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Thermophilic fungi: Their ecology and biocatalysts

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ABSTRACT

Thermophilic fungi represent an important group of extremophilic microbes inhabiting various natural and man-made habitats including composts, wood chip piles, nesting material of birds and other animals, municipal refuse and several others, and therefore, they are ubiquitous in their occurrence. The fungi that are capable of optimal growth at or beyond 40 °C are defined as thermophilic fungi, which form a diverse group of fungi capable of growth at elevated temperatures up to 60 °C. These fungi are capable of degrading organic materials efficiently with the help of extracellular enzymes with unique and desired properties suitable for various biotechnological applications. The enzymes are also useful in the treatment of industrial wastes and effluents that are rich in oil, heavy metals, anti-nutritional factors (e.g. phytic acid) and biomass conversion. The presence of genes encoding hydrolytic enzymes in the genomes of thermophilic fungi is an evidence for the decomposing potential of these fungi. Thermophilic fungi and their biocatalysts aid in environmental pollution management and play an important and critical role in mushroom composting. The utility of their biocatalysts in generating sugars from the bioconversion of agroresidues and their fermentation to biofuels is a major venture in the field of biotechnology. This article focuses on the ecology and biocatalysts of thermophilic fungi.

Keywords: Thermophilic fungi, hot environments, biocatalysts, biomass conversion, biofuels, genome

INTRODUCTION

Temperature is one of the most important ecological factors, which affects microbial activities and their distribution (Johri *et al.*, 1999; Maheshwari *et al.*, 2000). Microorganisms occur in a great variety of environments with extremes of temperature, pH, chemical contents and/or pressure due to their genetic and physiological adaptations (Cooney and Emerson, 1964; Johri *et al.*, 1999; Maheshwari *et al.*, 2000; Satyanarayana and Singh, 2004; Singh and Satyanarayana, 2009). Microorganisms appeared on the earth about 3.5 billion years ago at a time when temperatures were likely to be in extremes, which has been proven by the discovery of microorganisms from geothermal areas all over the world. Prokaryotes have a wide range of temperature tolerance, while in case of eukaryotic organisms, only a few species of fungi can tolerate up to 62 °C. These fungi have temperature maxima between 50 and 60 °C. Thermophilic fungi thrive in heaped masses of plant materials, piles of agricultural and forestry residues and other organic materials, which provide a suitable environment for their growth and development (Johri *et al.*, 1999; Satyanarayana and Singh 2004; Singh and Satyanarayana, 2009). Lindt (1886) was the first to describe a true thermophilic mould, *Mucor pusillus*. Thermophilic fungi are the group of fungi that are capable of growth optimally at or above 40 °C (Crisan, 1959; 1969). These are one of the major components of the microflora that develop in heaped masses of plant materials, which provide a suitable environment for their growth and development. Cooney and Emerson (1964) provided a taxonomic description of 13 species known at that time and about their habitats and biology. Presently a large number of thermophilic fungal species have been described (Mouchacca, 1997; 2000), which constitute a heterogeneous physiological group of various genera in *Zygomycetes*, *Ascomycetes* and *Basidiomycetes*. Thermophilic fungi can grow in simple media containing carbon and nitrogen sources and mineral salts, suggesting that they do not have any nutritional requirement for growth and are autotrophic for all vitamins (Cooney and Emerson, 1964).

Thermophilic fungi play an important role in the disposal of organic matter and toxic chemicals from domestic and industrial wastes (Singh and Satyanarayana, 2009). In the natural habitats, they colonize the organic matter and degrade the major components by the action of a number of hydrolytic enzymes. The hydrolyzed products are utilized by these moulds for their growth and development. Till now a large number of thermophilic fungi have been described and their physiology and enzymes have been discussed (Johri *et al.*, 1999; Satyanarayana and Johri 1992; Maheshwari *et al.*, 2000; Satyanarayana and Singh, 2004; Singh and Satyanarayana, 2009; 2011). In this review, we have focused on the ecology and biocatalysts of thermophilic fungi.

ECOLOGY OF THERMOPHILIC FUNGI

The thermophilic fungi occur in various types of soils and in habitats where decomposition of plant material takes place as in composts (Chang and Hudson, 1967), stored grains (Christensen, 1957), library books (Subrahmanyam, 1999), nesting materials of birds and animals (Cooney and Emerson, 1964; Satyanarayana *et al.*, 1985; Korni^{33}owicz-Kowalska and Kitowski, 2013) and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides congenial conditions for their

growth and development (Johri *et al.*, 1999; Singh and Satyanarayana, 2009) [**Table 1**]. In these habitats, thermophilic fungi may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions.

Tansey and Brock (1972) observed that the thermophilic fungi are much more common in acidic thermal habitats than those of neutral to alkaline pH. There are fewer than 50 species of thermophilic fungi which thrive at relatively high temperatures (Mouchacca, 1997). Thermophilic fungi have been isolated from manure, compost, industrial coal mine soils, beach sands, nuclear reactor effluents, Dead Sea valley soils, and desert soils of Saudi Arabia (Redman *et al.*, 1999). The occurrence of thermophilic fungi in aquatic sediment of lakes and rivers, as first reported by Tubaki *et al.* (1974), is mysterious in view of the low temperature (6-7 ^{o}C) and low level of oxygen available at the bottom of a lake. A few thermophilic fungi survive the stresses such as increased hydrostatic pressure, absence of oxygen and desiccation (Mahajan *et al.*, 1986). Cooney and Emerson (1964) published the first comprehensive account of the taxonomy, biology and activities of 13 thermophilic fungi and several additional thermophilic fungi have been discovered later. Subsequently a comprehensive account of thermophilic fungi was published by Mouchacca (1997) in which he attempted a critical reappraisal of the nomenclatural, and in some cases, also of the taxonomic status of known thermophiles. Latest valid names for all thermophilic fungal taxa have been elaborated and reframed by Mouchacca (2000). The original names of some of the taxa have been retained for their taxonomic value.

Thermophilic fungi are world-wide in distribution and most species do not show any geographical restriction (Johri *et al.*, 1999; Subrahmanyam, 1999) [**Table 1**]. The tropical sites are, however, more favorable due to elevated temperatures and a competitive microbial environment (Johri *et al.*, 1999; Subrahmanyam, 1999; Satyanarayana and Singh, 2004). They have been isolated from natural as well as man-made environments. Most significant natural habitat for these thermophilic fungi is the decomposing organic matter (Chang and Hudson, 1967; Bergman and Nilsson, 1967), for example, a newly built wood chip pile where temperature rapidly rises to ignition (Feist *et al.*, 1973). Solar heat in the tropics (Bilai, 1984) and moisture content (Festenstein, 1966) are the other factors that significantly influence the development of thermophilic fungi.

