BIODIVERSITY OF CELLULASE PRODUCING BACTERIA AND THEIR APPLICATIONS

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Bioconversion of cellulose, mainly by bacteria and fungi, plays a key role in the global carbon cycle. The biodiversity of these microorganisms in nature is due to diverse cellulosic substrates and geoclimatic conditions, but despite their abundance, only a few are efficiently involved in the biodegradation process. Bacterial cellulase is preferred over fungal one, owing to the higher bacterial growth rate, broad range of tolerance, synergy of the complex enzyme system, higher compatibility and feasibility towards genetic engineering. This paper reviews the diversity of aerobic and anaerobic cellulolytic bacteria, their cellulase activity, stability and application in biodegradation of lignocelluloses in various industries. Most of the cellulolytic bacteria belong to phyla Actinobacteria, Bacteroidetes, Fibrobacteres, Firmicutes and Proteobacteria. Among aerobic bacteria, members of Bacillaceae and Paenibacillaceae families mainly inhabit soil, cowdung and compost. However, anaerobic species of Clostridia have mostly been reported to originate from sediments of hot springs, soil and compost, whereas *Butyrivibrio*, *Fibrobacter*, *Halocella* and *Ruminococcus* are found in rumen. Purified bacterial cellulases generally have wide pH and temperature tolerance and a molecular mass in the range of 30-250 kDa. Efforts have been made to increase cellulase activity, all with limited success. Extremophilic bacteria, such as *Acidothermus cellulolyticus*, *Clostridium straminisolvens*, *Geobacillus pallidus*, *Sporotrichum thermophile*, *Thermobifida fusca*, *Thermomonospora curvata* and *Rhodothermus marinus*, have proved to be a better choice for cellulase production to improve the process economics for various industrial applications.

Keywords: *Bacillus* sp., biodiversity, cellulase, hemicellulose

INTRODUCTION

Cellulose, the most abundant, widely available, low cost, polymeric, renewable resource on Earth, has attracted recent worldwide concern in the search of alternative ways to meet future energy demands. Efforts have been made to reduce international dependence on conventional petroleum resources, and to minimize the impact of rising energy and feedstock material costs.[1-3] Complete and efficient hydrolysis of cellulose is the result of synergistic actions between different enzymes, which include exo-glucanases, endo-glucanases and β-glucosidase.

Cellulases belong to the glycoside hydrolase family of enzymes and act by cleaving β,1-4 glycosidic bonds of polysaccharides and oligosaccharides. Due to the ability of these enzyme systems to convert cellulosic materials into useful sugars, they have been considered to have great potential in biofuel production over the past few decades. Many types of organisms produce cellulases, which move through secretory pathways to reach the extracellular space, where enzymatic reactions take place.[4] Both fungi and bacteria have been widely used for their abilities to produce a wide variety of cellulases. More emphasis has been placed on the use of fungi due to their capability to produce copious amounts of cellulases, which can be easily extracted and purified, and are less complex than bacterial glycoside hydrolases. However, due to certain advantages, such as the higher growth rate of bacteria, synergy of their complex enzyme system, higher compatibility and feasibility towards genetic engineering, bacteria are preferred over fungi. In addition, bacteria inhabit a variety of environmental and industrial niches,

and their cellulolytic properties are extremely resistant to environmental stress.[5]

Over the years, culturable, cellulase producing bacteria have been isolated from diverse habitats, such as composting heaps, decaying plant materials, feces of ruminants and hot springs.[6] Bacteria show higher growth rates as compared to fungi, and have a good potential for use in cellulase production.[7] Among bacteria, recent and widespread concern regarding the need of an efficient cellulase producer targets extremophiles, which may evade harsh conditions during bioconversion processes. Cellulases provide a key opportunity for achieving tremendous benefits from biomass utilization,[8] contributing to satisfying the global industrial demand for enzymes. The cellulase market is expected to expand dramatically due to their applications in the hydrolysis of pretreated cellulosic materials to sugars and further to bioethanol and other bio-based products.[9]

The search is on for efficient cellulase producing bacteria, with high cellulase activity, which could be effective over a broad range of tolerance in order to achieve optimum utilization of these enzymes for use in biofuel and bioproduct industries. The current focus is now on screening and developing novel cellulose degrading enzymes with special characteristics.[10] The exploitation of bacteria in the search for improved enzymes provides a means to upgrade the feasibility of lignocellulosic biomass conversion, ultimately providing the means to a 'greener' technology.[5]

The current review envisages characterization of different cellulolytic aerobic and anaerobic bacteria and their biodiversity in nature. The paper discusses the benefits of bacteria over fungi and the trends in utilizing cellulosic substrates to produce cellulases in making the bioconversion process more efficient and compatible.

