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Cellulase Production by Bacteria: A Review

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Authors' contributions

This work was carried out in collaboration between all authors. Author SS has performed acquisition of data from different paper and involved in drafting and revising the manuscript. Author TKM has contributed drafting the manuscript, interpretation of data and necessary correction of the manuscript. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Cellulose is an abundant natural biopolymer on earth and most dominating Agricultural waste. This cellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value-added bioproducts. It can be degraded by cellulase produced by cellulolytic bacteria. This enzyme has various industrial applications and now considered as major group of industrial enzyme. The review discusses application of cellulase, classification of cellulase, quantification of cellulase, the types of cellulolytic bacteria and their screening. It describes the current knowledge of cellulase production by submerged fermentation and solid state fermentation, properties of cellulase and cloning and expression of cellulase gene. The biotechnological aspect of cellulase research and their future prospects are also discussed.

Keywords: Cellulytic bacteria; bioconversion; cellulases; endoglucanase; exoglucanase; β-glucosidase; cellulosome.

1. INTRODUCTION

About 200 gigatons of CO_2 are fixed of earth every year and the equivalent amount of organic material has to be degraded approximately 30 % by plants and animals to 70 % by microorganisms [1]. On average, cellulose accounts as 50% of the dry weight of plant

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biomass. Such plant biomass is the only foreseeable sustainable source of fuels and materials available to humanity. Agricultural residues are a great source of lignocellulosic biomass which is renewable, chiefly unexploited and inexpensive. These renewable resources are leaves, stems, and stalks from sources such as corn fiber, corn stover, sugarcane bagasse, rice straw, rice hulls, woody crops, and forest residues. Besides, there are multiple sources of lignocellulosic waste from industrial and agricultural processes, e.g., citrus peel waste, coconut biomass, sawdust, paper pulp, industrial waste, municipal cellulosic solid waste, and paper mill sludge. In addition, dedicated energy crops for biofuels could include perennial grasses such as Switch grass and other forage feed stocks such as *Miscanthus*, Elephant grass, Bermuda grass, etc [2].

Approximately 70% of plant biomass is locked up in 5- and 6-carbon sugars. These sugars are found in lignocellulosic biomass comprised of mainly cellulose (a homologous polymer of glucose linked by β 1.4 glycosidic bonds) hydrolyzed by a complex enzyme system named as cellulase (exoglucanase, endoglucanase and β glucosidase etc.); lesser hemicelluloses (heterologous polymer of 5- and 6-carbon sugars consists of pentoses D-xylose, D-arabinose and hexoses D-mannose, D-glucose, D-galactose with sugar acids); and least of all lignin (a complex aromatic polymer). In hardwoods hemicellulose contains mainly xylans, while in softwood mainly glucomannans are present. Briefly, xylan degradation requires endo-1-4,-β-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, as well as acetylxylan esterases. In glucomannan degradation β-mannanase and β-mannosidase are required to cleave the polymer backbone.

The limited nature of fossil fuels reserves which has been depleting at an alarming rate by civilized world. Burning of fossil fuels has also created a concern for unstable and uncertain petroleum sources, the rising cost of fuels and a concern with respect to global climate change. These concerns have shifted to utilize renewable resources for the production of a 'greener' energy replacement which can meet the high energy demand of the world. The Canadian renewable fuel standard has been raised and will contain 5% ethanol by 2010; the US Environmental Protection Agency raised their renewable fuel standard to 10.21% ethanol mixed fuels by 2009; while, the mandate for mixing ethanol in fuel for Brazil is 25% (set in 2007). Cellulases contribute to 8% of the worldwide industrial enzyme demands [3]. The cellulase market is expected to expand dramatically when cellulases are used to hydrolyzed pretreated cellulosic material to sugars, which can be fermented to bioethanol and biobased products on large scales. The cellulase market has been estimated in the United States to be as high as US $ 400million per year [4]. In the period 2004 -2014 an increase of approximately 100 % in the use of cellulase as a specialty enzyme is expected [5]. The biotechnology companies Genencor International and Novozymes Biotech have reported the development of technology that has reduced the cellulase cost for the cellulose-to-ethanol process from US$5.40 per gallon of ethanol to approximately 20 cents per gallon of ethanol [6], in which the two main strategies were (1) an economical improvement in production of cellulase to reduce US$ per gram of enzyme by process and strain enhancement, e.g., cheaper medium from lactose to glucose and alternative inducer system and (2) an improvement in the cellulase enzyme performance to reduce grams of enzyme for achieving equivalent hydrolysis by cocktails and component improvement [7].

