INDUSTRIAL AND BIOTECHNOLOGICAL POTENTIAL OF MICROBIAL CELLULASES

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Abstract: Biodegradation of plant cellulose is achieved through a concerted action of a group of enzymes of the cellulase system, synthesized by a diverse range of organisms. This biodegradation holds importance not only in the efficient recycling of cellulosic biomass within the biosphere, but also in a vast variety of industrial and biotechnological applications. In industrial and research arena, there is an increased interest in utilizing cellulases for lignocellulosic biomass conversion for the production of biobased products and bioenergy. This article presents an overview of cellulase producing microorganisms, along with the important applications of cellulases in the bioconversion of lignocellulosic biomass and in several industries like food, animal feed, brewery, wine, textile, laundry, paper and pulp.

Keywords: Cellulase system, Cellulosome, Industrial applications, Lignocellulose bioconversion

INTRODUCTION

Cellulose, a linear condensation polymer of β-1,4-linked glucose subunits, is the major carbohydrate synthesized by plants, where it forms highly tensile, insoluble, crystalline microfibrils that are resistant to enzymatic hydrolysis. Organisms that degrade cellulose, produce a group of cellulolytic enzymes that act synergistically with different specificities. These organisms play a pivotal role in the biodegradation and efficient recycling of cellulosic biomass within the biosphere (Béguin and Aubert, 1994). However, only a few microorganisms are capable of completely degrading native cellulose through the concerted action of the enzymes of the cellulase complex (Sharrock, 1988).

Cellulases are synthesized by a diverse array of organisms such as fungi, bacteria including actinomycetes, plants, and some invertebrates. The enzymatic cellulose hydrolysis (cellulolysis) involves cooperative interactions of three main components, endo-1,4-β-D-glucanase or endoglucanase (EC 3.2.1.4), exo-1,4-β-D-glucanase or cellobiohydrolase (CBH) (EC 3.2.1.91), and β-D-glucosidase or cellobiase (EC 3.2.1.21) (Kumar *et al.*, 2011), whereas some organisms also produce either 1,4-β-D-glucan glucohydrolase (EC 3.2.1.74), which catalyzes the removal of glucose residues from the non-reducing end of cello-oligosaccharides (McHale and Coughlan, 1980; Wood and McCrae, 1982), or cellodextrinase, which hydrolyses soluble cello-oligosaccharides into cellobiose Co(Huang and Forsberg, 1987) (Fig. 1). Endoglucanase randomly attacks cellulosic substrates in the amorphous regions and releases cello-oligosaccharides, then cellobiohydrolase acts on the non-reducing or reducing ends of the cello-oligosaccharides to remove cellobiose units (Vrsanska and Biely, 1992; Wood and Bhat, 1988), and finally β-D-glucosidase converts the cellobiose to glucose, thereby completing cellulolysis (Coughlan, 1985). Endoglucanase and cellobiohydrolase can synergistically act on crystalline cellulose to release cello-oligosaccharides and cellobiose (Mandels and Reese, 1964). End product inhibition may occur by cellobiose on endoglucanase and cellobiohydrolase, and by glucose on cellobiase (Goyal *et al.*, 1991). Aerobic bacterial and fungal cellulases are simpler in structure, whereas anaerobic bacterial and fungal cellulases are usually in the form of a multiple enzyme complex system known as cellulosomes (Zhang *et al.*, 2006).

Cellulases have attracted much interest owing to the diversity of their applications (Sakthivel *et al.*, 2010). Microbial cellulases have many potential industrial and biotechnological applications, and hence are in high demand (Kasana *et al.*, 2008). Biodegradation of cellulose by microorganisms is one of the most efficient ways to obtain small products with bioactivities of high value. An important application lies in the saccharification of lignocelluloses to fermentable sugars, which can be used to produce bioethanol and other useful products. Cellulases can be utilized for the hydrolysis of cellulosic portion of residual agrowastes to get glucose, and glucose thus obtained can be fermented by potential microbial strains to get alcohol (Singh and Mukerji, 1989). Several other applications of cellulases have become economically feasible and commercialized, like in food processing sector, brewery and wine industry, animal feed industry, textile processing, detergent market, paper industry, waste water treatment, etc. Industrial applications demand highly stable cellulases that can tolerate extreme temperatures and pH, and search for extremophiles is one of the means for meeting this demand (Ibrahim and El-diwany, 2007).

Sources of Cellulase

Cellulase producing organisms are found among extremely variegated taxonomic groups belonging to eubacteria, including actinomycetes, fungi, some plants and invertebrates; and they occur in mixed populations of synergistically interacting cellulolytic and non-cellulolytic species (Béguin and Aubert, 1994). Large number of microorganisms can produce cellulase, albeit, only a few of them are capable of producing significant quantities of cell-free bioactive enzymes that completely hydrolyze native crystalline cellulose, and hence fulfill the demands of industrial requirement (Bai *et al.*, 2012).