Lignocellulose and its composition

Cellulose, or β,1-4-glucan, is the most abundant and ubiquitous linear polymer of glucose with widespread industrial use. Cellulose microfibrils, roughly one third of the total mass of many plants, contain 500 to 14,000 glucose units linked by β,1-4 glycosidic bonds.[11,12] The cellulose fibrils are associated in the form of bundles[13] and attached to each other by hemicelluloses, amorphous polymers of different sugars, pectins and covered by lignin, which makes cellulose resistant to both biological and chemical treatment.[14] The dominant sugars in hemicelluloses are mannose in softwoods and xylose in hardwoods and agricultural residues.[15-17] These heteropolymers contain galactose, glucose, arabinose and a small amount of rhamnose, glucuronic acid, methyl glucuronic acid and galacturonic acid.[12,14] In contrast to cellulose, hemicelluloses have a random, amorphous and branched structure with little resistance to hydrolysis and they are more easily hydrolyzed by acids to their monomer components.[18-22] Lignin is a very complex, recalcitrant molecule constructed of phenylpropane units linked in a three-dimensional structure, which encapsulates cellulose and hemicelluloses, which, in turn, reduces the efficiency of hydrolysis.

Cellulase: The enzyme complex

Cellulases are inducible enzymes, which are synthesized by a large number of microorganisms, either cell-bound or extracellular during their growth on cellulosic materials.[23] Historically, cellulases have been divided into three major groups: endoglucanase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) and the synergistic actions of these enzymes are a widely accepted mechanism for cellulose hydrolysis.[10,24-27] Figure 1 presents a simplified schematic diagram of the enzymatic actions of cellulases, involving exoglucanase, endoglucanase and β-glucosidase on cellulose. Endoglucanase randomly cleaves the internal bonds in the amorphous region; exoglucanase cleaves the exposed chains produced by endoglucanases, whereas β-glucosidase hydrolyses the cellobiose unit produced by the action of exoglucanase. These enzymes can either be free in aerobic microorganisms, or grouped in a multicomponent enzyme complex, cellulosome, in anaerobic cellulolytic bacteria.[24] Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria and protozoans,[28] which acts as a biocatalyst for the conversion of cellulosic substrates to fermentable sugar; this process incurs major costs in a biorefinery.[29]

Cellulose has attracted attention as a renewable resource that can be converted into bioenergy and value-added bio-based products.[30] Enormous amounts of agricultural, industrial and municipal cellulosic wastes have accumulated or been used inefficiently due to the high cost of their utilization processes.[31,32] Therefore, it would be of considerable economic interest to develop

processes for effective treatment and utilization of cellulosic wastes as a cheap carbon source. These biomasses comprise leaves, stems and stalks from sources such as corncob, corn stover, sugarcane bagasse, rice, rice hulls, woody crops and forest residues. Besides, there are multiple sources of lignocellulosic waste from industrial and agricultural processes, some of these are: citrus peel waste, coconut biomass, sawdust, paper pulp, industrial waste, municipal cellulosic solid waste and paper mill sludge.[33]

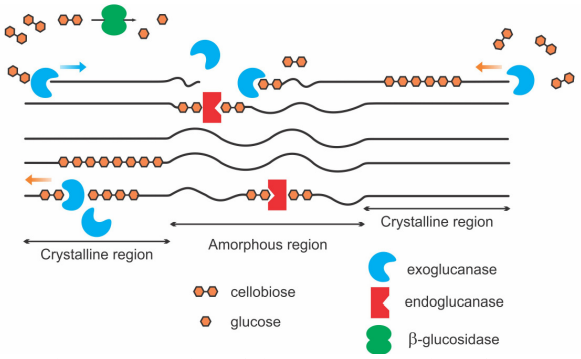

Figure 1: A simplified schematic representation of the enzymatic action of cellulase, involving exoglucanase, endoglucanase and β-glucosidase, on cellulose