Tansey and Jack (1976) and Thakre and Johri (1976) recovered several thermophilic fungi from different substrates. Besides these, some non-thermogenic environments like coal and moist soils in Australia, Antarctic and sub-Antarctic soils (Ellis, 1980a;b), aquatic sediments where bottom temperature never exceeds 6 or 7 ^{o}C and dust on sparingly used library books have also been found to harbor thermophilic fungi (Subrahmanyam, 1999). Salar and Aneja (2006) isolated thermophilic moulds from temperate soils of North India. The occurrence of *Chaetomium senegalense* (*Ascomycetes*) and *Myceliophthora fergusii* have been reported for the first time from India, and further explained the taxonomy and biogeography of some thermophilic fungi. Thermophilic fungi were isolated from grassland soils and litter, riparian litter, herbivore dung and biological soil crusts in an arid land ecosystem (Powell *et al.*, 2012). Korni^{33}owicz-Kowalska and Kitowski (2013) carried out an investigation on the isolation of thermophilic fungi from nests of wetland birds. In nests with the maximum weight, the number and diversity of thermophilic fungi were significantly greater than in other nests, with lower weight. The dominant species within the mycobiota under study was *Aspergillus fumigatus* inhabiting 95% of the nests under study. Thermophilic fungi from cultivated and desert soils, exposed continuously to cement dust particles were

Table 1. Occurrence and distribution of thermophilic fungi

Source	Reference/s
Thermogenic habitats	
Composts	Johri *et al.*, 1999; Singh and Satyanarayana, 2006a;b; 2008a;b;c;
Soil	Tansey and Jack, 1976; Hemida, 1992; Chadha *et al.*, 2004; Singh and Satyanarayana, 2006a; Salar and Aneja, 2006; 2007
Paddy straw compost	Johri *et al.*, 1999; Singh and Satyanarayana, 2006a;b
Wheat straw Compost	Chang and Hudson, 1967; Johri *et al.*, 1999
Municipal Compost	Crisan, 1959; 1969; Cooney and Emerson, 1964; Tansey and Jack, 1976; Johri *et al.*, 1999
Coal Spoil Tips	Thakre and Johri, 1976; Johri *et al.*, 1999
Stored grains	Johri *et al.*, 1999 ; Subrahmanyam, 1999; Christensen, 1957
Non-thermogenic habitats	
Cooling towers	Subrahmanyam, 1999
Dust of library books	Subrahmanyam, 1999
Arctic and Antarctic soils	Ellis, 1980a;b
Himalayan soil	Subrahmanyam, 1999
Human skin	Johri *et al.*, 1999; Subrahmanyam, 1999
Birds' Nests	Cooney and Emerson, 1964; Satyanarayana *et al.*, 1985; Korni^{33}owicz-Kowalska and Kitowski, 2013

isolated by Hemida (1992). The most dominant species among the fungi was *A. fumigatus*. Truly thermophilic fungi were also isolated that included *Chaetomium thermophile*, *Malbranchea pulchella* var. *sulfurea*, *Rhizomucor pusillus*, *Myriococcum albomyces*, *Talaromyces thermophilus* and *Torula thermophila*.

BIOCATALYSTS OF THERMOPHILIC FUNGI

Thermophilic fungi are ubiquitous in their occurrence which play an important role in the decomposition of organic matter. The presence of these fungi in decomposing materials provide sufficient evidence for the production of various hydrolytic enzymes responsible for the degradation of various components of organic materials such as starch, cellulose, xylan, lignin and other components. These polysaccharides are degraded by the action of extracellular enzymes of thermophilic fungi for their growth and development (Berka *et al.*, 2011). The fungi produce thermostable enzymes with desired properties for applications in various industries (**Fig. 1**). The research is focused mainly on the identification of suitable thermophilic fungal sources for the production and characterization of the desired enzymes for biotechnological applications. The thermophilic fungi help in environmental pollution management by decomposition of organic matter, effluent treatment and biosorption of heavy metals. *Rhizomucor pusillus* has been used in the biotransformation of antihelmintic drug albendazole to produce novel and active metabolites of commercial interest (Prasad *et al.*, 2011). Berka *et al.* (2011) carried out comparative genome sequence analysis of two thermophilic fungi, *Myceliophthora thermophila* and *Thiellavia terrestris*. They have revealed the presence of different hydrolytic enzymes responsible for the degradation of organic matter.

The thermophilic microbes have been considered as good sources of thermostable enzymes with high catalytic activity, greater resistance to denaturing agents, and lower incidence of contamination. Thermostable enzymes are receiving considerable attention because of their applicability in high-temperature catalysis of various enzymatic industrial processes. The thermophilic fungi produce a large number of enzymes which are useful in various industries such as food, textile, detergent, dairy, pharmaceuticals and others (**Fig. 1**). A variety of biocatalysts isolated from thermophilic fungi (**Table 2**) are briefly described below:

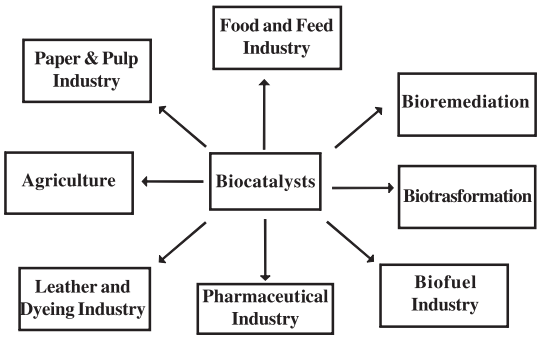

Fig. 1 Potential applications of thermophilic fungi and their biocatalysts

i) Phosphatases

Phosphatases are important group of hydrolytic enzymes responsible for making inorganic phosphorus available for organisms. The phosphoserine residues in caseins can be hydrolyzed by both alkaline and acid phosphatases. Bilai *et al.* (1985) screened a large number of thermophilic fungal strains for the production of acid phosphatases, and among them *Rhizopus micromyces, Rhizomucor pusillus, Talaromyces thermophilus, Populaspora thermophila, Thermomyces lanuginosus, Acremonium thermophile, Thermoascus aurantiacus* and *Chrysosporium thermophilum* grew very well on the medium, but only two fungal strains produced acid phosphatase. Satyanarayana *et al.* (1985) reported *Acremonium alabamensis* and *Rhizopus rhizoidiformis* to secrete only acid phosphatase, while other fungi produced acid and alkaline phosphatases. An extracellular (conidial) and an intracellular (mycelial) alkaline phosphatase from the thermophilic fungus *Scytalidium thermophilum* were purified by DEAE-cellulose and Concanavalin A-Sepharose chromatography (Guimarães *et al.*, 2001).

