed in *Pichia pastoris* (Topakas *et al.,* 2012). The recombinant esterase efficiently released ferulic acid from destarched wheat bran in combination with xylanase from *Trichoderma longibrachiatum.*

Pectinases: Pectic substances are heterogeneous group of polysaccharides present in plant biomass that are made largely of D-galacturonic acid (Kaur *et al.,* 2004). The enzymes hydrolyzing pectic substances are commonly called as pectinolytic enzymes or pectinases, which include polygalacturonase, pectin esterase, pectin lyase and pectate lyase on the basis of their mode of action (Kaur *et al.,* 2004). *S. thermophile* produced pectinase in submerged (Kaur and Satyanarayana, 2004) as well as solid state fermentations (Kaur *et al.,* 2004). The mould secreted high pectinase (250 U/g DMB) in SSF using wheat bran and citrus pectin at pH 7.0 and 45°C after 96 h (Kaur and Satyanarayana, 2004). Production of pectinase by *S. thermophile* in submerged fermentation was high as compared to static conditions (Kaur *et al.,* 2004). The combination of yeast extract and citrus pectin supported high pectinase production at pH 7.0, 200 rpm and 45°C. The enzyme was optimally active at pH 7.0 and 55°C showing K_m and V_{max} values of 0.416 mg ml^{-1} and 0.52 mol mg^{-1} min^{-1}, respectively.

Phytases: Phytases are the phosphatases hydrolyzing phytic acid, the stored form of organic phosphorus to myo-inositol and inorganic phosphate (Vohra and Satyanarayana 2003; Vats and Banerjee, 2004; Singh and Satyanarayana, 2011;2015). Phytic acid is a stored organic form of phosphorus in plants. It acts as an anti-nutritional factor by chelating metals such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} making them unavailable, complexing with proteins and thus affecting their digestion, and inhibiting enzymes such as a-amylase, trypsin, acid phosphatase and tyrosinase (Vats and Banerjee 2004; Singh and Satyanarayana, 2011;2015). Due to the lack of adequate levels of phytases in monogastrics (poultry, pigs, fishes and humans), phytic acid is excreted in faeces, which is degraded by soil microorganisms releasing phosphorus in the soil. This phosphorus reaches aquatic bodies, thus causing eutrophication. In order to overcome this problem, foods and feeds can be supplemented with phytases which improve nutritional value of foods and feeds. Phytase encoding genes from *Myceliophthora thermophila* was cloned and over-expressed in a mesophilic fungus *Aspergillus niger* (Mitchell *et al.,* 1997). The phytase of this mould is a monomeric glycoprotein with a molecular mass of 63 kDa. Hassouni *et al.*(2006) studied phytase production by *Myceliophthora thermophila* in solid-state fermentation using sugarcane bagasse; maximum phytase production was achieved at 45°C and pH 6.0 after 36 h of incubation at 70% moisture.

Phytase secretion by *M. thermophila* was the highest in SSF using sesame oil cake as substrate followed by wheat bran and mustard oil cake (Singh and Satyanarayana, 2006a). The mould secreted maximum phytase levels at 45°C, a substrate to moisture ratio of 1:2.5 and an a_w of 0.95 after 120 h. *Sporotrichum thermophile* secreted phytase in submerged fermentation too in synthetic medium containing starch, glucose, peptone and phytic acid along with micronutrients (Singh and Satyanarayana, 2008a) and in a cost-effective cane molasses medium at 45°C and at pH 5.0 (Singh and Satyanarayana, 2006b;2008c). Phytase of *S. thermophile* was purified to homogeneity using acetone precipitation followed by ion-exchange and gel-filtration chromatography (Singh and Satyanarayana, 2009). The enzyme displayed optimal activity at pH 5.0 and 60°C. The maximum hydrolysis rate (V_{max}) and apparent Michaelis-Menten constant (K_m) for sodium phytate were 83 nmoles mg^{-1} protein s^{-1} and 0.156 mM, respectively. Phytase was effective in the dephytinization of sesame oil cake, wheat flour, bread and soymilk with concomitant liberation of utilizable inorganic phosphate (Singh and Satyanarayana, 2006a;2008a;b;c). The enzyme also hydrolyzed insoluble phytates (Singh and Satyanarayana, 2010). Furthermore, both enzyme as well as thermophilic mould promoted the growth of wheat plants (Singh and Satyanarayana, 2010). The mould also secreted phytase in mixed substrate using wheat bran and sugarcane

bagasse in SSF (Kumari *et al.,* 2016). Recombinant phytase of *S. thermophile* was expressed in *Escherichia coli* (Ranjan *et al.,* 2015) and *Pichia pastoris* (Ranjan and Satyanarayana 2016).