In addition to this, the major industrial application of cellulases are in textile industry for bio-polishing of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness [8]. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juice and in baking, while de-inking of paper is yet another emerging application. A potential

challenging area where cellulases would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals [9]. Application of this enzyme in detergent, leather and paper industries demands identification of highly stable enzymes active at extreme pH and temperature. Some important applications of cellulases or cellulolytic bacteria are given in Table.1.

Table 1. Applications of cellulases or cellulolytic bacteria (Mandel, 1985) [10]

Application	
1.Removal of cell walls, crude fiber	a)Release cell contents Flavors Oils Spices Polysaccharides(agar) Proteins(seeds, leaves) b)Improve rehydratability of dried vegetables Soup mixes c)Oil seed cakes Straws Barley Mesquite d)Production of plant protoplasts Genetic engineering (higher plants)
2.Production of glucose, soluble sugars	a)Animal feed Molasses(direct or by-product) Increase nutritive value (add sugar to high-fiber feed Single–cell protein b)Industrial feedstock Glues, adhesives Solvents (ethanol, butanol, acetone.etc.) c)Raw material for fermentation industry Antibiotics Acetic acid,citric acid etc.
3.Production of lignin	Adhesives Resins Extenders Chemical raw materials
4.Miscellaneous food applications	a)Cell free protein High productivity High quality protein b)Addition of mycelia and extracellular protein Removal of crude fiber Conversion of fiber to sugar Removal of other unwanted compounds c)Protease production (e.g., meat tenderizer)
5.Decomposition of wastes and residues	Sewage treatments

The present review elucidated on bacterial cellulase production in both natural and technological context. Moreover, bacterial cellulase utilization from an integrative perspective

and diversity of cellulolytic bacteria and enzyme systems are described. Attempts are made to discuss the mode of action of cellulase in bacterial system and molecular biology of their regulation. In addition, the review also addressed cloning and expression of cellulase genes in heterologous hosts and how these rare cellulases can help some of the major bottlenecks in the biofuel industry and how some unique bacterial strategies in biotechnology can help in biorefining.

2. CLASSIFICATION OF CELLULASE

Microorganisms produced extracellular cellulases that are either free or cell associated to hydrolyze and metabolize insoluble cellulose. The biochemical analysis of cellulose systems from aerobic and anaerobic bacteria and fungi has been comprehensively reviewed during the past three decades. Following components of cellulase systems were classified based on their mode of catalytic action (Table 2).

2.1 Endoglucanases or Endo-1, 4-β-D-Glucan Glucanohydrolases (EC 3.2.1.4)

Endoglucanases cut at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends. It is generally active against acid-swollen amorphous cellulose, soluble derivatives of cellulose such as CMC, cellooligosaccharides [11].

2.2 Exoglucanase or 1, 4-β-D-Glucan Cellobiohydrolases (Cellobiohydrolases) (EC 3.2.1.91)

Exoglucanases act in a possessive manner on the reducing or non-reducing ends of cellulose polysaccharide chains, liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. These enzymes are active against crystalline substrate such as Avicel, amorphous celluloses and cellooligosaccharides. However, they are inactive against cellobiose or substituted soluble celluloses such as CMC.

2.3 Exoglucanases or 1, 4-β-D-Oligoglucan Cellobiohydrolases (Also Known as Cellodextrinases) (EC 3.2.1.74)

It catalyzes the removal of cellobiose from cellooligosaccharides or from p-nitrophenyl *-β* -D-cellobioside but inactive against amorphous cellulose or CMC.

2.4 β - Glucosidases or β-D-Glucoside Glucohydrolases (EC 3.2.1.21)

β -Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose from non-reducing end. It is inactive against crystalline or amorphous cellulose.