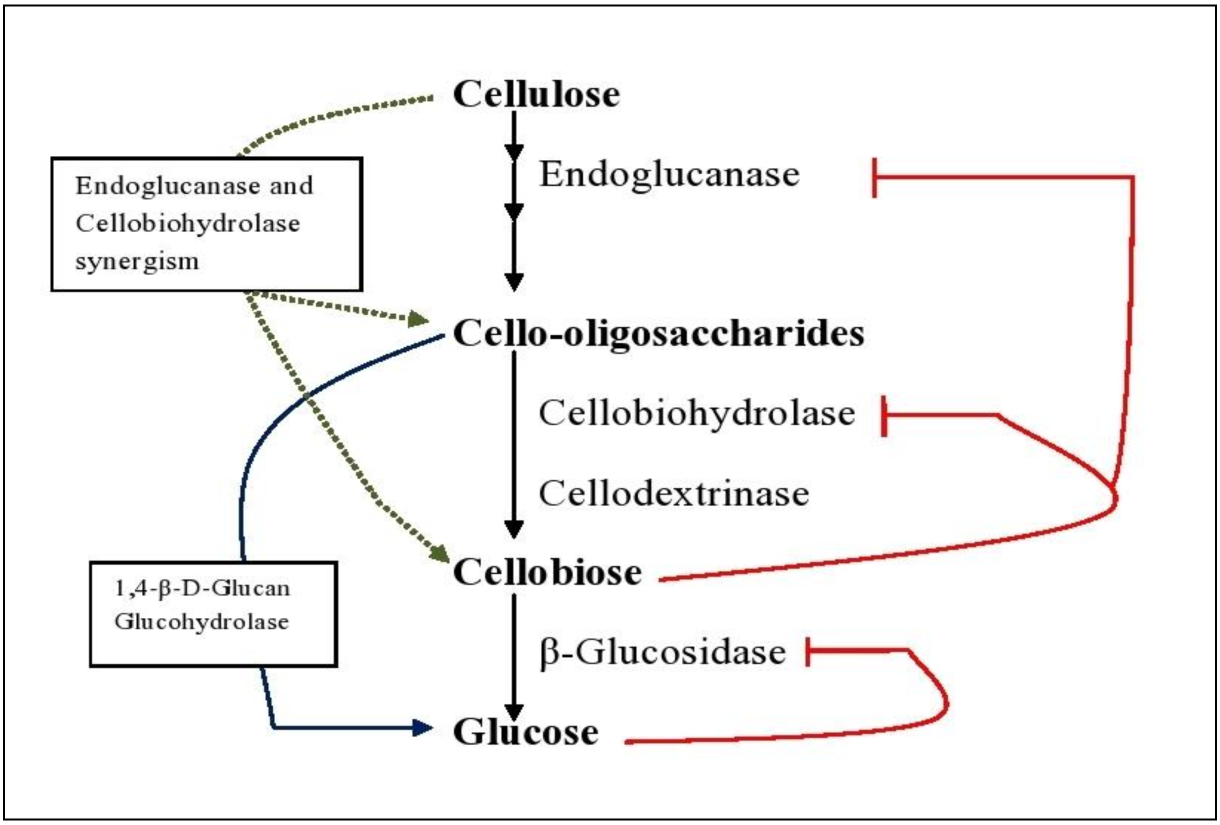

Fig. 1: General mechanism of cellulose hydrolysis (see text for details).

Among the many microorganisms, cellulases from the genera of *Clostridium*, *Cellulomonas*, *Thermonospora*, *Trichoderma* and *Aspergillus* have been the most extensively investigated (Kuhad *et al.*, 2011). Microorganisms like fungi and bacteria produce mainly three types of cellulolytic enzymes, namely endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase or 1,4-β-D-glucan cellobiohydrolase (CBH) and β-D-glucosidase, while some like *Penicillium funiculosum* and *Talaromyces emersonii* also produce 1,4-β-D-glucan glucohydrolase (Bhat and Bhat, 1997). Certain anaerobic cellulolytic fungi and bacteria produce a multi-component enzyme complex, known as the crystalline cellulose solubilizing factor (CCSF) and cellulosome (Lamed and Bayer, 1988; Wood *et al*., 1986). Cellulolytic microorganisms can be aerobic, anaerobic, mesophilic, thermophilic or psychrophilic, and some of them are mentioned in Table 1.

Cellulolytic organisms have been isolated from a wide variety of sources for the purpose of obtaining more efficient enzymes, such as from feces of ruminants (Akhtar *et al.*, 2012; Bai *et al.*, 2012; Kasana *et al*, 2008; Khan *et al.*, 2011), paper waste storage (Singh and Kumar, 1998), compost (Acharya *et al.*, 2012; Lv and Yu, 2013), decayed plant materials (Ponnambalam *et al.*, 2011; Sakthivel *et al.*, 2010), soil (Irfan *et al.*, 2012), water sample (Jayashree *et al.*, 2011), fish gut (Saha *et al.*, 2006), invertebrate gut (Gupta *et al.*, 2012; Shankar *et al.*, 2011), extreme environments like hot springs (Ibrahim and El-diwany, 2007), etc. Cellulolytic fungi occur in diverse niches with broad spectrum roles. Fungi of diverse classes hydrolyze cellulose through cellulolysis for various purposes. For instance, oomycetes (*Leptomitus*, *Pythium*) and ascomycetes (*Ceratocystis*) produce cellulase during mycelial wall extension, while rumen fungi help in dissociating plant cellulose for efficient ruminal digestion (Goyal *et al.*, 1991). *Trichoderma* cellulase system is one of the best characterized cellulase, based on biochemical and molecular biological approaches. There are multiple forms of the cellulase system in fungi, like for example *Trichoderma reesi* cellulase contains six endoglucanases, three cellobiohydrolases and one cellobiase (Beldman *et al.*, 1985). Such multiple forms usually arise from complexing of the protein with ampholyte carrier proteins (Farkas *et al.*, 1987) and with carbohydrates like lectin (Alluralde and Ellenrieder, 1984) or, also from the degree of glycosylation (Gum and Brown,

1977) and from proteolytically derived enzymes with new substrate specificities (Nakayama *et al.*, 1976).

Even though fungi are considered to be superior counterparts of cellulose degradation, still they do not produce a greatly superior cellulase system, and their cellulases are no better than those from certain bacteria (Gilkes *et al.*, 1991). Cellulolytic bacteria mostly produce endoglucanases, while some like *Microbispora bispora* also produce cell-bound cellobiohydrolase, and anaerobic bacteria like *Clostridium* sp. produce discrete cellulosome complex, comprising of different cellulolytic and other enzymes (Bhat and Bhat, 1997). Periplasmic cellodextrinases which degrade cellulooligomers have been found in some bacteria like *Bacteroides succinogenes* (Huang and Forsberg, 1987). *Persea americana* (Avocado) and *Dictyostelium discoideum* also produce cellulolytic enzymes during the maturation of their fruits and spores, respectively (Béguin and Aubert, 1994). Cellulase activity has also been reported in a number of invertebrates like annelids, molluscs and crustaceans, in their digestive gland secretions (Yokoe and Yasumasu, 1964).

Heat-tolerant enzymes from thermophiles have tremendous potential in industries and biotechnology due to their inherent capability to function under elevated temperatures (Kumar and Singh, 2011). Thermophilic cellulolytic microorganisms like *Thermonospora*, *Clostridium*, *Thermoascus*, etc. can produce stable cellulases that can ferment wide variety of substrates under a wide range of pH and temperature, and hence, have attracted considerable research interest due to their demand in industrial biotechnology (Bhat and Bhat, 1997). Moreover, certain psychrophilic microorganisms like *Acremonium alcalophilum*, *Arthrobacter* sp., etc., also produce cellulases at low temperatures, and are in huge demand in laundry market, environmental bioremediation, food industry and molecular biology (Kasana and Gulati, 2011). Considerable efforts are in progress worldwide to isolate microbial strains with efficient cellulolytic activity. Microbial cellulases are becoming increasingly important and their detailed investigation like three-dimensional structures and complete mechanism of cellulolysis, is necessary to improve their utility.

Cellulases in the Lignocellulosic Waste Bioconversion

Due to the global climate change and elevated fuel costs caused by excessive usage of fossil fuels, there have been several efforts to utilize natural renewable resources for the production of greener energy. Second generation fuels, based on non-edible crops like lignocellulosic biomass is gaining immense attention, because it is a potential resource for the production of biofuels, and also due to the fact that it is largely abundant, inexpensive and is environment friendly (Maki *et al.*, 2009). Plant biomass is essentially composed of lignocelluloses along with small portions of low molecular weight compounds, wherein lignocellulose is a complex of interacting primary polymers of cellulose, hemicellulose and lignin (McCarthy, 1987). The conversion of plant biomass is of immense ecological and biotechnological importance.