In the last two decades, research has been aimed at developing new technologies and microbial strains to reduce the cost of cellulase production and improve the bioconversion of cellulose, particularly for the biofuel industry. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization.[8] Currently, two significant points of these enzyme-based bioconversion technologies are reaction conditions and the production cost of the related enzyme system.[32] Therefore, much research has been aimed at obtaining new microorganisms producing celluloytic enzymes with higher specific activities and greater efficiency.[34-37] Cellulase yield appears to be dependent upon a complex relationship, involving a variety of factors like inoculum size, pH, temperature, presence of inducers, medium additives, aeration, and growth time.[38] China, India, South Korea and Taiwan have recently emerged as industrialized manufacturing centers with strong national research and development programs, and will play a great role in the world market of industrial cellulase production.[39]

Bacterial diversity among cellulase producers

Many microorganisms are able to produce and secrete cellulolytic, hemicellulolytic and lignolytic enzymes. These microorganisms are found among extremely variegated taxonomic groups inhabiting diverse habitats from extreme thermophillic conditions to polar regions and aerobic to anaerobic systems. Already by 1976, an impressive collection of more than 14,000 fungi active against cellulose and other insoluble fibres were reported.[24,40] Most of the cellulolytic bacteria fall within the phyla Actinobacteria, Bacteroidetes, Fibrobacteres, Firmicutes and Proteobacteria. Phyla Actinobacteria and Firmicutes constitute 80% of the isolated cellulose degrading bacteria.[24,41] Besides fungi, bacteria and protozoa, detached avocado fruit *Persea americana* and slime mould *Dictyostelium discoideum*[42] had also been reported for cellulolytic activity. An inspection of taxonomic diversity reveals that the ability to digest cellulose is widely distributed among many genera within the bacterial domain (Tables 1 and 2), although no cellulolytic members of the domain *Archaea* have been identified yet. Within the domain Eubacteria, there is a considerable concentration of cellulolytic capabilities among the predominantly aerobic order Actinomycetales (phylum Actinobacteria) and the anaerobic order Clostridiales (phylum Firmicutes).

The mechanisms through which microorganisms degrade cellulosic materials consist of production and secretion of enzymes, acting synergistically to release simple carbon to be used as a source of chemical energy. Efforts have continuously been made worldwide to increase the cellulase activity through strain improvement by mutagenesis techniques, optimization of physical and chemical components and expression of a desired gene into a suitable vector, all with limited success.

Table 1
Aerobic cellulolytic bacteria, their source, phylogenetic position and characteristics

Bacteria	Source	Phylum	Characteristics	References
Bacillus subtilis AS3	Cowdung	Firmicutes	Gram positive, rod, sporic	[43]
Bacillus sp.	Cowdung	Firmicutes	Gram positive, oval shape, sporic	[47]
Bacillus subtilis GN156	Soil	Firmicutes	Gram positive, rod, endosporic	[50]
Bacillus sp.	Soil	Firmicutes	Gram positive, motile, rod	[125]
Bacillus sp. C1	Cowdung	Firmicutes	Gram positive, motile, rod	[33]
Bacillus pumilus	Soil, dead plants	Firmicutes	Gram positive, rod, sporic	[126, 127]
B. amyloliquefaciens DL-3	Farm soil	Firmicutes	Gram positive, rod, endosporic, mesophillic	[32]
Bacillus licheniformis B-41361	--	Firmicutes	Gram positive, rod, mesophilic	[66]
Geobacillus sp.	Deep subsurface of goldmine	Firmicutes	Gram positive rod, sporic, mesophillic	[128]
Brevibacillus sp.	Deep subsurface of goldmine	Firmicutes	Gram positive, rod, sporic, mesophilic	[128]
Lysinibacillus sphaericus	Vermicompost	Firmicutes	Gram positive, rod	[129]
Paenibacillus barcinonensis MG 7	Soil	Firmicutes	Gram positive, rod, sporic	[49]
Paenibacillus sp.	Compost	Firmicutes	Gram positive, rod, endosporic, mesophillic	[128, 130]
Cellulomonas iranensis, C. persica	Forest humus soil	Actinobacteria	Gram positive, rod, alkalophillic	[131]
Cytophaga sp.	Soil	Bacteroidetes	Gram negative, rod, mesophillic, gliding,	[53, 54]
Acidothermus cellulolyticus	Acidic water, mud samples	Actinobacteria	Gram positive, rod, thermophillic	[41, 132, 133]
Sinorhizobium fredii	Soil	Proteobacteria	Gram negative, rod	[134]
Anoxybacillus flavithermus	Soil	Firmicutes	Gram positive, rod, endosporic, mesophillic	[49]
Pseudomonas aeruginosa	Soil	Proteobacteria	Gram negative, rod, mesophillic, flagellar	[56]