Phytases (*myo*-inositol hexakisphosphate phosphohydrolase) are the important class of phosphatases, which catalyze the hydrolysis of phytic acid to inorganic phosphate and *myo*-inositol phosphate derivatives (Singh and Satyanarayana, 2011; Singh *et al.*, 2011). These enzymes have potential applications in food and feed industries for mitigating their phytic acid and other organic phosphorus compounds to liberate utilizable inorganic phosphate and for improving the digestibility as a result of reduction of anti-nutrient components. Thermophilic fungi are known to secrete phytases in submerged as well as in solid state fermentations (Singh and Satyanarayana, 2011). Phytase from *Aspergillus fumigatus* was cloned and over-expressed in *A. niger* by Pasamontes *et al.* (1997). The phytase from *Thermomyces lanuginosus* was cloned and overexpressed in *Fusarium venenatum*, which was optimally active at 65 °C and pH 6.0 (Berka *et al.*, 1998). While Mitchell *et al.* (1997) screened a large number of thermophilic fungal strains for phytase production. *Rhizomucor pusillus* produced extracellular phytase in SSF using wheat bran optimally at 50 °C and pH 5.5 (Chadha *et al.*, 2004). Another thermophilic mould *Thermoascus aurantiacus* produced phytase in a medium containing glucose, starch and wheat bran (Nampoothiri *et al.*, 2004). *Sporotrichum thermophile* produced HAP-phytase in both solid state (Singh and Satyanarayana, 2006a; 2008b) and submerged (Singh and Satyanarayana, 2006b; 2008 a;c) fermentations.

Both phytases and acid phosphatases work in coordination; phytases cleave phytate in a selective manner, while acid phosphatases attack the inositol phosphate intermediates independently, and therefore, accelerate the total dephosphorylation process.

Table 2. Various biocatalysts produced by thermophilic fungi for different biotechnological applications

Enzyme	Source	Reference/s
Phosphatase	*Rhizopus micromyces, Rhizomucor pusillus, Talaromyces thermophilus, Populaspora thermophila, Thermomyces lanuginosus, Acremonium thermophile, Thermoascus aurantiacus Chrysosporium thermophilum Acremonium alabamensis Rhizopus rhizoidiformis*	Bilai *et al.*, 1985 Satyanarayana *et al.*, 1985
	Scytalidium thermophilum	Guimarães *et al.*, 2001
Phytases	*Aspergillus fumigatus*	Pasamontes *et al.*, 1997
	Thermomyces lanuginosus	Berka *et al.*, 1998
	Myceliophthora thermophila	Mitchell *et al.*, 1997
	Rhizomucor pusillus	Chadha *et al.*, 2004
	Thermoascus aurantiacus	Nampoothiri *et al.*, 2004
	Sporotrichum thermophile	Singh and Satyanarayana, 2006a;b; 2008a;b;c
Amylolytic enzymes	*Thermomyces lanuginosus*	Mishra and Maheshwari, 1996; Chadha *et al.*, 1997; Arnesen *et al.*, 1998; Petrova *et al.*, 2000; Nguyena *et al.*, 2002
	Thermomucor indicae-seudaticae	Kumar and Satyanarayana, 2003
	Scytalidium thermophilum	Roy *et al.*, 2000
	Malbranchea sulfurea	Gupta and Gautam, 1993
	Humicola grisea	Campos and Felix, 1995
	Humicola grisea var. *thermoidea*	Tosi *et al.*, 1993
Lipolytic enzymes	*Humicola lanuginosa*	Arima *et al.*, 1968; Omar *et al.*,1987
	Rhizomucor miehei	Boel *et al.*, 1988; Rao and Divaker, 2002
Cellulolytic enzymes	*Sporotrichum thermophile*	Bhat and Maheshwari, 1987
	Chaetomium thermophile var. coprophile	Venturi *et al.*, 2002
	Thermoascus aurantiacus	Gomes *et al.*, 2000; Parry *et al.*, 2001; Kalogeris *et al.*, 2003; de Palma-Fernandez *et al.*, 2002
	Thermomyces lanuginosus	Lin *et al.*, 1999
Xylanolytic enzymes	*Thermoascus aurantiacus*	Gomes *et al.*, 1994; Kalogeris *et al.*, 1998; dos Santos *et al.*, 2003
	Humicola lanuginosa	Grajek, 1987
	Melanocarpus albomyces	Prabhu and Maheshwari, 1999; Roy *et al.*, 2003; Narang *et al.*, 2001
	Sporotrichum thermophile	Katapodis *et al.*, 2003
	Thermomyces lanuginosus	Lin *et al.*, 1999; Singh *et al.*, 2000
	Chaetomium cellulolyticum	Baraznenoka *et al.*, 1999
	Thermoascus sp.	Matsuo *et al.*, 1998
Pectinolytic enzymes	*Penicillium duponti, Humicola stellata, H. lanuginosa, H. insolens, Mucor pusillus*	Craveri *et al.*, 1967
	Talaromyces thermophilus Sporotrichum thermophile, Mucor miehei, Populaspora thermophila,	Tong and Cole, 1975
	Thermoascus aurantiacus	Adams and Deploey, 1978
	Sporotrichum thermophile	Kaur *et al.*, 2004
Proteolytic enzymes	*Mucor pusillus*	Arima *et al.*, 1968
	Malbranchea pulchella var. *sulfurea*	Voordouw *et al.*, 1974; Stevenson and Gaucher, 1975
	Humicola lanuginosa	Stevenson and Gaucher, 1975; Shenolikar and Stevenson, 1982
	Thermomyces lanuginosus	Jensen *et al.*, 2002
	Scytalidium thermophilum	Ifrij and Ögel, 2002
	Myceliophthora sp.	Zanphorlin *et al.*, 2011

ii) Amylases

Thermostable amylolytic enzymes from thermophiles have many advantages in the starch processing industry. *Thermomyces lanuginosus* produces α-amylase and glucoamylase in the medium containing rice flour and corn steep liquor at 50 °C, 150 rpm and pH 5.5 after 72 h (Chadha *et al.*, 1997). Glucoamylase of *Scytalidium thermophilum* is optimally active at pH and temperature of 5.5/70 °C and

5.5/65 °C, respectively (Aquino *et al.*, 2001). Both α-amylase and glucoamylase from *T. lanuginosus* ATCC 34626 were purified to electrophoretic homogeneity (Nguyena *et al.*, 2002). The amylolytic enzymes were optimally active in the pH range between 4.4 and 6.6 at 70 °C. An extracellular neutral glucoamylase from *Thermomucor indicae-seudaticae* is optimally active at 60 °C and pH 7.0 (Kumar and Satyanarayana, 2003). An α-amylase was purified nine-fold from culture supernatant of *Scytalidium thermophilum* in a single step using fluidized bed chromatography, wherein alginate was used as the affinity matrix (Roy *et al.*, 2000).

