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REVIEW ARTICLE

CELLULOLYTIC MICROORGANISMS

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Abstract

Cellulose is biologically renewable resource widely found in industrial and municipal wastes and agriculture residues. The cellulosic waste material can be transformed to glucose and other soluble sugars by using cellulase enzymes of cellulolytic organisms. Now it is well known that hydrolysis of cellulose to reducing sugars, can be further used for the production of ethanol as biofuel. Within cellulolytic microorganisms, three major types of enzymatic activities are defined as cellulases which act synergistically on their substrate. At present, cellulases and related enzymes are widely used in different sectors (food, agriculture, animal feed, brewery and wine, textile and laundry, pulp and paper industries, and for research purposes). It has been thought that only a small percentage of microorganisms can degrade cellulose, probably because a wide range of cellulolytic organisms are not cultivable then unknown and not identified at present.

Keywords: cellulose, cellulases, fibrolytic organisms.

Introduction

Cellulose is the major polymeric component of plant matter and is the most abundant polysaccharide on Earth. It has been estimated that 7.2 x 1011 tonnes of cellulose is reserved in plants and that the yearly production of cellulose is 4 x 1010 tonnes (Coughlan, 1985). The half-life of cellulose at neutral pH in the absence of enzymes is estimated to be several million years so that microbial activity is responsible for most of the turnover of the carbon in cellulose although fire also plays a role (Falkowski et al. 2000).

Some cellulolytic bacteria and fungi work together with related microorganisms to convert insoluble cellulosic matter to soluble sugars (cellobiose and glucose), which are then assimilated by the cell. In order to catalyze this process, the cellulolytic microbes are able to produce several different enzymes, known as cellulases. Cellulolytic bacteria have been widely explored for cellulase production owing to their high growth rate, expression of multienzyme complexes, stability at extreme temperature and pH, lesser feedback inhibition, and ability to withstand variety of environmental stress (sharma et al. 2013).

At present, the best studied cellulose-degrading ecosystems are the rumen of herbivorous animals and compost systems. However, little is known about the microbial diversity during the composting of the organic fraction of source separated household wastes (i.e. vegetable, fruit and garden wastes, also called biowastes) (Ryckeboer et al. 2003)

Enzymatic hydrolysis is an economic process in the conversion of cellulose to easily fermentable low cost sugars (Muthuvelayudham and Viruthagiri, 2006).In case of commercial applications of industrial enzymes, microorganisms are the most important source of various enzymes (Ibrahim, 2008). Moreover, Thermostable enzymes are highly specific and thus have considerable potential for many industrial applications. The use of such enzymes those are important for industrial utilization because of the possible economic benefits of being able to degrade plant residues at elevated temperatures (Haki and Rakshit, 2003)

Cellulose biodegradation

Cellulose biogenesis results from the coordinated action of enzymatic polymerization, followed by the extrusion and crystallization of the nascent cellulose microfibrils (Brown, 1996). The combination of these events leads to the production of whisker-like crystalline microfibrils, wherein the cellulose chains are packed in parallel fashion (Hieta et al. 1984; Chanzy and Henrissat., 1985). T'he microfibrils are then assembled into superstructures, such as cell walls, fibers, pellicles and so on. Enzymatic hydrolysis of cellulose by microorganisms is a key step in the global carbon cycle.

Cellulose

Cellulose, representing more than 50% of the biomass, is the principal component of plant cell wall. It is also synthesised by some fungi (*Allomyces* and oomycetes), algae (Valonia), protozoa (*Dyctostelium, Discoideum*), bacteria (Acetobacter *xylinum, Rhizobium, Agrobacterium and Sarcinia*). Thus, some animals are able to produce cellulose, particularly in the tests of ascidians (where the cellulose was historically termed "tunicine") although it is also a minor component of mammalian connective tissue (Endean, 1961)

Cellulose is composed of linear chains of D-glucose linked by ß-1,4-glycosidic bonds (Figure1). Each D-anhydroglucopyranose unit possesses hydroxyl groups at C2, C3, and C6 positions. The molecular structure imparts cellulose with its characteristic properties: hydrophylicity, chirality, degradability, and broad chemical variability initiated by the high donor reactivity of hydroxyl groups. Cellulose is much more crystalline compared to other saccharides. to be amorphous in water cellulose requires a temperature of 320 °C and pressure of 25 MPa (Shigeru et al. 2006)

Cellulose could be found in the forme of different crystalline structures (according to the location of hydrogen bonds between and within strands). Natural cellulose is cellulose I (Iα produced by bacteria and algae and Iβ by higher plants). Cellulose II consiste in regenerated cellulose. With various chemical treatments it is possible to produce the structures cellulose III and cellulose IV (Pérez and Mackie, 2001)