Table 2
Anaerobic cellulolytic bacteria, their source, phylogenetic position and characteristics

Bacteria	Source	Phylum	Characteristics	References
Clostridium thermocellum	Sewage soil	Firmicutes	Gram positive, rod, flagellar, thermophillic	[135-137]
C. acetobutylicum	Soil, grass	Firmicutes	Gram positive, rod, flagellar	[138, 139]
C. stercorarium	Compost	Firmicutes	Gram positive, rod, flagellar	[140]
C. cellulovorans	Dairy farm soil	Firmicuts	Gram positive, rod, flagellar, thermophillic	[141]
C. hungatei	Soil	Firmicutes	Gram positive, rod, flagellar, thermophillic	[142]
C. cellulolyticum	Compost	Fermicutes	Gram positive, rod, flagellar mesophillic	[143-144]
C. josui	Compost	Fermicutes	Gram positive, rod, flagellar, mesophillic	[145]
C. herbivorans	Deep soil	Firmicutes	Gram positive, rod, mesophillic	[146]
C. papyrosolvens	Paper mills	Firmicutes	Gram positive, rod, flagellar, mesophillic	[147]
C. aldrichii	Wood digester	Fermicutes	Gram positive, rod, flagellar, mesophillic	[148]
C. cellobioparum	Mud	Firmicutes	Gram positive, rod, flagellar, thermophilic	[149-150]
Ruminococcus albus	Rumen	Firmicutes	Gram positice, coccus, mesophillic	[64, 149, 151]
R. flavefaciens	Rumen	Firmicutes	Gram positice, coccus, mesophillic	[149, 151-153]
Bacteroides cellulosolvens	Sewage sludge	Firmicutes	Gram negative, non-endosporic, mesophillic	[154, 155]
Acetivibrio cellulolyticus	Sewage sludge	Firmicutes	Flagellar, mesophillic	[156, 160]
Butyrivibrio fibrisolvens	Rumen	Firmicutes	Gram positive, rod, mesophilic	[149, 161]
Thermotoga neapolitana	Deep soil	Firmicutes	Gram positive, rod, thermophillic	[162]
Halocella cellulolytica	Rumen	Firmicutes	Gram positive, rod, mesophilic	[163]
Fibrobacter succinogenes	Rumen	Fibrobacteres	Gram positive, rod, flagellar, mesophillic	[149, 151, 164, 165]
F. succinogenes	Intestinal track	Fibrobacteres	Gram positive, rod, flagellar, mesophillic	[65]

The co-cultivation strategies of bacteria and fungi[43,44] have also increased the desirable components of cellulase and resulted in improved saccharification of lignocellulosic biomasses.

Aerobic cellulase producing bacteria

Aerobic bacteria produce numerous individual, extra-cellular enzymes, most of them contain the carbohydrate binding module (CBM) joined to one end of the catalytic domain for different cellulose conformations. Eubacteria are now becoming widely exploited for purification and production of various novel glycoside hydrolases. The higher growth rate than that of fungi, enhanced production of cellulases, simple nutritional requirements, the broad range of environmental tolerance and the enormous range of environmental and industrial habitats are the key reasons to hunt bacteria for increased function and synergy. Currently, *Bacillus amyloliquefaciens*, *B. licheniformis*, *B. pumilus*, *B. subtilis*, *Paenibacillus barcinonensis*, *Psedomonas aeruginosa* and *P. fluorescens* are known as potent cellulase producers isolated from soil, compost, cowdung, goldmine, acidic water and mud (Table 1). Of these, about 70% of the isolated cellulolytic aerobic bacteria are of genus Bacillus and Paenibacillus. The best-studied species of cellulolytic aerobic bacteria belong to the genera *Cellulomonas* and *Thermobifida* (formerly *Thermomonospora*).[24]