2.5 Cellobiose Phosphorylase or Cellobiose: Orthophosphate Alfa–D-Glucosyl Transferase (EC 2.4.1.20)

It catalyzes the reversible phosphorolytic cleavage of cellobiose. It was first discovered by Ayers [12] in cells of *Ruminococcus flavefacience.*

$$\text{Cellobiose} + H_3PO_4 = \text{alfa} - D - \text{glucose 1-P} + \text{glucose}$$

2.6 Cellodextrin Phosphorylase Or 1,4-B-D-Oligoglucan Orthophosphate Alfa – D-Glucosyl Transferase (EC 2.4.1.49)

It was found in cells of *Clostridium thermocellulam* [13]. It does not act on cellobiose but catalyze the reversible phosphorylytic cleavage of cellodextrins ranging from cellotriose to cellohexose.

$$(1,4 – \beta\text{-D-glucosyl})_n + H_3PO_4 = (1,4 – \beta\text{-D-glucosyl})_{n-1} + \text{alfa –D-Glucose-1-P}$$

2.7 Cellobiose Epimerase (EC 5.1.3.11)

It was first reported in cells of *Ruminococcus albus* [14]. It catalyzes the following reaction:

Cellobiose = 4-O- β-D-glucosylmannose

3. SCREENING OF CELLULASE PRODUCING BACTERIA

Screening for bacterial cellulase activity in microbial isolates is typically performed on plates containing crystalline cellulose or microcrystalline cellulose such as Avicel in the agar at a final concentration of 0.1-0.5 %(w/v). After incubation of a suitable period, a zone of clearing surrounding the colonies will be indicated that cellulose producer [15]. The colonies of cellulolytic *Cytophaga* spp. did not shown any clearing zone [16]. So the diameter of the clearing zone may not accurately reflect the true cellulase activity.

For a rapid screening of cellulase producing bacteria, after the incubation of the agar medium are containing 0.5% (W/V) carboxymethyl cellulose (CMC) as sole carbon source and flooded with 1% (W/V) Congo red [17]. After 20 minutes, the dye is decanted and the plates are again flooded with 5M NaCl which is decanted after 20-30 minutes. Positive colonies are detected to be surrounded by a pale orange to clear zone against red background. The cellulolytic bacteria can be screened directly on such plate, but replica plating from master plate is preferred for isolation of active colonies as flooded reagent impairing isolation. Plant et al. [18] has reported a semi-quantitative assay for cellulase activity in bacteria by using cellulose-azure into the upper two layers of agar tubes. The dye released from the substrate is determined densitometrically. Kasana et al. 2008 found that Gram's iodine for plate flooding in place of hexadecyltrimethyl ammonium bromide or Congo red, gave a more rapid and highly discernable result [19].

However, plate-screening methods using dyes are not quantitative method for the poor correlation between enzyme activity and halo size. This problem solved by the development of short cellooligosaccharides possessing modified reducing terminal with chromogenic/fluorogenic groups e.g. fluorescein, resorufin and 4-methylumbelliferone for higher sensitivity and quantification [20]. But a major limitation of the use of fluorescent substrates into agar plates is the tendency for hydrolysis products to diffuse widely and therefore are not as readily used such compounds. So, new substrates, 2-(2'-benzothiazolyl)-phenyl (BTP) cellooligosaccharides were synthesized for the screening of cellulolytic microorganisms in plate assays [21].

Table 2. Bacterial cellulase enzyme system

Enzyme	E. C. number	Reaction	Other Names	Family
i)Endo -1,4 β-D-glucan-glucanohydrolase	E. C. 3. 2. 1. 4	Cut at random at internal amorphous sites of cellulose generating oligosaccharides of various lengths. It acts on Endo-1, 4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans.	Endoglucanse, Endo-1,4-β –glucanse, Carboxymethyl cellulase, β -1,4-endoglucon hydrolase, Endocellulase	5, 6, 7, 8, 10, 12, 44, 51, 61, 74
ii)Exoglucanase or 1,4-β-D-glucan cellobiohydrolases (cellobiohydrolases)	E.C.3.2.1.91	Hydrolysis of 1,4-beta-D-glucosidiclinkages in cellulose and cellotetraose, releasing cellobiose from the non-reducing ends of the chains	Exoglucanase, Exocellobiohydrolase, 1, 4- β-cellobiohydrolase.	5, 6, 7, 9, 10, 48,
iii) Exoglucanases or 1,4-β-D-oligoglucan cellobiohydrolases	EC 3.2.1.74	Removal of cellobiose from cellooligosaccharide or from p-nitrphenyl-β-D-cellobioside	Cellodextrinases	-
iv) β - Glucosidases or β-D-glucoside gluco-hydrolases	E.C.3.2.1.21 .	Hydrolysis of terminal non-reducing beta-D-glucose residues with release of beta-D-glucose.	Gentobiase, Cellobiase, Amygdalase.	1, 3, 9
v) Cellobiose: orthophosphate alfa–D-glucosyl transferase	E.C. 2.4.1.49	It catalyzes the reversible phosphorolytic cleavage of cellobiose	Cellobiose phosphorylase	-
vi) 1,4-β-D-oligoglucan:orthophosphate alfa –D-glucosyl transferase	E.C. 2.4.1.20	It catalyzes the reversible phosphorolytic cleavage of cellodextrins ranging from cellotriose to cellohexoses.	Cellodextrin phosphorylase	-
vii) Cellobiose 2- epimerase	EC 5.1.3.11	It catalyzes the cellobiose into 4-O- β-D-glucosylnannose.	Cellobiose 2- epimerase	-
viii) Complete Cellulase system	-	Catalyzes extensive hydrolysis of crystalline cellulose	Total cellulase	-