Lignocellulosic biomass is produced in increasing amounts in the form of municipal and industrial wastes, agricultural residues, etc., and their degradation is effected by the cooperative action of mixed populations of microorganisms in nature. Unfortunately on a global scale, much of the lignocellulosic waste is discarded by burning, which causes pollution. Hydrolysis of lignocellulose is complicated by the complexing of cellulose with hemicellulose, lignin and other components, because of which, its biodegradation is not only dependent on the environmental conditions but also on the ability of the microorganisms to effectively catalyze degradation (Waldrop *et al.*, 2000). Lignocellulose hydrolysis requires efficient degradation of cellulose, hemicellulose and lignin. For cellulose hydrolysis, a concerted action of endoglucanase, exoglucanase and β-glucosidase is essential (Tomme *et al.*, 1995); whereas hemicellulose being more heterogeneous, requires a variety of other enzymes (Kuhad *et al.*, 1997). The rate-limiting step of lignin degradation is difficult, and it requires an oxidative degradation that includes several enzymes like lignin peroxidases, manganese peroxidases and laccases (Kuhad *et al.*, 1997; Leonowicz *et al.*, 1999).

Fungi and bacteria have been heavily exploited for their abilities to degrade lignocellulose, and most emphasis has been on fungal biodegradation, because of their ability to produce and secrete copious amounts of less complex cellulases and hemicellulases (Maki *et al.*, 2009). Several anaerobic fungi of the genus *Piromyces*, *Neocallimastix*, *Orpinomyces*, etc., produce highly active cellulases and hemicellulases like xylanase, and thus, can be effectively utilized in biotechnology (Hodrova *et al.*, 1998). Whereas, white rot fungi like *Phanerochaete chrysosporium*, *Pleurotus aureus*, *Tramtes versicolor*, etc., are some of the most powerful degraders of lignin, thereby overcoming this rate-limiting step (Edler and Kelly, 1994). Nowadays, bacterial enzymes are being widely exploited due to their higher growth rate, extreme resistance to environmental stress and production of stable multi-enzyme complexes that perform with increased function and synergy (Maki *et al.*, 2009). Some novel bacterial and fungal feruloyl and *p*-coumaroyl esterases act synergistically with hemicellulases to degrade hemicellulose-lignin association (Borneman *et al.*, 1990; Kuhad *et al.*, 1997). A diverse range of both mesophilic and thermophilc species of *Streptomyces*, *Micromonospora*, *Microbispora*, *Thermonospora*, *Actinomadura*, *Pseudonocardia*, *Saccharomonospora*, *Nocardia* and *Rhodococcus* also exhibit activity against lignocellulose (McCarthy, 1987).

Successful exploitation of lignocelluloses for commercial purposes is limited by many physical and chemical barriers of waxes and cuticles of the intact plant epidermis along with the recalcitrance of the lignified tissues, due to which, there is a need for suitable initial mechanical, chemical, thermal and biological treatments of the substrates for increasing the accessibility of the microbial enzymes to the substrate (Fan *et al*., 1982). Hence, biological conversion of lignocelluloses requires the development of cheap substrate pre-treatment techniques, along with improved cellulolysis and efficient cellulolytic product's fermentation (Béguin and Aubert, 1994). Usually, enzymatic cellulolysis is performed prior to the fermentation step, but the process can be simplified through simultaneous cellulolysis and fermentation by engineering organisms with good cellulolytic efficiency to have improved fermentative properties (Hogsett *et al*., 1992).

Industrial bioconversion of lignocelluloses requires multifunctional cellulases with broad substrate utilization and efficiency at wide temperature and pH ranges used in industrial conditions (Bai *et al*., 2012). Complete cellulose hydrolysis into glucose, which could be fermented into ethanol, isopropanol or buatnol is not yet economically feasible, albeit, development of efficient processes are required to generate fuels from cellulose, to fulfill the need to provide suitable alternatives to the depleting fossil fuels and increasing greenhouse gases (Béguin and Aubert, 1994). Hence, there is a substantial growing interest in developing adept processes for utilizing cellulases for the proficient treatment of the inexpensive cellulosic wastes, which can offer tremendous advantages of biomass utilization.

Industrial Potential of Cellulases

Cellulases were utilized in the early 1980s in the animal feed and food industries, followed by their use in the textile, wine, brewery, laundry and paper industries (Bhat, 2000), and is briefly illustrated in Table 2. Today, cellulases along with hemicellulases and pectinases account for about 20% of the world enzyme market (Mantyla *et al*., 1998). Cellulases have important applications in:-

a) **Animal feed industry:** Cellulases have a wide range of potential applications in animal feed industry, which is an important sector of agribusiness comprising of ruminants, poultry, pigs, pet foods and fish farming (Bhat, 2000). Cellulases help in eliminating the antinutritional factors present in grains or vegetables, and in improving the nutritional value of feed by degrading certain cereal compounds (Galante *et al*., 1998b). β-glucanases hydrolyze cereal β-glucans, which helps in decreasing intestinal viscosity and releasing nutrients from grains, thereby markedly improving the digestion and absorption of feed components and weight gain by broiler chickens and hens (Cowan, 1996; Hesselman *et al*., 1982). Cellulases have also been used to improve the feed utilization, milk yield and body weight gain by ruminants (Bhat, 2000).

b) **Food industry:** Cellulases help in improving the nutritive quality of fermented foods, homogeneous water absorption by cereals and dried vegetables, and in the production of low-calorie food ingredient oligosaccharides (Béguin and Aubert, 1994; Bhat and Bhat, 1997; Mandels, 1985). Cellulases in association with pectinases are used to release antioxidants from fruit and vegetable pomace, which helps in controlling coronary heart disease, atherosclerosis, and in reducing food spoilage (Meyer *et al*., 1998). Cellulases with pectinases and hemicellulases are used to macerate fruit pulps to maximum possible liquefaction, which results in higher and more nutritive juice yield with better stability and reduction of processing time, and they are also used in the extraction of olive oil with higher levels of antioxidants, vitamin E, and slower induction of rancidity (Bhat, 2000). Vacuum infusion of the cellulase component, β-glucosidase is used to increase the aroma and volatile characters of fruits and vegetables (Bhat, 2000).

c) **Brewery and wine industry:** Inclusion of cellulases and related polysaccharidases has been known to improve the efficient production of high quality beer and wine. In brewery, endoglucanases are used to overcome the gel or precipitate formation, along with the low extract yield of beers, caused due to the use of unmalted or poor quality barley during malting and fermentation (Galante *et al*., 1998b). In wine production, use of cellulases and other enzymes help in hydrolyzing the plant cell wall polysaccharides, which considerably improves skin maceration, color extraction of grapes, quality, stability, clarification and aroma of wines (Caldini *et al*., 1994; Galante *et al*., 1998b; Gunata *et al*., 1990).

d) **Textile industry:** Cellulases have been utilized successfully in textile industries worldwide, because of their ability to improve the fabric quality through controlled modification of cellulosic fibres. Important applications are in bio-stoning of denim garments, bio-polishing of non-denim fabrics and in defibrillation of lyocell containing fabrics (Bhat, 2000). During bio-stoning of denim garments, use of cellulases provides a less work intensive and safer working conditions without causing any environmental pollution. Cellulases break-off small fibre ends on the cotton fabric, which eventually causes loosening of the indigo dye after washing, leading to the highly desired aged effect of the denim garments (Galante *et al.*, 1998a). In bio-polishing of non-denim garments, cellulases remove the excess of the short microfibrils and surface fuzziness, which produces a smooth glossy appearance with improved color brightness and uniformly improved finishing (Galante *et al.*, 1998a).

e) **Laundry industry:** Cellulases are used in laundry to improve the production of high quality fabrics. They are utilized in household washing powders to enhance the detergent performance by removing small fuzzy fibrils extruding from fabric surfaces, which leads to an improved color brightness along with the restoration of softness in cotton fabrics and better removal of trapped dirt particles in the microfibril network (Galante *et al.*, 1998a; Godfrey, 1996).