Cellulomonas spp. are coryneform bacteria that produce at least six endoglucanases and one exoglucanase (Cex).[45] Three endoglucanases (E1, E2, and E5), two exoglucanases (E3 and E6) and an unusual cellulase with both endoglucanase and exoglucanase activity (E4) have also been reported from *Thermobifida fusca*, a thermophilic filamentous bacterium from soil.[24] The latter enzyme has high activity on bacterial microcrystalline cellulose and exhibits synergism with endoglucanases and exoglucanases of *T. fusca.*[46] Rumen acts as a chemostat for many cellulolytic bacteria to enhance the digestibility of cellulosic feed. In the past five years, many bacteria from ruminants, such as *B. subtilis* AS3,[43] *Bacillus* sp. C1,[33] *Bacillus* sp.[47] from cowdung and *B. amyloliquefaciens* SS35[48] from rhinoceros dung, have been isolated and found effective for utilizing various cellulosic substrates. Among different bacteria inhabiting the soil, potential cellulose degraders are *Anoxybacillus flavithermus*,[49] *Bacillus amyloliquefaciens* DL-3,[32] *B. subtilis* GN156,[50] *B. pumilus*,[51] *Cytophaga huthincini*,[52-54] *Paenibacillus barcinonensis*[55] and *Pseudomonas aeruginosa.*[56]

Thermophilic and hyperthermophilic prokaryotes represent a unique group of microorganisms that grow at temperatures that may generally exceed 45 °C or even 100 °C. Several cellulolytic hyperthermophiles have been isolated during the past decade.[57] Thermophillic bacteria, such as *Clostridium straminisolvens*, *Sporotrichum thermophile*, *Thermonospora curvata*,[58,59] *Rhodothermus*,[24] *Geobacillus pallidus*[60] and an actinomycete *Acidothermus cellulolyticus*[24] are well-known to produce cellulase for the biodegradation of cellulosic substrates. Moving forward, thermophilic organisms may be used as potent sources of thermostable cellulases for efficient hydrolysis of lignocellulosic biomasses.

Anaerobic cellulase producing bacteria

Many anaerobic bacteria possess a unique extracellular multi-enzyme complex, called cellulosome.[61,62] Only a few of the enzymes in cellulosomes contain a CBM, but the protein to which they are attached (called scaffoldin) does contain a CBM, which binds the complex to cellulose.[62] The majority of the cellulolytic anaerobic bacteria of genus Clostridium are *Clostridium acetobutylicum*, *C. aldrichii*, *C. cellulolyticum*, *C. cellovioparum*, *C. cellulovorans*, *C. herbivorans*, *C. hungatei*, *C. josui*, *C. papyrosolvens*, *C. stercorarium* and *C. thermocellum* (Table 2). Although cellulolytic *Acetovibrio cellulolyticus*, *Bacteroides cellulosolvens*, *B. succinogenees*, *Butyrivibrio fivrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. falvefaciens* have also been reported from different ecological niches. Cellulosomes from *Clostridium* and *Ruminococcus* species have been exhaustively studied during the last decade. The architecture of cellulosomes is similar among these organisms, although cellulosome composition varies from species to species. The cellulosome of the thermophilic *C. thermocellum* is discussed and briefly compared to those of the mesophilic *C. cellulolyticum*, *C. cellulovorans* and *R. albus.*[61] However, *C. stercorarium* is the only species for which no cellulosome has been observed.[61] Ruminal bacteria of the genus *Ruminococcus* are phylogenetically related to, but do not fall within, the family Clostridiaceae.[24] Recently, the presence of dockerin-like sequences in at least seven of the cellulase and xylanase genes of

Ruminococcus flavefaciens and the production of 1.5-MDa cellulosome-like structures on the *R. albus* cell surface in the presence of cellobiose and organic acids (phenylacetic and phenylpropionic acid) suggested that *Ruminococcus* spp. indeed produce cellulosomes.[63] The structure of the *R. albus* cellulosomes differs from that of the clostridial, suggesting an independent evolutionary path.[64] *Fibrobacter succinogenes* S85 is another efficient cellulolytic bacterium isolated from rumen, which actively adheres to cellulose.[65] Although the cellulases of *F. succinogenes* are cell associated, no cellulosome structures have been identified.