Laccases: Laccases are the copper containing enzymes that catalyze the oxidation of phenolic compounds. Laccases have been reported in *S. thermophile.* Laccase gene from *S. thermophile* was cloned and expressed in *A. oryzae* (Berka *et al.,* 1997). The recombinant enzyme was different from native one with respect to isoforms, high molecular weight, and three-fold higher specific activity. The pure enzyme was optimally active at 60°C and pH 6.5. Covalently immobilized laccase of *S. thermophile* on Eupergit C and Eupergit C 250L was used for the removal of Acid Green 27 dye in a packed bed reactor (PBR) [Lloret *et al.,* 2012a]. The dye was decolorized from 57 to 88 % during repeated batch cycles. A continuous PBR with immobilized biocatalyst was used in the treatment of endocrine disrupting chemicals resulting in their degradation up to 80 %. In enzymatic fed batch reactor for the removal of estrogens by free laccase of *S. thermophile*, more than 90 % oxidation was attained due to process optimization (Lloret *et al*., 2012b). An enzymatic membrane reactor was also designed for the degradation of estrogens up to 97%. Different combinations of laccases, xylanase and cellulase used in biobleaching of eucalyptus pulp led to improvement in pulp properties (Valls *et al.*, 2013). *Trametes villosa* and *S. thermophile* laccases were used in combination with mediator. Furthermore, pulp properties were improved by including a xylanase pretreatment, but no significant effect was observed after the cellulase pretreatment. The partial heat capacity of *S. thermophile* laccase was determined by calorimetric scans in the 4.5-10.0 pH range by Toledo-Núñez *et al.* (2012). His residues have been shown to play an important role in the stability of enzyme. The brightness of eucalyptus pulp was increased after treatment with laccase of *S. thermophile*, but the highest improvements were attained with methyl syringate as laccase mediator with a concomitant decrease in kappa number (Babot *et al.*, 2011).

Directed evolution improved eight-fold expression of *S. thermophile* laccase in *Saccharomyces cerevisiae* (Bulter *et al.,* 2003). The molecular mass of mutant expressed in *S. cerevisiae* was 30 % higher (110 kDa) as compared to that expressed in *S. thermophile* (85 kDa) as a result of glycosylation. The thermophilic mould is also known to secrete a large number of other enzymes (**Table 1**).

BIOMOLECULES

S. thermophile produces a large number of biomolecules having various biotechnological applications. Estatins A and B are thiol protease inhibitors isolated from the culture filtrate of *S. thermophile* M4323 by Yaginuma *et al.,* (1989). These are basic and water-soluble inhibitors having molecular formula of $C_{18}H_{25}N_5O_5$ and $C_{18}H_{25}N_5O_6$ for A and B, respectively. They specifically inhibited thiol proteases like papain, ficin and bromelain.

Oligosaccharides are a group of short chain nondigestible polysaccharides widely distributed in plants. These are not digested by human beings and other animals but are beneficial for the growth of probiotic gut microbiota. Fructooligosaccharides (FOS) were synthesized by cultivating *S. thermophile* in a sucrose rich medium (Katapodis *et al.,* 2004). Submerged fermentation with sucrose concentration of 250 g/L resulted in the production of 12.5 g FOS/L that contained three sugars, namely 1-kestose, 6-kestose and neokestose. These sugars were fractionated by gel filtration chromatography and analyzed by HPLC. An endoxylanase of *S. thermopile* liberates xylooligosaccharides (XOS) from birchwood xylan (Katapodis *et al.,* 2003). Aldopentauronic acid was the main acidic XOS separated from the hydrolysate by anion-exchange and size exclusion chromatography. Its structure was determined by 13C NMR spectroscopy. The aldopentauronic acid yield was 25% (w/w), which caused increase in both the number of cucumber regenerants and their fresh weight (Katapodis *et al.,* 2003). Acidic oligosaccharides were obtained from birchwood xylan by treatment with xylanse of *S. thermophile* (Christakopoulos *et al.,* 2003). The xylanase liberated an aldopentauronic acid separated from the hydrolysate by anion-exchange and size exclusion chromatography. Primary structure was determined by 13C NMR spectroscopy. Aldopentauronic acid was found more active against the Gram positive bacteria and *Helicobacter pylori* than Gram negative bacteria.

The feruloyl esterase of *S. thermophile* generated feruloylated derivative of D-arabinose by transfering feruloyl group to D-arabinose using a mixture of n-hexane, t-butanol and water (Vafiadi *et al.,* 2007). This feruloylated compound had an MIC value of 25 g/ml against *Mycobacterium bovis* BCG (Vafiadi *et al.,* 2007).