Researchers have now focused to cellulase genes from unculturable microorganisms in extreme environments with hopes that the enzymes isolated will be novel and have specific applications in the biorefining industry due to a higher resistance to harsh environmental conditions. To identify novel cellulases from all species present, culturable and nonculturable in a swift manner, a metagenomic clone library should be prepared and then functionally screened.

4. CELLULASE PRODUCTION USING THE SUBMERGED FERMENTATION (SmF) AND SOLID STATE FERMENTATION (SSF) OR CULTIVATION (SSC)

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms. It has been widely used for the production of cellulase for their wide uses in industry. Over the years, fermentation techniques have gained immense importance due to their economic and environmental advantages. Two broad fermentation techniques have emerged as a result of this rapid development: Submerged Fermentation (SmF) and Solid State Fermentation (SSF).

4.1 Solid-State Fermentation (SSF) / Solid-State Cultivation (SSC)

SSF utilizes solid substrates, like bran, bagasse, paddy straw, other agricultural waste and paper pulp [22]. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as cheaper substrates. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high water activity, such as bacteria [23].

4.2 Submerged Fermentation (SmF)/Liquid Fermentation (LF)

SmF utilizes free flowing liquid substrates, such as molasses and broth [22]. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content. An additional advantage of this technique is that purification of products is easier.

4.3 A Comparison between SmF and SSC Method

Cellulases are produced using the submerged fermentation (SmF) method traditionally, in which the cultivation of microorganisms occurs in an aqueous solution containing nutrients. An alternative to this traditional SmF method is the solid state cultivation (SSC) method, which involves the growth of microorganisms on solid materials in the absence of free liquids [24]. Since SSC involves relatively little liquid when compared with SmF, downstream processing from SSC is theoretically simpler and less expensive (Fig. 1 and Table 3. During the past ten years, a renewed interest in SSC has developed due, in part, to the recognition that many microorganisms, including genetically modified organisms (GMO), may produce their products more effectively by SSC [25]. SSC has three advantages viz. i) lower consumption of water and energy, ii) reduced waste stream and iii) more highly concentrated product [26]. Moreover, The biosynthesis of cellulases in SmF process is strongly affected by catabolic and end product repressions [27] and on the overcoming of these repressions to significant extent in solid state fermentation (SSF) system [28], therefore, are of economic importance. The amenability of SSF technique to use up to 20-30% substrate, in contrast to the maximum of 5% in SmF process, has been documented [29].

The SSF is generally preferred as it offers many advantages such as two-three times higher enzyme production as well as protein rate, higher concentration of the product in the medium, direct use of air-dried fermented solids as source of enzyme which lead to elimination of expenses on downstream processing, employment of natural cellulosic wastes as substrate in contrast to the necessity of using pure cellulose in submerged fermentation (SmF) and the possibility of carrying out fermentation in non-aseptic conditions [30]. Some example of cellulase producing bacteria with their method of fermentation is given in Table 4.