Characterization of bacterial cellulases

Enzymes show their own pH sensitivity, temperature optima, thermal stability, substrate specificity, kinetics and different chemicals that can have an effect on their activity. The broad range of pH tolerance and thermal stability are among the promising characteristics of cellulases for the biofuel and bio-based industries. Many researchers have purified and characterized cellulases from different bacteria, such as *Bacillus licheniformis* WBS1,[39] *B. licheniformis*,[66] *B. sphaericus* JS1,[67] *B. subtilis* AS3,[68] *Bacillus* sp. C1,[33] *Bacillus* sp. HSH-810,[69] *Bacillus* sp. M9,[70] *Bacillus* sp. WBS3,[39] *Cellulomonas* sp. YJ5,[71] *Melanocarpus* sp. MTCC 3922,[72] *Paenibacillus barcinonensis* MG7,[49] *Pyrococcus horikoshi*[73] and *Thermomonospora* sp.[67]

The molecular weight of purified cellulase has been reported in the range of 30-250 kDa from different bacterial strains and purification strategies have led to up to 280-fold enhancements of the activity (Table 3). Purified cellulases from bacteria were found stable in a pH range of 4-7.5, thermally stable up to 90 ℃ for *Bacillus* sp. M9,[70] with an optimum temperature of 35-50 ℃ for different bacteria, however the optimum activity at 60 ℃ or more was observed for *Cellulomonas* sp. ASN2[75] and *Caldibacillus cellulovorans.*[76]

The characterization of cellulases became inevitable for the production of recombinant cellulase or for *in situ* mass production using bacterial strains to reduce the need of multiple enzyme production during the hydrolysis mechanism. Multifunctional cellulase (CelAB) with two catalytic carbohydrate domains, cellobiohydrolase and β-1,4(3) endoglucanase activity responsible for degrading multiple complex polysaccharides, showed an optimum pH and temperature of 6 and 42 °C, respectively, and was found able to reduce the viscosity of CMC by 40% in 25 min.[77] A bifunctional endoglucanase/endoxylanase was also isolated from *Cellulomonas flavigena*, and showed optimum cellulase and xylanase activity at pH 6 and 9, respectively, with a general optimum temperature of 50 °C,[77] exhibiting potential for use in different industrial processes. Similarly, in 2007, a multifunctional enzyme was found to be produced by *Terendinibacter turnerae* T7902, which is a bacterial symbiont isolated from the wood-boring marine bivalve *Lydrodus pedicellatus.*[5]

Purification of bacterial cellulase by ion-exchange chromatography, such as DEAE (diethylaminoethanol) sepharose and sepharyl chromatography has been extensively used at a pilot scale. It is also used for direct recovery of proteins and other charged molecules, as the technique is known to have high resolving power, high capacity, is simple to operate, highly robust, generic and economical.[78]

The purified cellulase from Bacilli revealed a molecular mass of 54 kDa from *B. amyloliquefeciens* DL-3,[32] 61-80 kDa from *Bacillus* sp. AC1,[79] 97 kDa from *Bacillus* sp. C1,[33] 80 kDa from *Bacillus* sp. HSH-810,[69] *Bacillus* sp. M-9[70] and 37 kDa from *B. licheniformis* B-41361.[66] Similar molecular mass of purified cellulase was reported for other groups of bacteria, namely 36, 38 and 43.7 kDa for *Pseudomonas fluorescens*,[80] *Thermomonospora* sp.[74] and *Cellulomonas* sp. YJ5,[71] respectively (Table 4).

More recently, modifications to bacterial cellulases through protein engineering by adopting rational design and directed evolution[5] have taken stage in the production of efficient hydrolytic enzymes. The design and construction of self-assembling protein complexes and the architecture of cellulosomes as templates have shown new ways towards cellulose degradation.

Biodegradation of lignocellulosic biomass

Microorganisms have developed distinct well-adapted cellular machineries in order to derive energy from plant biomass for production and secretion of carbohydrate-active enzymes by their saprophytic lifestyle, which involves living on dead or decaying organic matter.[81]

Table 3
Molecular weight of purified cellulases and enzymatic activity for different cellulolytic bacteria from diverse habitats