Cellulases

The enormous structural variety and rigidity of cellulosic matters have given rise to a phenomenal diversity of degradative enzymes, the cellulases. There is a wide spectrum of microorganisms which can produce the variety of enzymes like cellulases, hemicellulases, ligninases, pectinases, esterases, oxidoreductases and proteases (Aslam et al. 2009; Chandra et al. 2010; Chidi et al. 2008). Although a large number of microorganisms can degrade cellulose, only a few them produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose (Koomnok, 2005)

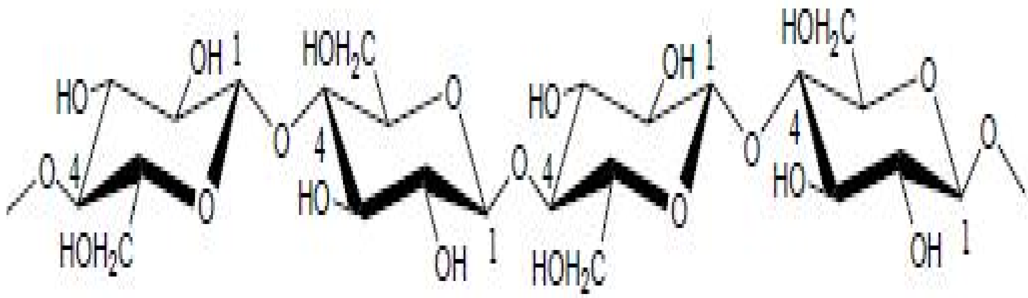
Components of cellulase systems were first classified based on their mode of catalytic action and have more recently been classified based on structural properties (Henrissat et .al., 1998). Three major types of cellulases are known, endoglucanases, exoglucanases and β-glucosidases. These enzymes can either be free or grouped in a multicomponent enzyme complex (cellulosome) found in anaerobic cellulolytic bacteria (Mosier et al. 1999).

Biotechnology of cellulases and hemicellulases began in early 1980s, first in animal feed followed by food applications (Chesson, 1987; Thomke et al. 1980; Voragen, 1992; Voragen et al. 1980, 1986). Cellulases have versatile applications in textile, laundry, pulpand paper, fruit juice extraction, and animal feed additives (Das et al. 2010). In addition, they find use in saccharification of lignocellulosic agroresidues to fermentable sugars which can be used for production of bioethanol, lactic acid, and single-cell protein (Tae et al ., 2000; Sanchez and Cardona, 2008).

Cellulolytic microorganisms diversity

Originally it was thought that only microorganisms produced cellulases but it is now clear that some insects, mollusks, nematodes, and protozoa also produce cellulases (Watanabe and Tokuda, 2001) At present, It appears that some animal species,

Figure 1. The structure of cellulose



including termites and crayfish, produce their own cellulases, which differ substantially from those of their indigenous microflora (Watanabe and Tokuda, 2001). Moroever, even when termites, ruminants or shipworms utilize cellulose as an energy source, microorganisms usually are involved in its degradation (Weimer, 2009; Distel et al. 2002).

In the rumen, forage degradation and fermentation in assimilable compounds for host animals are carried out by a strict anaerobic microbial population made up of numerous species of bacteria, protozoa and fungi organised in a trophic chain (Bhat, 2000). Indeed the fibrolytic agents in the digestive tracts of ruminants are essentially represented by both bacteria and Chytridomycete fungi. The number of bacteria is more important, and in lowfiber diets the fungi are often absent (Lee et al. 1997). However, the fungi appear to enhance degradation via physical penetration and weakening of the plant cell walls (Akin et al. 1990; 1989; Ho et Abdullah, 1999). Moroever, among the bacteria, there is a distinct difference in cellulolytic strategy between the aerobic and anaerobic groups. With relatively few exceptions (Rainey et al. 1994; Svetlichnyi et al. 1990).

No cellulolytic microorganism of domain *Archaea* have yet been discovered (Lynd et al. 2002). Whereas, there is considerable number of cellulolytic microorganisms within the eubacteria among the predominantly aerobic order *Actinomycetales* (phylum *Actinobacteria*) and the anaerobic order *Clostridiales* (phylum *Firmicutes*) (Lynd et al. 2002).

The cellulolytic bacteria (Table 1) comprise diverse physiological groups but only a few species within are actively cellulolytic:

Group (1) aerobic gram-positive bacteria (*Cellulomonas* and *Thermobifida*);

Grou p (2) aerobic gliding bacteria (*Cytophaga*, and *Sporocytophaga*).

Grou p (3) fermentative anaerobes, (*Clostridium, Ruminococcus*, and *Caldicellulosiruptor*) containing a few gram-negative species, most of which are phylogenetically related to the *Clostridium* assemblage (*Butyrivibrio* and *Acetivibrio*) but some of which are not (*Fibrobacter*).