Thermomyces lanuginosus produced α-amylase in shake flasks after 120 h with low molecular weight dextran as carbon source supplemented with Tween 80 (Arnesen *et al.*, 1998). While *Malbranchea sulfurea* produced α-amylase (extracellular) and α-glucosidase (cell bound) during submerged fermentation (Gupta and Gautam, 1993). Campos and Felix (1995) described a glucoamylase from *Humicola grisea* having molecular mass and pI of 74 kDa and 8.4, respectively. Glucoamylase from *H. grisea* var. *thermoidea* was purified and characterized (Tosi *et al.*, 1993). It is a glycoprotein with pH and temperature optima of 5.0 and 55 °C. Another mould *T. lanuginosus* IISc91 produced α-amylase and glucoamylase during growth which were purified near to homogeneity (Mishra and Maheshwari, 1996). α-Amylase was a dimeric glycoprotein of 42 kDa, which displayed optimal activity at pH 5.6 and 65 °C. Petrova *et al.* (2000) purified two α-amylases from *Thermomyces lanuginosus* ATCC 34626 by precipitation and chromatographic techniques, which were optimally active at pH 5.0. A novel α-amylase from the thermophilic fungus, *Malbranchea cinnamomea* was purified, characterized and crystallized by Han *et al.* (2013). The purified enzyme of 60.3 kDa exhibited maximal activity at pH 6.5 and 65 °C. It was capable of hydrolyzing amylose, starch, amylopectin, pullulan, cyclodextrins and maltooligosaccharides. á-Amylase gene comprised 1,476-bp open reading frame encoding 492 amino acids. The crystal structure of enzyme solved at a resolution of 2.25 Å revealed three domains with ten α helices and 14 β strands.

iii) Lipases

Lipases (EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerols and long chain fatty acids. In detergents, the alkaline lipases with a temperature range of 30-60 °C are preferred. Arima *et al.* (1968) purified an extracellular lipase from *Humicola lanuginosa* strain Y-38, isolated from compost. The fungi produced lipase in a medium containing soybean oil, starch, corn steap liquor, and antifoaming agent. *Humicola lanuginosa* produced protease in a medium containing sorbitol, corn steap liquor, silicone oil as an antifoaming agent and whale or castor oil as an inducer (Omar *et al.*, 1987). A lipase from *Humicola lanuginosa* was cloned and overexpressed in *Aspergillus oryzae* (Huge-Jensen *et al.*, 1989). An extracellular 32 kDa lipase from *Rhizomucor miehei* was purified by anion exchange chromatography followed by affinity and hydrophobic interaction chromatography (Boel *et al.*, 1988). Rao and Divaker (2002) studied the esterification of α-terpineol with acetic anhydride or propionic acid mediated by *Rhizomucor miehei* lipase. An extracellular lipase gene from thermophilic fungus *Thermomyces lanuginosus* HSAUP0380006 was cloned through RT-PCR and RACE amplification (Zheng *et al.*, 2011). Its coding sequence predicted a 292 residue protein with a signal peptide of 17 amino acids. High level expression for recombinant lipase was achieved in *Pichia pastoris* GS115 under the control of strong AOX1 promoter. The purified enzyme has a molecular mass of 33 kDa and was stable at 60 °C and pH 9.0. Lipase activity was stimulated by Ca^{2+} and inhibited by Fe^{2+}, Zn^{2+}, K^{+}, and Ag^{+}. The enzyme hydrolyzed and synthesized esters efficiently.

Novo Nordisk A/S introduced the first lipase from *Humicola lanuginosa* in detergents in 1988. The cDNA encoding lipase of *Humicola lanuginosa* was cloned and expressed in *Aspergillus oryzae* and the recombinant lipase is produced on a large scale.

iv) Cellulases and hemicellulases

Cellulose is the major component of plant cell wall made of β-1, 4-linked D-glucosyl units. Cellulases are enzymes that catalyse the hydrolysis of cellulose to smaller oligosaccharides. Bhat and Maheshwari (1987) demonstrated that the endo- and exoglucanase production by *Sporotrichum thermophile* was about 10-fold lower and the β-glucosidase activity was about 1.6-fold lower than *Trichoderma reesei*. *Sporotrichum thermophile* degraded cellulose faster than *Trichoderma reesei* despite its lower enzyme activities. Kalogeris *et al.* (2003) studied the production of extracellular cellulolytic enzymes in solid state fermentation by the thermophilic fungus *Thermoascus aurantiacus*. The fermentation medium was optimized for simultaneous production of endoglucanase and β-glucosidase by *Thermoascus aurantiacus* using statistical factorial designs in shake flask (Gomes *et al.*, 2000). The optimized medium containing solka floc and soymeal produced high enzyme titers after an incubation time of 264 h. Endoglucanase and β-glucosidase were optimally active at pH 4.5 and pH 5 and 75 °C.

An extracellular β-D-glucosidase produced by *Thermomyces lanuginosus*-SSBP was purified to near homogeneity that contained two identical subunits with a native molecular mass of 200 kDa (Lin *et al.*, 1999). The enzyme exhibited optimal activity at 65 °C and pH 6.0. An extracellular β-glucosidase secreted by *Thermoascus aurantiacus* was purified to homogeneity by DEAE-Sepharose, Ultrogel AcA 44 and Mono-P column chromatography by Parry *et al.* (2001). The enzyme was a homotrimer, with a monomeric mass of 120 kDa. The trimer was optimally active at 80 °C and pH 4.5 with the lowest K_m towards p-nitrophenyl-β-D-glucoside (0.1137 mM) and

the highest K_{cat} towards cellobiose and β-trehalose (17052 min^{-1}). The thermophilic fungus *Chaetomium thermophilum* var. *coprophilum* produced large amounts of extracellular and intracellular β-glucosidase when grown on cellulose or cellobiose (Venturi *et al.*, 2002). β-Glucosidase from the fungus *Thermoascus aurantiacus* grown on semi-solid fermentation medium containing corncobs was partially purified in 5 steps—ultrafiltration, ethanol precipitation, gel filtration and two anion exchange chromatography runs followed by characterization (de Palma-Fernandez *et al.*, 2002). An endoglucanase gene from the thermophilic fungus *Myceliophthora thermophila* was functionally expressed in methylotrophic yeast *Pichia pastoris* (Karnaouri *et al.*, 2013). The purified enzyme had a molecular mass of 65 kDa and exhibited a high activity on substrates containing β-1,4-glycosidic bonds (carboxymethyl cellulose, barley β-glucan, cello-oligosaccharides) as well as activity on xylan-containing substrates (arabinoxylan and oat spelt xylan).