Bacteria	Source	Molecular weight (kDa)	Enzyme activity in crude cellulase	Enzyme activity in purified cellulase	Fold-purification	Reference
Bacillus sp. CH43	Hot springs	40	--	--	--	[166]
Bacillus sp.	Cow dung	33	4 U/mg	6.6 U/mg	--	[47]
B. amyloliquefaciens DL-3	Soil	53	292 U/ml	6070 U/ml	21	[32]
B. pumilus EB3	--	30-65	0.57 U/ml	2 U/ml	1	[127]
Bacillus sp.	Soil	58	16 U/ml	246 U/mg	15	[125]
Bacillus sp. C1	Cowdung	97	8 U/ml	68 U/ml	8	[33]
Bacillus sp. AC1	Fermented foods	33	24 U/mg	7001U/mg	289	[79]
B. licheniformis B-41361	--	37	3 U/ml	183 U/mg	60	[66]
Paenibacillus barcinonensis MG7	Soil	59	--	17 U/mg	--	[55]
Caldibacillus cellulovorans	Compost	85	0.27 U/ml	32 U/ml	117	[76]
Clostridium thermocellum	--	125	1 U/ml	3.6 U/ml	2.7	[167]
C. thermocellum	--	85-210	13 U/ml	100 U/ml	15	[136]
Ruminococcus albus F-40	Rumen	40-250	--	--	--	[64]
Rhizobium fredii J1	Indigenous soil	97	0.42 U/mg	3.8 U/mg	10	[134]

U/mg: Specific enzyme activity; U/ml: Enzyme activity

Table 4
Physiological conditions for cellulases produced by different bacteria

Bacteria	Optimum pH	Optimum temperature (ºC)	Thermal stability (ºC)	Reference
Bascilus sp. C1	7	50	20-70	[33]
Bacillus sp.	6	50	--	[125]
Bacillus thuringiensis	4	40	20-70	[168]
B. subtilis YJ1	6	50-60	<50	[66]
B. subtilis DLG	4.8	55	--	[38]
B. pumilus EB3	6	60	30-70	[127]
B. coagulans Co4	7.5	60	35-75	[169]
Bacillus sp. M-9	5	60	40-90	[70]
Paenibacillus barcinonensis MG7	7	35	25-50	[55]
Caldibacillus cellulovorans	6.5-7.0	80	65-89	[76]
Cellulomonas sp. ASN2	7.5	60	40-80	[75]
Nocardiopsis sp. KNU	5	40	--	[170]
Cellvibrio gilvus	7.6	<40	--	[171]
Rhizobium fredii J1	7	35	30-45	[134]

To initiate the production of industrially important products from cellulosic biomass, bioconversion of the cellulosic components into fermentable sugars is necessary.[82] Therefore, the enzymatic conversion of these biomasses into fermentable sugars at a pilot scale has potential applications in the bioethanol industry. Although extensive work has been carried out to meet the future challenges of bioethanol production, there is no self-sufficient process or technology available to convert the lignocellulosic biomass for bioenegy generation. Fungi and some bacteria are mainly accountable for the biodegradation of lignocellulosic substrate. Low lignin content biomass is favoured by bacterial degradation owing to a limited release of lignin degrading enzymes, such as lignin peroxidise (LiP), laccase (Lac), manganese peroxidise (MnP), versatile peroxidise (VP) and H_2O_2, generating enzymes such as glyoxal oxidase (GLOX) and aryl alcohol oxidase. Among fungi, *Phanerochaete chrysosporium* and *Phlebia radiata* are well-known producers of extracellular peroxidases,[83] as well as *Coriolus tersicolor*, which is known to produce intracellular peroxidase.[84] A white-rot basidiomycete, *Rigidoporous lignosus*, is also known to secrete two oxidative enzymes, Lac and MnP, responsible for solubilizing the lignin in a synergistic way.[85] *Ceriporiopsis subvermispora*, *Phaenerochaete chrysosporium*, *Phlebia subserialis* and *Pleurotus ostreatus* are able to metabolize lignin in a variety of lignocellulosic biomasses.[86,87] In addition, some species of bacteria (such as *Azospirillum lipoferum* and *Marinomonas mediterranea*), a brown-rot fungus *Postia placenta*[81] and saprophytic homobasidio-mycete *Pycnoporous cinnabarinus*[88] have also been reported for the mineralization of lignin. Among white-rot fungi, *Cyathus bulleri* and *C. cinnabarinus* have also played a potential role in lignin degradation.[89,90] A number of studies have aimed to increase the production potential of microbial laccase for biotechnological applications.[91-93]