F) **Paper and pulp industry:** Cellulases have been used along with other enzymes in the paper and pulp industry for bio-mechanical pulping, bio-modification of fibres, removing of ink coating and toners from paper, improving drainage of the paper mills, and manufacturing of soft papers like paper towels and sanitary papers (Saranraj *et al*., 2012). In bio-mechanical pulping, use of cellulases have led to the reduced high-energy consumption and improved fibre properties (Leatham *et al*., 1990). In bio-modification of fibres, cellulases along with hemicellulases have been used to improve the pulp beatability, paper sheet density and runnability, leading to highly productive and trouble free printing process (Noe *et al*., 1986).

CONCLUSION

Since cellulose is an exuberant renewable natural biological resource, the production of bio-based products from them is imperative, and for that, reduction in cellulase production cost, enhancement in cellulase performance along with specific activities, and increased sugar yields are required for efficiency. Advancement of technologies for effectively converting less costly agricultural and forest residues to highly useful products can, not only provide an improved environmental quality, but also a sustainable energy resource. Finally, it is necessary to increase the volumetric production of stable cellulases with greater catalytic efficiency on native cellulosic substrates, and because of their numerous practical applications at industrial level along with constantly increasing demand of these enzymes, there is an urgent need to explore new environments for the isolation of cellulolytic microorganisms.

Table1: Some important microbial sources of cellulase. Here, 'M/T/P*' represents either mesophilic (M), thermophilic (T) or psychrophilic (P), and 'CC#' represents cellulase components, where 1- endo-1,4-β-D-glucanase or endoglucanase, 2- exo-1,4-β-D-glucanase or cellobiohydrolase (CBH), 3- β-D-glucosidase or cellobiase, 4- 1,4-β-D-glucan glucohydrolase, 5- cellobiose dehydrogenase, 6- cellulosome complex, and 7- cellodextrinase.

Organism	M/T/P*	CC#	References
Fungi			
(Aerobic)			
Sporotrichum pulverulentum Fusarium	M	1,2,3	Eriksson, 1978
solani	M	1,2,3	Wood and McCrae, 1977
Penicillium funiculosum	M	1,2,3,4	Wood and McCrae, 1982
Penicillium pinophilum	M	1,2,3	Wood and McCrae, 1986
Talaromyces emersonii	T	1,2,3,4	McHale and Coughlan, 1980
Trichoderma koningii	M	1,2,3	Wood and McCrae, 1972 & 1982
Trichoderma reesi	M	1,2,3	Kubicek, 1992
Trichoderma viride	M	1,2,3	Bauchop, 1979
Myceliophthora thermophila [*Sporotrichum thermophile*]	T	1,2,3	Bhat and Maheshwari, 1987
Thermoascus aurantiacus	T	1,2,3	Khandke *et al*., 1989
Chaetomium thermophile	T	1,2,3	Bhat and Bhat, 1997
Humicola insolens	T	1,2,3,5	Bhat and Bhat, 1997
Acremonium alcalophilum	P	1	Hayashi *et al*., 1996
Rhodotorula glutinis (Yeast)	P	1	Oikawa *et al*., 1998
(Anaerobic)			
Neocallimastix frontalis	M	6	Wood *et al*., 1986
Piromonas communis	M	6	Wood, 1992
Sphaeromonas communis	M	6	Wood, 1992
Orpinomyces sp. PC-2	M	6	Ljungdahl, 2008
Bacteria			
(Aerobic)			
Bacillus subtilis			

Bacillus pumilus	M	1,2,3	Chan and Au, 1987
Bacillus circulans	M	1,3	Ariffin *et al.*, 2006
Pseudomonas sp. strain CL3	M	1,2,3	Kim and Kim, 1993
Pseudomonas fluorescens subsp.	M	1,2,3	Cheng and Chang, 2011
cellulosa	M	1,7	Ferreira *et al.*, 1991; Hazlewood *et al.*,
Acinetobacter anitratus			1992
Branhamella sp.	M	1,3	Ekperigin, 2007
Eubacterium cellulosovens	M	1,3	Ekperigin, 2007
Paenibacillus curdlanolyticus strain B-6	M	1,3	Anderson and Blair, 1996
Salinivibrio sp. strain NTU-05	M	1,2,3	Pason *et al.*, 2006
Arthrobacter sp. strain C2-2			
Acidothermus cellulolyticus 11B	M	1	Wang *et al.*, 2009
Erwinia chrysanthemi strain 3665	P	3	Benesova *et al.*, 2005
(Anaerobic)	T	1,2,3	Barabote *et al.*, 2009
Butyrivibrio fibrosolvens	M	1	Boyer *et al.*, 1984
Ruminococcus flavefaciens			
Ruminococcus albus	M	6,7	Berger *et al*, 1990
Clostridium cellulovorans	M	6	Aurilia *et al.*, 2000
	M	6	Ohara *et al.*, 2000
Clostridium cellobioparum	M	6	Shoseyov and Doi, 1990; Tamaru *et*
Clostridium papyrosolvens			*al.*, 2000
Clostridium josui	M	6	Lamed *et al.*, 1987
Clostridium cellulolyticum	M	6	Pohlschröder *et al.*, 1995
	T	6	Kakiuchi *et al.*, 1998
Clostridium thermocellum	M	6	Bélaich *et al.*, 1997; Pagés *et al.*, 1997
Clostridium cellulofermentas			Lamed and Bayer, 1988
Bacteroides cellulosovens	T	6	Yanling *et al.*, 1991
Fibrobacter succinogenes	M	6	Lamed *et al.*, 1991
	M	6	Huang and Forsberg, 1987; Schellhorn
Acetovibrio cellulolyticus	M	6, 7	and Forsberg, 1984
Paenibacillus sp. strain C7			Ding *et al.*, 1999
Paenibacillus sp. BME-14	M	6	Shipkowski and Brenchley, 2005
Pseudoalteromonas sp. MB-1	P	3	Fu *et al.*, 2010
Pseudoalteromonas sp. DY3	P	3	You and Wang, 2005
Shewanella sp. G5	P	1	Zeng *et al.*, 2006
	P	1	Cristobal *et al.*, 2008
Actinomycetes	P	3	
Cellulomonas biazotea NCIM-2550			
Streptomyces lividans			Saratale *et al.*, 2010
	M	1,2,3	
Streptomyces flavogriseus			Kluepfel *et al.*, 1986; Moldoveanu and
Micromonospora melanospora	M	1,3	Kluepfel, 1983
Micromonospora bispora			MacKenzie *et al.*, 1984
Thermonospora sp.	M	1,2,3	Van Zyl, 1985
	M	1,2,3	McCarthy, 1987
	T	1,2,3	Hagerdal *et al.*, 1980
	T	1,2,3	

Table 2: Some important industrial applications of cellulases (Modified from Bhat, 2000)

Industry	Major Applications
Animal Feed	a) Improvement of the monogastric and ruminant feed nutritional quality. b) Improvement in the digestion, absorption and weight gain of broiler chickens and hens.
Food	a) Extraction and clarification of fruit and vegetable juices, along with the production of fruit nectars and purees. b) Alteration of the sensory properties of fruits and vegetables through vacuum infusion, and extraction of olive oil.