Xylanases of thermophilic fungi are receiving considerable attention due to their application in biobleaching of pulp in the paper industry, where the enzymatic removal of xylan from lignin-carbohydrate complexes facilitates the leaching of lignin from the cell wall fibre, obviating the need of chlorine for pulp bleaching in the brightening process. They also have application in animal feed to improve its digestibility. Wheat bran supported maximum enzyme production in *Thermoascus aurantiacus* and *Humicola lanuginosa* (Grajek, 1987). Kalogeris *et al.* (1998) observed that xylanase production by *Thermoascus aurantiacus* was enhanced by optimization of various physicochemical factors in SSF. *Melanocarpus albomyces* isolated from compost produced cellulase-free xylanase in the medium containing bagasse as carbon source (Prabhu and Maheshwari, 1999). Xylanase production by *Thermoascus aurantiacus* was optimized in shake-flasks using Box-Wilson method and a central composite design that resulted in optimum levels of hemicellulases (Gomes *et al.*, 1994). The xylanase and β-xylosidase displayed optimal activity at pH 5.0 and 75-80 °C.

Sporotrichum thermophile ATCC 34628 xylanase was purified to homogeneity by Q-Sepharose and Sephacryl S-200 column chromatography methods by Katapodis *et al.* (2003). The enzyme has a molecular mass of 25 kDa, an isoelectric point of 6.7 with optimal activity at pH 5 and 70 °C. Xylanase was produced in solid-state fermentation by *Thermoascus aurantiacus* using bagasse as substrate after 6 days supplemented with rice bran extract (dos Santos *et al.*, 2003). Immobilization of xylanase of *Melanocarpus albomyces* IIS68 on Eudragit L-100 inreased K_m and V_{max} values (Roy *et al.*, 2003). The immobilized enzyme was reused up to ten times without any affect in activity. An extracellular xylanase of *Thermomyces lanuginosus*-SSBP was purified by ammonium sulphate precipitation followed by DEAE-Sephadex A25 and quaternary aminoethyl (QAE)-Sephadex A25 column chromatography techniques (Lin *et al.*, 1999). The molecular mass of the purified xylanase was 23.6 kDa with a pI value of 3.8. Among eight strains of *Thermomyces lanuginosus* isolated from different geographical regions, strain SSBP produced the highest xylanase activity on corn cob medium at 40-50 °C and pH 6.0-7.0 (Singh *et al.*, 2000). The enzyme was optimally active at pH 6 or 6.5 and 70 °C having a molecular mass of 24.7 kDa and pI value of 3.9. *Chaetomium cellulolyticum* produced three xylanases with molecular weights of 25, 47, and 57 kDa and pIs of 8.9, 8.4, and 5.0, respectively (Baraznenoka *et al.*, 1999). Both the 25- and 47-kDa xylanases were related to the family of G and the 57-kDa xylanase to the family of F. These xylanases had a neutral pH optimum within the range of 6.0-7.0. A β-xylosidase of *Thermoascus* sp. was purified by ultrafiltration, ethanol precipitation, and chromatography with DEAE-Toyopearl 650M, Mono Q HR5/5, and Phenyl Superose HR5/5 (Matsuo *et al.*, 1998). The molecular mass of the enzyme was recorded as 107 kDa by gel filtration chromatography-Superdex 200 HR and 100 kDa by SDS-PAGE. The enzyme was optimally active at 55 °C and pH 4.5. *Melanocarpus albomyces* IIS68 produced xylanase in SSF using wheat straw as substrate (Narang *et al.*, 2001). The optimum levels of the variables optimized by response surface methodology resulted in xylanase activity of 7760 U g^{-1} initial dry substrate. Two novel xyloglucanase genes were cloned from the thermophilic fungus *Rhizomucor miehei* (Song *et al.*, 2013). Both genes contained open reading frames of 729 bp encoding 242 amino acids. The two genes, without signal peptides, were cloned and successfully expressed in *Escherichia coli* with similar molecular masses of 25.6 and 25.9 kDa. Enzyme A showed optimal activity at pH 6.5 and 65 °C while B at pH 5.0 and 60 °C.

Humicola brevis var. *thermoidea* was cultivated under solid state fermentation using wheat bran as a substrate for â-glucosidase and xylanase production (Masui *et al.*, 2012). After optimization using response surface methodology the levels of xylanase and β-glucosidase were increased. Optimum pH for β-glucosidase and xylanase activities was 5.0 and 5.5, respectively. β-Glucosidase activity was stimulated by xylose up to 1.7-fold. Three xylanase genes (xynA, xynB, xynC) were identified in *Humicola insolens* Y1 (Du *et al.*, 2013). Recombinant enzymes produced in *Pichia pastoris* showed optimal activities at pH 6.0–7.0 and 70–80 °C. XynA exhibited better alkaline adaptation and thermostability, and had higher catalytic efficiency and wider substrate specificity. Two xylanases were purified from the thermophilic fungus *Sporotrichum thermophile* grown in submerged culture using wheat straw as carbon source (Vafiadi *et al.*, 2010). The enzymes (StXyn1 and StXyn2), have molecular masses of 24 kDa and 48 kDa, respectively and were optimally active at pH 5.0 and at 60 °C.

vi) Pectinases

Pectin is a heteropolysaccharide mainly composed of polygalacturonic acid linked by α-1,4 linkages and it plays

an important role in plants by maintaining the integrity of the cell wall and middle lamella. Pectinase is the major pectin hydrolyzing enzyme which helps in the decomposition of vegetable matter. Thermophilic fungi are also known to produce pectinases. Craveri *et al.* (1967) described pectinase from *Penicillium duponti, Humicola stellata, H. lanuginosa, H. insolens* and *Mucor pusillus.* The thermophilic mould *Talaromyces thermophilus* produced pectinase in stationaty liquid culture using sodium polypectate as the substrate (Tong and Cole, 1975). The enzyme showed optimal activity at 50 °C. Adams and Deploey (1978) observed the secretion of pectinolytic enzymes by *Sporotrichum thermophile*, *Mucor miehei, Populaspora thermophila, Talaromyces leycettanus* and *Thermoascus aurantiacus.* Whitehead and Smith (1989) studied the production of pectinase by *S. thermophile* in both static as well as submerged fermentation. Static culture had a higher yield than shake flask culture. *Sporotrichum thermophile* is also known to produce pectinase in solid state as well as submerged fermentation using citrus peal as substrate (Kaur and Satyanarayana, 2004; Kaur *et al.*, 2004).