Cellulase: Application and future prospects

Cellulases from microorganisms have been exploited for a broad range of industrial applications, such as animal feed, textile, detergent, paper and wine industries, including sustainable production of many chemicals and enzymes. Cellulases have been commercially available for more than 30 years, and these enzymes have represented a target for both academic and industrial research.[94,95] The enzyme complex is the most successful in finishing cellulose-based textiles,[96,97] which can remove partially detached microfibrils from cotton or cotton-blended garments and restore a smooth surface and the original colour to the garments.[96,98] The application of cellulase in paper and pulp industries has increased considerably during the last decade.[5] Cellulase, along with hemicellulases, has also been used for biomodification of fiber properties with the aim of improving drainage and beatability in the paper mills before or after beating of pulp.[99] Enzymatic saccharification of lignocellulosic materials, such as sugarcane bagasse, corncob, rice straw, *Prosopis juliflora*, *Lantana camara*, switch grass, saw dust, and forest residues, by cellulases for biofuel production is perhaps the most popular application being investigated.[100-102] Bioconversion of lignocellulosic materials into useful and high value products normally requires multi-step processes.[101,103,104] Technologies are currently available for all steps in the bioconversion of lignocellulosic biomass to ethanol and other value-added chemical products.[105-108] However these technologies must be improved to produce renewable biofuel and other by-products at prices that can compete with more conventional production systems. Not only the recalcitrance of the substrate, but also several other factors limit cellulase efficiency during the hydrolysis process, which includes end product inhibition, thermal deactivation of the native protein, nonspecific binding to lignin[109] and irreversible adsorption of the enzymes to the heterogeneous substrate.[110] Strategies for recycling and reuse of the enzymes may also be used to reduce the cost involved in enzymatic hydrolysis.[105,108,111,112] The recovery of enzyme is largely influenced by the adsorption of the enzymes onto the substrate, especially to lignin and enzyme inactivation. There are several reports where the nonspecific and irreversible adsorption of cellulase to lignin has been observed.[109,113]

Microbial glucanases and related polysaccharides play important roles in fermentation processes to produce alcoholic beverages including beers and wines.[95,100,114,115] Cellulases also have an important application as part of a macerating enzyme complex (cellulases, xylanases and pectinases) used for extraction and clarification of fruit and vegetable juices to increase the yield of juices.[116,117] The cellulases and hemicellulases are responsible for partial

hydrolysis of lignocellulosic materials, dehulling of cereal grains, hydrolysis of β-glucans and better emulsification and flexibility of feed materials, which result in the improvement in the nutritional quality of animal feed.[114,118] Various enzyme preparations consisting of different combinations of cellulases, hemicellulases and pectinases have potential applications in agriculture for enhancing the growth of crops and controlling plant diseases.[10,119] Cellulases and related enzymes are capable of degrading the cell wall of plant pathogens in controlling the plant disease.[10] These enzymes are also used to decrease olive paste viscosity in olive oil production and to intensify the process of extracting the polyphenolic substances contained in the olive fruit.[120] The use of cellulases, along with protease and lipase, in detergents is a more recent innovation in this industry.[95] Agro-industrial and forest wastes contain a large amount of unutilized or underutilized cellulose and their disposal has raised severe environmental concerns.[121,122] Nowadays, these wastes are judiciously utilized to produce valuable products, such as enzymes, sugars, biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds, and human nutrients.[97,101,102,106,123,124]

CONCLUSION

The development of rapid, reliable and inexpensive methods for characterization of the bacterial community allows us to explore a greater number of efficient cellulase producers. Despite the monopoly in producing various types of cellulases by fungi, bacteria have gained importance due to their higher growth rate, broad range of pH and temperature, and capability to degrade diverse cellulosic substrates with great ease. An improved activity of cellulase can be achieved by optimizing physical and chemical parameters, using a rational design strategy and random mutagenesis techniques. Studies have been heavily oriented towards cellulases with better catalytic performances, thus requiring lower enzyme loading with reduced reaction time in lignocellulose hydrolysis. Habitats, such as soil, compost, decaying plant materials, bovine rumen, sewage sludge, invertebrate guts, forest waste piles, wood processing plants, rotten leaf litters, animal faeces, paper mills and waste water, have been successfully explored as sources of novel cellulolytic microorganisms. The ability of these microbes may hold a great prospect to improve the competitiveness of the whole process for the production of second generation biofuel.

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