Brewery and Wine	a) Overcoming of precipitate formation and improvement of low extract yield of beers. b) Improvement of quality, stability, clarification and aroma of wines.
Textile	a) Bio-stoning of denim garments. b) Bio-polishing of cotton and non-denim fabrics. c) Defibrillation of lyocell containing fabrics.
Laundry	a) Improvement in the color brightness of clothes. b) Better dirt removal from cotton fabrics.
Paper and Pulp	a) Bio-mechanical pulping and bio-deinking. b) Bio-improvement of drainage properties of paper mills. c) Bio-modification and characterization of pulp fibres.

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REFERENCES

Acharya, A.; Joshi, D.R.; Shrestha, K. and Bhatta, D.R. (2012). Isolation and screening of thermophilic cellulolytic bacteria from compost piles. *Scientific World*, **10**(10): 43-46.

Akhtar, N.; Sharma, A.; Deka, D.; Jawed, M.; Goyal, D. and Goyal, A. (2012). Characterization of cellulase producing *Bacillus* sp. for effective degradation of leaf litter biomass. *Environmental Progress and Sustainable Energy*, doi: 10.1002/ep.11726.

Alurralde, J.L. and Ellenrieder, G. (1984). Effect of attached carbohydrates on the activity of *Trichoderma viride* cellulases. *Enzyme and Microbial Technology*, **6**: 467-470.

Anderson, K.L. and Blair, B.G. (1996). Regulation of the cellulolytic activity of *Eubacterium cellulosolvens* 5494: a review. *SAAS Bulletin, Biochemistry and Biotechnology*, **9**: 57-62.

Ariffin, H.; Abdullah, N.; Umi Kalsom, M.S.; Shirai, Y. and Hassan, M.A. (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology*, **3**(1): 47-53.

Aurilia, V.; Martin, J.C.; McCrae, S.I.; Scot, K.P.; Rincon, M.T. and Flint, H.J. (2000). Three multidomain esterases from the cellulolytic rumen anaerobe *Ruminococcus flavefaciens*17 that carry divergent dockerin sequences. *Microbiology*, **146**: 1391-1397.

Bai, S.; Kumar, M.R.; Kumar, D.J.M.; Balashanmugam, P.; Balakumaran, M.D. and Kalaichelvan, P.T. (2012). Cellulase production by *Bacillus subtilis* isolated from cow dung. *Archives of Applied Science Research*, **4**(1): 269-279.

Barabote, R.D.; Xie, G.; Leu, D.H.; Normand, P.; Necsulea, A.; Daubin, V.; Me´digue, C.; Adney, W.S.; Xu, X.C.; Lapidus, A.; Parales, R.E.; Detter, C.; Pujic, P.; Bruce, D.; Lavire, C.; Challacombe, J.F.; Brettin, T.S. and Berry, A.M. (2009). Complete genome of the cellulolytic thermophile *Acidothermus cellulolyticus* 11B provides insights into its ecophysiological and evolutionary adaptations. *Genome Research*, **19**(6): 1033-1043.

Bauchop, T. (1979). Rumen anaerobic fungi of cattle and sheep. *Applied and Environmental Microbiology*, **38**: 148-158.

Béguin, P. and Aubert, J.P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, **13**(1): 25-58.

Bélaich, J.P.; Tardif, C.; Bélaich, A. and Gaudin, C. (1997). The cellulolytic system of *Clostridium cellulolyticum*. *Journal of Biotechnology*, **57**: 3-14.

Beldman, G.; Searle-Van Leeuwen, M.F.; Rombouts, F.M. and Voragen, F.G.J. (1985). The cellulase of *Trichoderma viride*: Purification, characterization and comparison of all detectable endoglucanases, exoglucanases and β-glucosidases. *European Journal of Biochemistry*, **146**: 301-308.

Benesova, E.; Markova, M. and Kralova, B. (2005). α-Glucosidase and β-glucosidase from psychrotrophic strain *Arthrobacter* sp. C2-2. *Czech Journal of Food Sciences*, **23**: 116-120.

Berger, E.; Jones, W.A.; Jones, D.T. and Woods, D.R. (1990). Sequencing and expression of a cellodextrinase (*ced1*) gene from *Butyrivibrio fibrisolvens* H17c cloned in *Escherichia coli*. *Molecular Genetics and Genomics*, **223**: 310-318.

Bhat, K.M. and Maheshwari, R. (1987). *Sporotrichum thermophile*: Growth, cellulose degradation and cellulase activity. *Applied and Environmental Microbiology*, **53**: 2175-2182.

Bhat, M.K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, **18**: 355-383.

Bhat, M.K. and Bhat, S. (1997). Cellulose degrading enzymes and their potential industrial applications. *Biotechnology Advances*, **15**: 583-620.

Borneman, W.S.; Hartley, R.D.; Morrison, W.H.; Akin, D.E. and Ljundahl, L.G. (1990). Feruloyl and *p*-coumaroyl esterase from anaerobic fungi in relation to plant cell wall degradation. *Applied Microbiology and Biotechnology*, **33**: 345-351.

Boyer, M.H.; Chambost, J.P.; Magnan, M. and Cattanéo, J. (1984). Carboxymethyl-cellulase from *Erwinia chrysanthemi*. I. Production and regulation of extracellular carboxymethyl-cellulase. *Journal of Biotechnology*, **1**: 229-239.

Caldini, C.; Bonomi, F.; Pifferi, P.G.; Lanzarini, G. and Galante, Y.M. (1994). Kinetic and immobilization studies on fungal glycosidases for aroma enhancement in wine. *Enzyme and Microbial Technology*, **16**: 286-291.

Chan, K.Y. and Au, K.S. (1987). Studies on cellulase production by a *Bacillus subtilis*. *Antonie van Leeuwenhoek*, **53**: 125-136.

Cheng, C.L. and Chang, J.S. (2011). Hydrolysis of lignocellulosic feedstock by novel cellulases originating from *Pseudomonas* sp. CL3 for fermentative hydrogen production. *Bioresource Technology*, **102**: 8628-8634.

Coughlan, M.P. (1985). Cellulases: Production, properties and applications. *Biochemical Society Transactions*, **13**: 405-406.