Fungi are well-known agents of decomposition of organic matter in general and of cellulosic substrates in particular (Carlile and Watkinson., 1997; Montegut, 1991). Fungal cellulose utilization is distributed across the entire kingdom, from the primitive, protist-like Chytridomycetes to the advanced Basidiomycetes. A number of species of the most primitive group of fungi, the anaerobic Chytridomycetes, are well known for their ability to degrade cellulose in gastrointestinal tracts of ruminant animals. Cellulolytic capability is also well represented among the remaining subdivisions of aerobic fungi. Within the approximately 700 species of Zygomycetes, only certain members of the genus *Mucor* have been shown to possess significant cellulolytic activity, although members of this genus are better known for their ability to utilize soluble substrates. By contrast, the much more diverse subdivisions Ascomycetes, Basidiomycetes, and Deuteromycetes (each of which number over 15,000 species (Carlile and Watkinson., 1997), contain large numbers of cellulolytic species. Members of genera that have received considerable study with respect to their cellulolytic enzymes and/or wood-degrading capability

Table 1. Morphological features of some cellulolytic strains of bacteria (Lynd et al., 2002)

Oxygen relationship	Representative species	Gram reaction	Morphology	Motility	References
Aerobic	*Acidothermus cellulolyticus*	+	Rod		(Bergquist et al. 1999)
	Bacillus pumilis	+	Rod	Flagellar	(Gordon et al. 1973)
	Caldibacillus cellovorans	+	Rod		(Bergquist et al. 1999)
	Cellulomonas flavigena, C. uda	+	Rod	Flagellar	(Bagnara et al. 1987)
	Cellvibrio fulvus, C. gilvus	-	Curved rod	Flagellar	(Shafer et King, 1965)
	Cytophaga hutchinsonii	-	Rod	Gliding	(Kauri et Kushner, 1985)
	Erwinia carotovora	-	Rod	Flagellar	(Barras et al. 1994)
	Micromonospora	+	Filamentous rod	Nonmotile	(Gallagher et al. 1996)
	Pseudomonas fluorescens var. *cellulosa*	-	Rod	Flagellar	(Kim, 1987)
	Sporocytophaga myxococcoides	-	Rod	Gliding	(Vance et al. 1980)
	Rhodothermus marinus		Rod		(Bergquist et al. 1999)
	Streptomyces reticuli	+	Filamentous rod	Nonmotile	(Wachinger et al. 1989)
	Thermobifida fusca	+	Filamentous rod	Nonmotile	(Zhang et al. 1998)
Anaerobic	*Acetivibrio cellulolyticus*	-	Curved rod	Nonmotile	(Khan et al. 1994)
	Anaerocellum thermophilum	+	Rod	Flagellar	(Svetlichnyi et al. 1990)
	Butyrivibrio fibrisolvens	+	Curved rod	Flagellar	(Hungate, 1966)
	Caldicellulosiruptor saccharolyticum	-	Rod	Flagellar	(Rainey et al. 1994)
	Clostridium thermocellum, C.cellulolyticum	+	Rod	Flagellar	(Ljungdahl et al. 1981)
	Eubacterium cellulosolvens	+	Rod	Nonmotile	(Gylswyk et al. 1986)
	Fervidobacterium islandicum	-	Rod	Flagellar	(Huber et al 1990)
	Fibrobacter succinogenes	-	Rod	Nonmotile	(Hungate, 1966)
	Halocella cellulolytica	-	Rod	Flagellar	(Simankova et al. 1993)
	Ruminococcus albus, R. flavefaciens	+	Coccus	Nonmotile	(Hungate, 1966)
	Spirochaeta thermophila	+	Spiral		(Aksenova et al. 1992)
	Thermotoga neapolitana	-	Rod		(Bergquist et al. 1999)

include *Bulgaria, Chaetomium*, and *Helotium* (Ascomycetes); *Coriolus, Phanerochaete, Poria, Schizophyllum* and *Serpula* (Basidiomycetes); and *Aspergillus, Cladosporium, Fusarium, Geotrichum, Myrothecium, Paecilomyces, Penicillium*, and *Trichoderma* (Deuteromycetes) (Lynd et al; 2002).

The nutrient requirements for growth of cellulolytic species include available nitrogen, phosphorus, and sulfur, plus standard macro- and microminerals and various vitamins. Although additional nutrients present in complex media (e.g., peptones and yeast extract) are not usually required, they often stimulate the growth of individual strains, sometimes dramatically (Lynd et al; 2002).

Conclusion

The increase in human population with Industrial development and biotechnology progression enabled easier daily production of enormous residues and urbain wastes which contains several kinds of polymers, an important biologically renewable resource. Basic and applied research regarding fibrolytic organisms producing a wide variety of enzymes (cellulases, hemicellulases and pectinases) has not only enhanced our scientific knowledge but has also revealed their enormous potential in different sectors. However, until now the number of cellulolytic microorganisms clearly identified and characterized is low compared to the high number of organisms and the enormous complexity of cellulose-degrading ecosystems. Accordingly, our researches about such microorganisms should be multiplied again.

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