vii) Proteases

Proteases are protein degrading enzymes which are classified on the basis of a critical amino acid required for the catalytic function (e.g., serine protease), the pH optimum of their activity (acidic, neutral, or alkaline), their site of cleavage (e.g., aminopeptidases, which act at the free N terminus of the polypeptide chain, or carboxypeptidases, which act at the C terminus of the polypeptide chain), or their requirement of a free thiol group (e.g., thiol proteinase). Proteases have been used in the food, dairy, and detergent industries and for leather processing. Arima *et al.*, (1968) obtained a soil isolate of *Mucor pusillus* that produced an enzyme with a high ratio of milk-clotting to proteolytic activity after screening of 800 microorganisms.

Malbranchea pulchella var. *sulfurea* and *Humicola lanuginosa* both produced inducible proteases in the presence of casein (Stevenson and Gaucher, 1975). The production of protease by *Malbranchea pulchella* var. *sulfurea* was repressed by glucose, peptides, amino acids and yeast extract (Voordouw *et al.*, 1974). The enzyme was classified as a serine protease showing optimal activity at pH 8.5 and was stable over a broad pH range. The molecular mass was recorded 11 to 17 kDa by gel filtration chromatography and 32 to 33 kDa by SDS-PAGE.

Shenolikar and Stevenson (1982) purified an alkaline protease of *Humicola lanuginosa* in one step based on its specific binding to an organomercury-Sepharose column, from which the enzyme was selectively eluted with a buffer containing mercuric chloride. Both gel filtration and sedimentation analyses showed that the enzyme had a molecular mass of 237 kDa. The production of extracellular enzymes by the thermophilic fungus *Thermomyces lanuginosus* was studied in chemostat cultures at a dilution rate of 0.08 h-1 in relation to variation in the ammonium concentration in the feed medium (Jensen *et al.*, 2002). Extracellular proteases produced by *Scytalidium thermophilum*, grown on microcrystalline cellulose, were most active at pH 6.5–8.0 and 37–45 °C when incubated for 60 min (Ifrij and Ögel, 2002). A novel alkaline protease enzyme from a thermophilic fungus *Myceliophthora* sp. was purified and characterized by Zanphorlin *et al.* (2011). The molecular mass of the enzyme was determined 28.2 kDa by MALDI-TOF MS and it was inhibited by PMSF revealing it a serine-protease. The enzyme was optimally active at pH 9.0 and 45 °C. Mg^{2+} was the only cation that increases protease activity. The N-terminal sequence of the purified protease (GVVGVC) presented identity and homology wih other proteases from fungi.

viii) Miscellaneous Biocatalysts

Beside the above, thermophilic fungi are also known to secrete other biocatalysts too. From the mycelium extract of *Humicola insolens* (Sugiyama *et al.*, 1993), protein disulphide isomerase (EC 5.3.4.1) was purified. The glycosylated enzyme was a dimmer with a molecular mass of 120 kDa with identical subunits of 60 kDa and with a pI of 3.5.

Among eight thermophilic fungi screened for mannanases and galactanases (Araujo and Ward, 1990), highest mannanase titres were recorded in *Talaromyces byssochlamydoides* and *Talaromyces emersonii.* Mannanases were induced by locust bean gum except in the case of *Thermoascus aurantiacus*, where mannose had a greater inducing effect. Highest mannanase activity was recorded in *Talaromyces* sp. when peptone was used as nitrogen source where as sodium nitrate promoted maximum production of this enzyme by *Thielavia terrestris* and *Thermoascus aurantiacus.* The pH optima of mannanases and galactanases from thermophilic fungi were in the range of 5.0-6.6 and 4.0-5.8, respectively. The mannanase from *Talaromyces emersonii* and galactanase from *Thielavia terrestris* were the most thermostable, retaining 100% activity after 3h at 60°C. An endo-1,4-β-mannanase gene was cloned from the thermophilic fungus *Rhizomucor miehei* for the first time and expressed in *Escherichia coli* (Katrolia *et al.*, 2013). The gene has an open reading frame of 1330 bp encoding 378 amino acids and contained four introns. The purified enzyme is a monomer of 43 kDa. The enzyme displayed optimal activity at 55 °C and pH 7.0. The enzyme displayed the highest specificity for locust bean gum, followed by guar gum and konjac powder.

The precipitate from spent culture fluid of *Thielavia terrestris* were resolved into four â-D-mannanase and one β-mannosidase (Araujo and Ward, 1991). The pH optima for the enzyme were in the range of 4.5-5.5.

Chitin is a bipolymer found in the cell wall of fungi and in the exoskeleton of marine invertebrates and arthropods.

It is composed of β-1,4 linked 2-acetamido-2-deoxy-D-glucose residues, which are also named as N-acetyl-D-glucosamine residues. For the complete hydrolysis of chitin many microorganisms, plants and animals have an enzyme system composed of two hydrolytic enzymes: Chitinase (EC 3.2.1.14) and N-acetyl β-glucosamidase also named as chitobiase (EC 3.2.1.30). In cell walls of fungi , the chitin may be accompanied by chitosan which is a similar bipolymer but lacking N-acetyl group. The chitin chitosan complex occurring in the fungal cell walls is not a mixture of the two pure polymer but is represented by chitin chains which are deacetylated to a variable degree (Kauss *et al.*, 1982). An extract from the thermophilic fungi *Mucor miehei* contained an enzyme, chitin deacetylase (EC 3.5.1.41) catalyzing this reaction with optimal activity around pH 5.5 and a 65% inhibition by 10mM sodium acetate. This activity was found predominantly in the cell extract.