Cowan, W.D. (1996). Animal feed. In: Godfrey, T. and West, S. (ed.) Industrial Enzymology, 2nd edition, Macmillan Press, London, pp: 360-371.

Cristobal, H.A.; Breccia, J.D. and Abate, C.M. (2008). Isolation and molecular characterization of *Shewanella* sp. G5, a producer of cold-active beta-D-glucosidases. *The Journal of Basic Microbiology*, **48**: 16-24.

Ding, S.Y.; Bayer, E.A.; Steiner, D.; Shoham, Y. and Lamed, R. (1999). A novel cellulosomal scaffoldin from *Acetovibrio cellulolyticus* that contains a family 9 glycosyl hydrolase. *Journal of Bacteriology*, **181**: 6720-6729.

Edler, D.J. and Kelly, D.J. (1994). The bacterial degradation of benzoic acid and benzoid compounds under anaerobic conditions: unifying trends and new perspectives. *FEMS Microbiology Reviews*, **13**: 441-468.

Ekperigin, M.M. (2007). Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp. *African Journal of Biotechnology*, **6**(1): 28-33.

Eriksson, K.E. (1978). Enzyme mechanisms involved in cellulose hydrolysis by the rot fungus *Sporotrichum pulverulentum*. *Biotechnology and Bioengineering*, **70**: 317-332.

Fan, L.T.; Lee, Y.H. and Gharpuary, M.M. (1982). The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. *Advances in Biochemical Engineering/Biotechnology*, **23**: 157-187.

Farkas, V.; Kolarova, N. and Labudovfi, I. (1987). Complexation with carrier ampholytes as the possible source of artifacts during isoelectric focusing of cellulase. *Biologia* (Bratislava), **42**: 327-333.

Ferreira, L.M.A.; Hazlewood, G.P.; Barker, P.J. and Gilbert, H.J. (1991). The cellodextrinase from *Pseudomonas fluorescens* subsp. *cellulosa* consists of multiple functional domains. *The Biochemistry Journal*, **279**: 793-799.

Fu, X.; Liu, P.; Lin, L.; Hong, Y.; Huang, X.; Meng, X. and Liu, Z. (2010). A novel endoglucanase (Cel9P) from a marine bacterium *Paenibacillus* sp. BME-14. *Applied Biochemistry and Biotechnology*, **160**: 1627-1636.

Galante, Y.M.; De Conti, A. and Monteverdi, R. (1998a). Application of *Trichoderma* enzymes in textile industry. In: Harman, G.F. and Kubicek, C.P. (ed.) *Trichoderma* & *Gliocladium*- Enzymes, biological control and commercial applications, Taylor & Francis, London, 2: 311-326.

Galante, Y.M.; De Conti, A. and Monteverdi, R. (1998b). Application of *Trichoderma* enzymes in textile industry. In: Harman, G.F. and Kubicek, C.P. (ed.) *Trichoderma* & *Gliocladium*- Enzymes, biological control and commercial applications, Taylor & Francis, London, 2: 327-342.

Gilkes, N.R.; Kilburn, D.G.; Miller, R.C., Jr. and Warren, R.A.J. (1991). Bacterial cellulases. *Bioresource Technology*, **36**: 21-35.

Godfrey, T. (1996). Textiles. In: Godfrey, T. and West, S. (ed.) Industrial enzymology, 2nd edition, Macmillan Press, London, pp: 360-371.

Goyal, A.; Ghosh, B. and Eveleigh, D. (1991). Characteristics of fungal cellulases. *Bioresource Technology*, **36**: 37-50.

Gum, E.K. and Brown, R.D., Jr. (1977). Comparison of four purified extracellular 1,4-β-D-glucan cellobiohydrolase from *Trichoderma viride*. *Biochimica et Biophysica Acta*, **492**: 225-231.

Gunata, Y.Z.; Bayonove, C.L.; Cordonnier, R.E.; Arnaud, A. and Galzy, P. (1990). Hydrolysis of grape monoterpenyl glycosides by *Candida molischiana* and *Candida wickerhamii* β-glucosidases. *Journal of the Science of Food and* Agriculture, **50**: 499-506.

Gupta, P.; Samant, K. and Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*, doi: 10.1155/2012/578925.

Hagerdal, B.; Ferchak, J.D. and Kendall Pye, E. (1980). Saccharification of cellulose by the cellulolytic enzyme system of *Thermomonospora* sp. I. Stability of cellulolytic activities with respect to time, temperature and pH. *Biotechnology and Bioengineering*, **22**: 1515-1526.

Hayashi, K.; Nimura, Y.; Ohara, N.; Uchimura, T.; Suzuki, H.; Komagata, K. and Kozaki, M. (1996). Low-temperature active cellulase produced by *Acremonium alcalophilum* JCM 7366. *Seibutsu-kogaku Kaishi*, **74**: 7-10.

Hazlewood, G.P.; Laurie, J.I.; Ferreira, L.M. and Gilbert, H.J. (1992). *Pseudomonas fluorescens* subsp. *cellulosa*: an alternative model for bacterial cellulase. *Journal of Applied Bacteriology*, **72**(3): 244-251.

Hesselman, K.; Elwinger, K. and Thomke, S. (1982). Influence of increasing levels of β-glucanase on the productive value of barley diets for broiler chickens. *Animal Feed Science and Technology*, **7**: 351-358.

Hodrova, B.; Kopecny, J. and Kas, J. (1998). Cellulolytic enzymes of rumen anaerobic fungi *Orpinomyces joyonii* and *Caecomyces communis*. *Research in Microbiology*, **149**: 417-427.

Hogsett, D.A.; Ahn, H.J.; Bernardez, T.D.; South, C.R. and Lynd, L.R. (1992). Direct microbial conversion- Prospects, progress, and obstacles. *Applied Biochemistry and Biotechnology*, **34**: 527-541.

Huang, L. and Forsberg, C.W. (1987). Isolation of a cellodextrinase from *Bacteroides succinogenes. Applied and Environmental Microbiology*, **53**: 1034-1041.

Ibrahim, A.S.S. and El-diwany, A.I. (2007). Isolation and identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. *Australian Journal of Basic and Applied Sciences*, **1**: 473-478.

Irfan, M.; Safdar, A.; Syed, Q. and Nadeem, M. (2012). Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. *Turkish Journal of Biochemistry*, **37**(3): 287-293.

Jayashree, S.; Lalitha, R.; Vadivukkarasi, P.; Kato, Y. and Seshadri, S. (2011). Cellulase production by pink pigmented facultative methylotrophic strains (PPFMs). *Applied Biochemistry and Biotechnology*, **164**(5): 666-680.

Kakiuchi, M.; Isui, A.; Suzuki, K.; Fujino, T.; Fujino, E.; Kimura, T.; Karita, S.; Sakka, K. and Ohmiya, K. (1998). Cloning and DNA sequencing of the genes encoding *Clostridium josui* scaffolding protein CipA and cellulase CelD and identification of their gene products as major components of the cellulosome. *Journal of Bacteriology*, **180**: 4303-4308.

Kasana, R.C. and Gulati, A. (2011). Cellulases from psychrophilic microorganisms: a review. *Journal of Basic Microbiology*, **51**: 572-579.