GENOME AND SECRETOME OF *SPOROTRICHUM THERMOPHILE*

The genome of *S. thermophile* is 38.7 Mb containing seven telomere-to-telomere chromosomes with 51.4% GC content (Berka *et al.,* 2011). Their telomeres comprise TTAGGG repeats commonly found in telomeres of filamentous fungi. The protein coding fractions of the genomes include 9,110 genes with largest gene families of transporters and signaling proteins (Berka *et al.,* 2011). The genome of *S. thermophile* encodes an array of hydrolytic and oxidative enzymes besides CAZymes, enabling the mould to utilize non-carbohydrate substrates too (Berka *et al.,* 2011). The thermophilic mould harbors large numbers (>210) of glycoside hydrolases and polysaccharide lyases covering most of the recognized families. The mould is rich in pectin and pectate lyases (five PL1, one PL3) and relatively poor in polygalacturonases (two GH28). Pectin lyases are most active at neutral to alkaline pH, whereas GH28 pectin hydrolases are most active in acidic pH. The mould grows best on pectin under neutral to alkaline conditions (Berka et al., 2011). The secretome of *S. thermophile* is predicted to comprise 683 proteins, of which 569 are homologs. The predicted extracellular proteins include about 180 CAZymes, 40 peptidases, >65 oxidoreductases and >230 proteins of unknown function (Berka *et al.,* 2011).

Various biotechnologically important enzymes from *S. thermophile* have been cloned and expressed in homologous and heterologous systems. A novel phytase from a *M.*

thermophila was isolated and over-expressed in *A. niger* (Mitchell *et al.,* 1997). The encoded *phyA* phytase protein showed 48% identity with *phyA* of *A. niger* and has 21-29% identity compared to other histidine acid phosphatases. Phytase of *S. thermophile* was cloned and expressed in *E. coli* (Ranjan *et al.,* 2015). The pure recombinant phytase has the molecular mass of 55 kDa with K_m and V_{max}, k_{cat} and k_{cat}/K_m values of 0.143 mM, 185.05 nmoles mg^{-1} s^{-1}, 5.1 × 103 s^{-1}, and 3.5 × 107 M^{-1} s^{-1}, respectively. Recombinant enzyme was stimulated by Mg^{2+} and Ba^{2+} but inhibited by other ions to a varied extent. The enzyme was resistant to both pepsin and trypsin, and dephytinized tandoori and naan (unleavened flat Indian breads), and bread. Phytase of *S. thermophile* was also expressed in *Pichia pastoris* under AOX promoter (Ranjan and Satyanarayana, 2016). Recombinant phytase production was 40-fold higher than that of the native fungal strain. The pure recombinant phytase has the molecular mass of 70 kDa with K_m, V_{max}, k_{cat} and k_{cat}/K_m values of 0.147 mM, 183 nmol/mg s, 1.3×10^3 /s and 8.84×10^6 /M s, respectively.

Gene encoding extracellular laccase of *M. thermophila* showed homology with laccases from diverse fungal genera (Berka *et al.,* 1997). The recombinant laccase expressed in *A. oryzae* under transcriptional control of -amylase gene promoter and terminator, was purified to homogeneity by anion-exchange chromatography. The molecular mass was approximately 100 to 140 kDa by gel filtration and to be 85 kDa by SDS-PAGE containing 40 to 60% glycosylation. Recombinant enzyme had optimal activity at pH 6.5 and retained nearly 100% of activity when incubated at 60°C for 20 min. An endoglucanase from *M. thermophila* was functionally expressed in *Pichia. pastoris* (Karnaouri *et al.,* 2014). The purified recombinant enzyme showed a molecular mass of 65 kDa and exhibited high activity on substrates containing β-1, 4-glycosidic bonds such as carboxymethyl cellulose, barley β-glucan, and cello-oligosaccharides as well as activity on xylan-containing substrates like arabinoxylan and oat spelt xylan. A glucuronoyl esterase from the thermophilic fungus *S. thermophile* was functionally expressed in *Pichia pastoris* under the transcriptional control of the alcohol oxidase promoter (Topakas *et al.,* 2010). The enzyme was optimally active at pH 7.0 and 55°C on substrates containing glucuronic acid methyl ester. A ferulic acid esterase from *M. thermophila* was functionally expressed in *Pichia pastoris* (Topakas *et al.,* 2012). The pure recombinant enzyme had a molecular mass of 39 kDa. The enzyme released ferulic acid efficiently from destarched wheat bran along with xylanase. Mannan hydrolyzing enzymes form *M. thermophila* C1 were cloned, expressed in heterologous host (Dotsenko *et al.,* 2012).

Sporotrichum thermophile is an efficient decomposer of organic matter due to secretion of an array of hydrolytic enzymes. The enzymes secreted by the mould are thermostable and catalytically more efficient than their mesophilic counterparts. The bioactive molecules of the mould are of pharmaceutical and therapeutic value. Further efforts are, however, needed for heterologous/homologous expression of hydrolytic enzymes for large scale applications in various industries.

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