Trehalose (α-D-glucopyransoyl-α-glucopyranoside) is widely distributed in fungi. The first study of trehalase (EC 3.2.1.28) from a thermophilic fungus was performed by Prasad and Maheshwari (1978), and trehalase was shown to be one of the most active glucosidase in the homogenate of the mycelium from *Thermomyces lanuginosus.* The purified enzyme was optimally active at 50 °C and pH 5.5 with a high specificity for trehalose. The molecular weight was 170kDa. An extracellular trehalase was purified from *Humicola grisea* var. *thermoidea* (Zimmermann *et al.*, 1990). This enzyme was a glycoprotein, which migrated as a single polypeptide band during PAGE under non-denaturing conditions. The apparent molecular weight was 580 kD by gel filtration chromatography. The enzyme was separable into three polypeptide bands of 105kD, 98kD and 84kD on SDS-PAGE. The results indicated that the native enzyme may be a hexameric protein composed of three classes of polypeptides of different molecular weights. The purified enzyme was optimally active at 60 °C and pH 5.6 with a high specificity for trehalose (apparent K_m=2.3mM) and its activity was not inhibited by other disaccharides. The enzyme was activated by Ca^{+2}, Co^{+2} and Mn^{+2} and inhibited by inorganic phosphate, AMP, ADP, and ATP. The enzyme bound irreversibly to Con-A and had a high carbohydrate content (Sorensen and Crisan, 1974). From a study of *Scytalidium thermophilum* with starch as the carbon source, Kadowaki *et al.* (1998) found large extracellular trehalase level and an intracellular and an extracellular trehalase with molecular masses of 370 and 398kDa. The analysis by SDS-PAGE suggested that the native enzymes were composed of five subunits. The carbohydrate contents of intra- and extracellular enzymes were 51 and 81% and pI 3.7 and 3.4, respectively. Both enzymes had optimum pH at 6.0 but their temperature optima differed slightly. Km values were in the range of other trehalose (0.2-20mM). The trehalases were activated by divalent cations. A trehalase was purified from the mycelium of *Chaetomium thermophilum* var. *corpophilum.* The enzyme composed of two identical polypeptides of apparent molecular mass of 98 kDa. The pI of the enzyme was about 3.9. The purified trehalase was stimulated by calcium, manganese and cobalt and inhibited by EDTA, ADP and ATP. The enzyme exhibited a K_m of 0.63 mM, optimum pH of 6.5, and optimum temperature of 55 °C. Trehalases from a thermophilic fungus *Thermomyces lanuginosus* and a mesophile *Neurospora crassa* were purified to compare their thermal characteristics and kinetic constants. Both trehalases were maximally active at 50 °C, had an acidic pH optimum and were glycoproteins. At their temperature optimum, their K_m was similar but the V_{max} of *Neurospora crassa* enzyme was nine times higher than of *Thermomyces lanuginosus* enzyme. The catalytic efficiency (k_{cat} / $K_{m)}$ for *Neurospora crassa* trehalase was one order of magnitude higher (6.2 x 10^6 M^{-1} s^{-1}) than of *Thermomyces lanuginosus* trehalase (4 x 10^5 M^{-1} s^{-1}) [Bharadwaj and Maheshwari, 2000]. The thermophilic fungus *Scytalidium thermophilum* produced large amounts of intracellular and extracellular trehalase activity when grown on starch as the sole carbon source (Kadowaki *et al.*, 1998). The apparent molecular mass of the native enzyme was 370 kDa (extracellular trehalase) and 398 kDa (intracellular trehalase). The analysis by SDS-PAGE showed unique polypeptide bands of approx. 82 kDa (extracellular trehalase) and 85 kDa (intracellular trehalase), suggesting that the native enzymes were composed of five subunits. The carbohydrate content of extracellular and intracellular trehalases was 81% and 51%, respectively. Electrofocusing indicated a pI of 3.7 and 3.4, for the extracellular and intracellular enzymes, respectively. Both trehalases were highly specific for trehalose and were stimulated by Ca^{2+} and MN^{2+}. Inhibition was observed in the presence of aluminium, mercury, copper, zinc, EDTA, ADP, and ATP. Apparent Km values for the extracellular and intracellular trehalases were 3.58 mM and 2.24 mM, respectively. The optimum pH for the extracellular and the intracellular trehalases were 6.0, and the optimum of temperature 60 °C and 65 °C**.**

Sucrose (β-D-Fructofuranosyl-β-D-glucopyranoside) occurs specially in the juices from plants and is by far the most abundantly distributed of the sugars. Invertase (EC.3.2.1.26) hydrolyses sucrose to glucose and fructose. The ability to produce invertase was studied in shake flasks with different strains of four thermophilic fungi viz. *H. lanuginosa, Chrysosporium thermophilum, Thermomyces lanuginosus* and *Malbranchea pulchella* var. *sulfurea* (Kirillova and Shchepankevich, 1989). *Thermomyces lanuginosus* utilized sucrose earlier than it did glucose, but at 30 °C the two sugars were utilized at nearly comparable rates (Maheshwari and Balasubramanyam, 1988). The purified invertase from *Thermomyces lanuginosus* has a molecular mass of 37 kDa (Chaudhuri and Maheshwari, 1996) but was unusually unstable.

α-Galactosidase (EC.3.2.1.22) is specific for β-galactoside bond, which occurs in compounds from plant tissues, e.g. in raffinose, rhamnose, etc. *Sporotrichum thermophile* and

Thermomucor indicae-seudaticae were shown to produce α-Galactosidase extracellularily (Satyanarayana *et al.,* 1985), whereas intacellular activity was found in *M. pulchella* var. *sulfurea, Rhizopus microsporus, Talaromyces emesonii, Thermoascus aurantiacus* and *Torula thermophila.* The activity from mycelium of *Penicillium duponti* had been partly purified (Arnaud *et al.*, 1976). In the fraction with activity against 2-nitrophenyl-α-D-galactoside(2-NPG) one major and two minor bands were found PAGE stained with Coomasie blue, but the activity was found in the major band, which had molecular weight of approximately 500 kDa. The optimum temperature for activity with stachyose and raffinose was 55 °C and with 2-NPG the activity was still raising at 70 °C. The optimum pH for activity was 7.0-8.0 with stachyose and 7.5 and 8.5 with 2-NPG α-Galactosidase gene from *Rhizomucor miehei* was cloned and expressed in *Escherichia coli* (Katrolia *et al.*, 2012). The gene has an open reading frame of 2256bp encoding 751 amino acid residues. It was optimally active at pH 4.5 and 60 °C and displayed remarkable resistance to proteases. The enzyme completely hydrolyzed raffinose and stachyose present in soybeans and kidney beans at 50 °C within 60 min.