Kasana, R.C.; Salwan, R.; Dhar, H.; Dutt, S. and Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Current Microbiology*, **57**: 503-507.

Khan, J.A.; Ranjan, R.K.; Rathod, V. and Gautam, P. (2011). Deciphering cow dung for cellulase producing bacteria. *European Journal of Experimental Biology*, **1**(1): 139-147.

Khandke, K.M.; Vithayathil, P.J. and Murthy, K.S. (1989). Purification of xylanase, β-glucosidase, endocellulase and exocellulase from thermophilic fungus, *Thermoascus aurantiacus*. *Archives of Biochemistry and Biophysics*, **274**: 491-500.

Kim, C.H. and Kim, D.S. (1993). Extracellular cellulolytic enzymes of *Bacillus circulans* are present as two multiple-protein complexes. *Applied Biochemistry and Biotechnology*, **42**: 83-94.

Kluepfel, D.; Shareck, F.; Mondou, F. and Morosoli, R. (1986). Characterization of cellulase and xylanase activities of *Streptomyces lividans*. *Applied Microbiology and Biotechnology*, **24**: 230-234.

Kubicek, C.P. (1992). The cellulase proteins of *T. reesei*: structure, multiplicity, mode of action and regulation of formation. *Advances in Biochemical Engineering/Biotechnology*, **45**: 1-27.

Kuhad, R.C.; Gupta, R. and Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme Research*, doi: 10.4061/2011/280696.

Kuhad, R.C.; Singh, A. and Eriksson, K.E.L. (1997). Microorganisms and enzymes involved in the degradation of plant fibre cell walls. *Advances in Biochemical Engineering/Biotechnology*, **57**: 45-125.

Kumar, A. and Singh, V.P. (2011). Thermophilic bacteria and their potential for industrial applications. *Journal of Plant Development Sciences*, **3**(1&2): 19-30.

Kumar, A.; Singh, A. and Singh, V.P. (2011). Industrial enzymes of microbial origin. In: Trivedi, P.C. (ed.) Biotechnology: A New Approach, Agrobios, India, pp: 291-308.

Lamed, R. and Bayer, E.A. (1988). The cellulosome of *Clostridium thermocellum*. *Advances in Applied Microbiology*, **33**: 1-46.

Lamed, R.; Morag, E.; Moryosef, O. and Bayer, E.A. (1991). Cellulosome-like entities in *Bacteroides cellulosolvens*. *Current Microbiology*, **22**: 27-34.

Lamed, R.; Naimark, J.; Morgenstern, E. and Bayer, E.A. (1987). Specialized cell surface structures in cellulolytic bacteria. *Journal of Bacteriology*, **169**: 3792-3800.

Leatham, G.; Myers, G. and Wegner, T. (1990). Biochemical pulping of aspen chips: energy savings resulting from different fungal treatments. *Tappi Journal*, **73**: 197-200.

Leonowicz, A.; Matuszewska, A.; Luterek, J.; Ziegenhagen, D.; Wojtas-Wasilewska, M.; Cho, N.S.; Hofrichter, M. and Rogalski, J. (1999). Biodegradation of lignin by white rot fungi. *Fungal Genetics and Biology*, **27**: 175-185.

Ljungdahl, L.G. (2008). The cellulase/hemicellulase system of the anaerobic fungus *Orpinomyces* PC-2 and aspects of its applied use. *Annals of the New York Academy of Sciences*, **1125**: 308-321.

Lv, W. and Yu, Z. (2013). Isolation and characterization of two thermophilic cellulolytic strains of *Clostridium thermocellum* from a compost sample. *Journal of Applied Microbiology*, **114**(4): 1001-1007.

MacKenzie, C.R.; Bilous, D. and Johnson, K.G. (1984). *Streptomyces flavogriseus* cellulase: evaluation under various hydrolysis conditions. *Biotechnology and Bioengineering*, **26**: 590-594.

Maki, M.; Leung, K.T. and Qin, W. (2009). The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass.

International Journal of Biological Sciences, **5**(5): 500-516.

Mandels, M. (1985). Applications of cellulases. *Biochemical Society Transactions*, **13**: 414-415.

Mandels, M. and Reese, E.T. (1964). Fungal cellulases and the microbial decomposition of cellulose fabric. *Developments in Industrial Microbiology*, **5**: 5-20.

Mantyla, A.; Paloheimo, M. and Suominen, P. (1998). Industrial mutants and recombinant strains of *Trichoderma reesei*. In: Harman, G.F. and Kubicek, C.P. (ed.) *Trichoderma* & *Gliocladium*- Enzymes, biological control and commercial applications, Taylor & Francis, London, 2: 291-309.

McCarthy, A.J. (1987). Lignocellulose-degrading actinomycetes. *FEMS Microbiology Reviews*, **46**: 145-163.

McHale, A. and Coughlan, M.P. (1980). Synergistic hydrolysis of cellulose by components of the extracellular cellulase system of *Talaromyces emersonii*. *FEBS Letters*, **117**: 319-322.

Meyer, A.S.; Jepsen, S.M. and Sorensen, N.S. (1998). Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. *Journal of Agricultural and Food Chemistry*, **46**: 2439-2446.

Moldoveanu, N. and Kluepfel, D. (1983). Comparison of β-glucosidase activities in different *Streptomyces* strains. *Applied and Environmental Microbiology*, **46**: 17-21.

Nakayama, M.; Tomita, Y.; Suzuki, H. and Nisizawa, K. (1976). Partial proteolysis of some cellulase components from *Trichoderma viride* and substrate specificity of the modified products. *The Journal of Biochemistry*, **79**: 955-966.

Noe, P.; Chevalier, J.; Mora, F. and Comtat, J. (1986). Action of enzymes in chemical pulp fibres. Part II: enzymatic beating. *Journal of Wood Chemistry and Technology*, **6**: 167-184.

Ohara, H.; Karita, S.; Kimura, T.; Sakka, K. and Ohmiya, K. (2000). Characterization of the cellulolytic complex (cellulosome) from *Ruminococcus albus*. *Bioscience, Biotechnology and Biochemistry*, **64**: 254-260.

Oikawa, T.; Tsukagawa, Y. and Soda, K. (1998). Endo-β-glucanase secreted by a psychrotrophic yeast: Purification and characterization. *Bioscience, Biotechnology, and Biochemistry*, **62**: 1751-1756.

Pagés, S.; Gal, L.; Bélaich, A.; Gaudin, C.; Tardif, C. and Bélaich, J.P. (1997). Role of scaffolding protein CipC of *Clostridium cellulolyticum* in cellulose degradation. *Journal of Bacteriology*, **179**: 2810-2816.

Pason, P.; Kyu, K.L. and Ratanakhanokchai, K. (2006). *Paenibacillus curdlanolyticus* strain B-6 xylanolytic-cellulolytic enzyme system that degrades insoluble polysaccharides. *Applied and Environmental Microbiology*, **72**(4): 2483-2490.

Pohlschröder, M.; Canale-Parola, E. and Leschine, S.B. (1995). Ultrastructural diversity of the cellulase complexes of *Clostridium papyrosolvens* C7. *Journal of Bacteriology*, **177**: 6625-6629.

Ponnambalam, A.S.; Deepthi, R.S. and Ghosh, A.R. (2011). Qualitative display and measurement of enzyme activity of isolated cellulolytic bacteria. *Biotechnology, Bioinformatics and Bioengineering*, **1**(1): 33-37.

Saha, S.; Roy, R.N.; Sen, S.K. and Ray, A.K. (2006). Characterization of cellulase-producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica* (Peters) and grass carp, *Ctenopharyngodon idella* (Valenciennes). *Aquaculture Research*, **37**: 380-388.

Sakthivel, M.; Karthikeyan, N.; Jayaveny, R. and Palani, P. (2010). Optimization of culture conditions for the production of extracellular cellulase from *Corynebacterium lipophiloflavum*. *Journal of Ecobiotechnology*, **2**(9): 6-13.

Saranraj, P.; Stella, D. and Reetha, D. (2012). Microbial cellulases and its applications: A review. *International Journal of Biochemistry and Biotech Science*, **1**: 1-12.

Saratale, G.D.; Saratale, R.G.; Lo, Y.C. and Chang, J.S. (2010). Multicomponent cellulase production by *Cellulomonas biazotea* NCIM-2550 and its applications for cellulosic biohydrogen production. *Biotechnology Progress*, **26**(2): 406-416.

Schellhorn, H.E. and Forsberg, C.W. (1984). Multiplicity of extracellular β-(1,4)-endoglucanases of *Bacteroides succinogenes* S85. *Canadian Journal of Microbiology*, **30**: 930-937.

Shankar, T.; Mariappan, V. and Isaiarasu, L. (2011). Screening cellulolytic bacteria from the mid-gut of the popular composting earthworm, *Eudrilus eugeniae* (Kinberg). *World Journal of Zoology*, **6**(2): 142-148.

Sharrock, K.R. (1988). Cellulase assay methods: a review. *Journal of Biochemical and Biophysical Methods*, **17**(2): 81-105.

Shipkowski, S. and Brenchley, J.E. (2005). Characterization of an unusual cold-active beta-glucosidase belonging to family 3 of the glycoside hydrolases from the psychrophilic isolate *Paenibacillus* sp. strain C7. *Applied and Environmental Microbiology*, **71**: 4225-4232.

Shoseyov, O. and Doi, R.H. (1990). Essential 170-kDa subunit for degradation of crystalline cellulose by *Clostridium cellulovorans* cellulase. *Proceedings of the National Academy of Sciences, USA*, **87**: 2192-2195.

Singh, V.K. and Kumar, A. (1998). Production and purification of an extracellular cellulase from *Bacillus brevis* VS-1. *Biochemistry and Molecular Biology International*, **45**(3): 443-452.

Singh, V.P. and Mukerji, K.G. (1989). Microbes in biotechnology. In: Mukerji, K.G.; Singh, V.P. and Garg, K.L. (ed.), Frontiers in Applied Microbiology, Rastogi and Company, Meerut, 3: 61-84.

Tamaru, Y.; Karita, S.; Ibrahim, A.; Chan, H. and Doi, R.H. (2000). A large gene cluster for the

Clostridium cellulovorans cellulosome. *Journal of Bacteriology*, **182**: 5906-5910.

Tomme, P.; Warren, R.A. and Gilkes, N.R. (1995). Cellulose hydrolysis by bacteria and fungi. *Advances in Microbial Physiology*, **37**: 1-81.

Van Zyl, W.H. (1985). A study of the cellulases produced by three mesophilic actinomycetes grown on bagasse as substrate. *Biotechnology and Bioengineering*, **27**(9): 1367-1373.

Vrsanska, M. and Biely, P. (1992). The cellobiohydrolase I from *Trichoderma reesei* QM 9414: action on cello-oligosaccharides. *Carbohydrate Research*, **227**: 19-27.

Waldrop, M.P.; Balser, T.C. and Firestone, M.K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry*, **32**: 1837-1846.

Wang, C.Y.; Hsieh, Y.R.; Ng, C.C.; Chan, H.; Lin, H.T.; Tzeng, W.T. and Shyu, Y.T. (2009). Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU-05. *Enzyme and Microbial Technology*, **44**: 373-379.

Wood, T.M and McCrae, S.I. (1986). The cellulase of *Penicillium pinophilum*: Synergism between enzyme components in solubilizing cellulose with special reference to the involvement of two immunologically distinct cellobiohydrolases. *The Biochemical Journal*, **234**: 93-99.

Wood, T.M. (1992). Fungal cellulases. *Biochemical Society Transactions*, **20**: 45-53.

Wood, T.M. and Bhat, M.K. (1988). Methods for measuring cellulase activities. *Methods in Enzymology*, **160**: 87-112.

Wood, T.M. and McCrae, S.I. (1972). The purification and properties of the C1 component of *Trichoderma koningii* cellulases. *The Biochemical Journal*, **128**(5): 1183-1192.

Wood, T.M. and McCrae, S.I. (1977). Cellulase from *Fusarium solani*: purification and properties of the C1 component. *Carbohydrate Research*, **57**: 117-133.

Wood, T.M. and McCrae, S.I. (1982). Purification and some properties of a (1,4)-β-D-glucan glucohydrolase associated with the cellulase from the fungus *Penicillium funiculosum. Carbohydrate Research*, **110**: 291-303.

Wood, T.M. and McCrae, S.I. (1982). Purification and some properties of the extracellular β-glucosidase of the cellulolytic fungus, *Trichoderma koningii. Journal of General Microbiology,* **128**: 2973-2982.

Wood, T.M.; Wilson, C.A.; McCrae, S.I. and Joblin, K.N. (1986). A highly active extracellular cellulase from the anaerobic rumen fungus *Neocallimastix frontalis*. *FEMS Microbiology Letters*, **34**: 37-40.

Yanling, H.; Youfang, D. and Yanquan, L. (1991). Two cellulolytic *Clostridium* species: *Clostridium cellulosi* sp. nov. and *Clostridium cellulofermentans* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, **41**: 306-309.

Yokoe, Y. and Yasumasu, I. (1964). The distribution of cellulase in invertebrates. *Comparative Biochemistry and Physiology*, **13**: 323-338.

You, Y.W. and Wang, T.H. (2005). Cloning and expression of endoglucanase of marine cold-adapted bacteria *Pseudoalteromonas* sp. MB-1. *Wei Sheng Wu Xue Bao*, **45**: 142-144.

Zeng, R.; Xiong, P. and Wen, J. (2006). Characterization and gene cloning of a cold-active cellulase from a deep-sea psychrotrophic bacterium *Pseudoalteromonas* sp. DY3. *Extremophiles*, **10**: 79-82.

Zhang, Y.H.P.; Himmel, M.E. and Mielenz, J.R. (2006). Outlook for cellulase improvement: Screening and selection strategies. *Biotechnology Advances*, **24**: 452-481.