β-Galactosidase (EC.3.2.1.23) converts lactose to galactose and glucose and can thereby raise the sweetness of dairy products (e.g. milk) or lower the content of lactose in milk formulated for people with lactose-intolerance. This enzyme is also known as lactase. Among 54 strains of thermophilic fungi representing 15 species of 12 genera were tested for their ability to produce lactase (Sorensen and Crisan, 1974). Strains of *Chaetomium thermophile* var. *coprophile, Humicola grisea* var. *thermoidea, Humicola lanuginosa, Malbranchea pulchella* var. *sulfurea, Mucor pusillus, Mucor miehei, Sporotrichum thermophile* and *Torula thermophila,* exhibited good growth on semisolid lactose medium at pH 6.6 and 37 °C. *Rhizomucor pusillus* produced extracellular β-galactosidase with optimum pH and temperature of 2.5 and 60°C respectively (Tomoda *et al.*, 1976). The enzyme is stable at pH 3.0-9.0 but inactivated if exposed to temperatures higher than 60 °C at pH 7.0 for 30 min, and inhibited by Fe^{3+}, Sn^{2+}, $Pb^{2+}$and Hg^{2+}. Satyanarayana *et al.* (1985) studied β-galactosidase production in 13 thermophilic fungi and found both extra- and intracellular activity in *Humicola insolens, Malbranchea pulchella* and *Talaromyces emersonii* whereas only intracellular activity was found in *Acremonium alabamensis, Chaetomium thermophile, Humicola lanuginosa,* and *Sporotrichum thermophile* . Using lactose in the medium for cellulase production by *Talaromyces emersonii,* a β-galactosidase with Km of 20mM for lactose and 17mM for p-nitrophenyl- β-D-galactoside had been found in the mycelial extract (McHale and Morrison, 1986). The optimum pH and temperature for the activity was 6.0 and 33 °C, respectively.

Laccases (EC1.10.3.1) are the copper containing enzymes that catalyze the oxidation of phenolic compounds that is accompanied by reduction of oxygen to the water. The range of substrate oxidized varies from one laccase to another. Laccase like activity has been demonstrated in compost (Chefetz *et al.*, 1998) Laccases were also found in *Scytalidium thermophilum* and *Myceliophthora thermophila*. The gene encoding laccase of *Myceliophthora thermophila* was cloned and expressed in *Aspergillus oryzae* and the recombinant enzyme was purified from culture broth with a two to four-fold-higher yield (11-19 mg l^{-1}) than that of native laccase (Berka *et al.*, 1997). The recombinant enzyme differed from the native in three respects: multiplicity of isoforms, high molecular weight (85 versus 80kDa), and three-fold higher specific activity. The optimum pH for the activity was 6.5 and the enzyme retained full activity when incubated at 60 °C for 20 min. Chefetz *et al.* (1998) purified a laccase from the culture filtrate of a thermophilic fungus *Chaetomium thermophilum* by ultrafiltration, anion-exchange chromatography and affinity chromatography. The enzyme was a glycoprotein of 77kDa showing stability in a broad pH range from 5.0-10.0 at a temperature of 50 °C, but its optimum pH was 6.0. Its half-life was 12h at 50 °C. Cu-chelating agents inhibited the activity. It was suggested that laccase from this fungus is involved in the humification process during composting.

Keratinase production by *Myceliophthora thermophila* GZUIFR-H49-1 was optimized by Liang *et al.* (2011) with the help of statistical designs that resulted in 6.4-fold enhancement in production.. Among all the medium components tested, the soluble starch, urea, sodium thiosulfate and $CaCl_2$ affected keratinase production significantly.

A low molecular mass cutinase from *Thielavia terrestris* was purified and biochemically characterized (Yang *et al.*, 2013). The mould secreted a highly active cutinase in the medium containing wheat bran as the carbon source. The cutinase was purified 19-fold with a recovery yield of 4.8 %. The molecular mass of the purified enzyme was 25.3 and 22.8 kDa using SDS-PAGE and gel filtration, respectively. It displayed optimal activity at pH 4.0 and 50 °C. The enzyme was highly stable in organic solvents. The K_m values for this enzyme towards *p*-nitrophenyl acetate, pNP butyrate, and pNP caproate were 7.7, 1.0, and 0.52 mM, respectively.

THERMOPHILIC FUNGAL GENOMES

The genome of *Myceliophthora thermophila* has been sequenced by Berka *et al.* (2011). The finished 38.7 Mb genome of *Myceliophthora thermophila* contains seven telomere-to-telomere chromosomes with 51.4% GC content (Berka *et al.*, 2011). Their telomeres comprise TTAGGG repeats commonly found in 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 comparison of proteins encoded in the genome of *Myceliophthora* with other fungi revealed that mould harbor large number (>210) of glycoside

hydrolases and polysaccharide lyases covering most of the recognized families (Berka *et al.*, 2011). *Myceliophthora thermophila* 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 genome of *Myceliophthora thermophila* encodes an array of hydrolytic and oxidative enzymes besides CAZymes, enabling the mould to utilize non-carbohydrate substrates also (Berka *et al.*, 2011). The secretome of *Myceliophthora thermophila* 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).

Thielavia terrestris is a thermophilic and acidophilic fungus found even in geothermal environments. It can grow at temperatures up to 55 °C (Berka *et al.*, 2011). The 36.9 Mb genome of *T. terrestris* contains six telomere-to-telomere chromosomes with 54.7% GC content (Berka *et al.*, 2011). Their telomeres comprise TTAGGG repeats commonly found in filamentous fungi. The major difference occurs in chromosome (Ch)1 of *T. terrestris* that harbor most of the genes located on Ch2 and Ch4 of *Myceliophthora thermophila*. The protein coding regions of the genome contain 9,813 genes with largest gene families of transporters and signaling proteins higher than *Myceliophthora thermophila* (Berka *et al.*, 2011). The mould is poor in pectin and pectate lyases and relatively rich in polygalacturonases in contrast to *Myceliophthora thermophila*. The genome analysis suggests that the two thermophiles can be considered as good decomposers due to the secretion of a battery of hydrolytic and oxidative enzymes.

CONCLUSIONS AND FUTURE PERSPECTIVES

Thermophilic fungi are ubiquitous in their occurrence in thermogenic as well as non-thermogenic environments. These fungi are capable of degrading organic matter efficiently due to their extracellular enzymes. Thermophilic fungal biocatalysts are thermostable and sturdy, and therefore, are useful for several industrial applications. The thermophilic fungi play an important role in environmental management because of their ability to degrade the materials present in industrial effluents, heavy metal biosorption and in producing bioethanol from lignocellulosic residues. The analysis of genome sequences of the thermophilic fungi provided evidence for the presence of various hydrolytic enzymes. Efforts are underway to further understand the diversity of thermophilic fungi and their biocatalysts using both culture-dependent and culture-independent approaches.

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