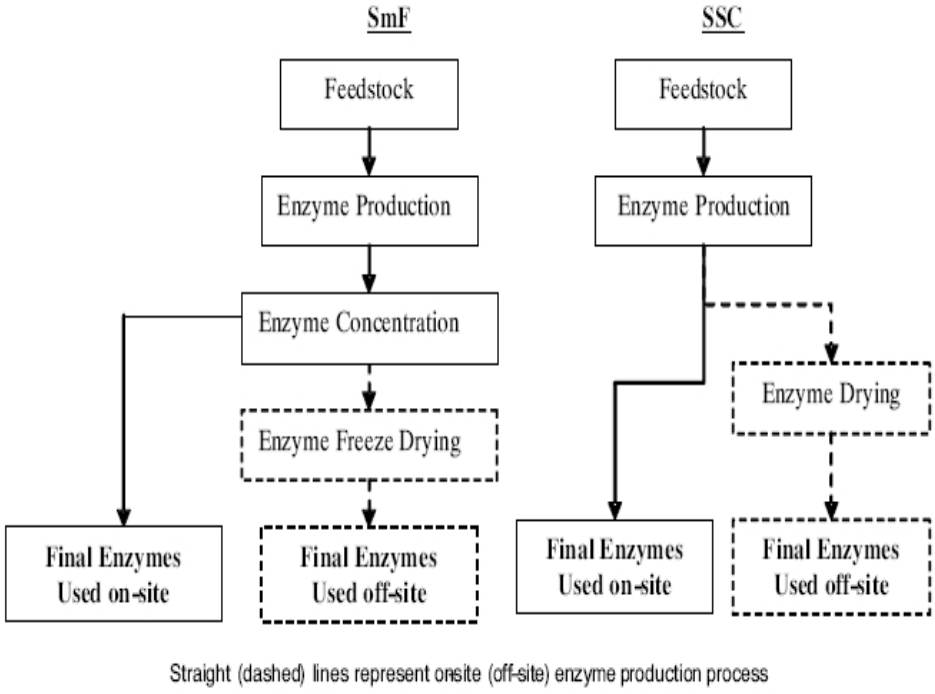

Fig. 1. Flow chart of enzyme production using the traditional SmF method compared to the SSC method. [26]

Table 3. Comparison of characteristics for SmF and SSC methods

Factor	SmF	SSC
Water	High volumes of water consumed and effluents discarded	Limited consumption of water and no effluent
Mechanical agitation	Good homogenization	Static conditions preferred
Scale up	Industrial equipment available	New design equipment needed
Energy	High energy consuming	Low energy consuming
Equipment Volume	High volumes and high costs	Low volumes and lost costs
Concentration	30-80g/l	100-300g/l

Table 4. Fermentative production of cellulase by bacteria

Name of the bacteria	Tempera-ture	Types of Substrates used	pH	Type	References
Anoxybacillus flavithermus EHP2	75°C	CMC	7.5	SmF	[31]
Anoxybacillus sp. 527	70°C	Crystalline cellulose	6.0	SmF	[32]
Bacillus sp.AC-1	70°C	CMC	4.5–6.5	SmF	[33]
Bacillus sp. LFC15	50°C		9–10	SmF	[34]
Bacillus subtilis	37°C	CMC	7.0	SmF	[35]
Bacillus thuringiensis	40ºC	Soluble cellulose, CMC, Insoluble crystalline cellulose	4.0	SmF	[36]
Bacillus sp	50°C	Sugar Cane Bagasse	4.5-5.5	SSF	[37]
Bacillus sp. NZ	50°C	agricultural residues	9–10	SSF	[38]
Bacillus sp	-	Round nut shell	-	SSF	[39]
Bacillus Cereus		Palm Kernel Cake		SSF	[40]
Bacillus licheniformis MVS1	50-55	CMC, Filter paper	6.5 to 7.0	SSF	[41]
Bacillus sp. MVS3					
Cellulomonas cellulans MTCC 23	-	Paddy Straw	-	SmF	[42]
Clostridium thermocellum		Cellulose and paper pulp		SmF and SSF	[26]
Cytophaga hutchinsonii NCIM 2338	-	Paddy Straw	-	SmF	[42]
Streptomyces sp. BRC1	26°C	CMC	7.0-7.5	SmF	[43]
Streptomyces sp. BRC2					
Microbacterium sp. MTCC 10047	37°C	CMC	7.0	SmF	[44]
Bosea sp. MTCC 10045	37°C	CMC	7.0	SmF	[45]

5. METHODS FOR QUANTIFICATION OF CELLULASES

All existing cellulase activity assays can be divided into three types: (1) the accumulation of products after hydrolysis, (2) the reduction in substrate quantity, and (3) the change in the physical properties of substrates. The majority of assays involve the accumulation of hydrolysis products, including reducing sugars, total sugars, and chromophores are given in the Table 5.

Table 5. The common colorimetric sugar assays (modified from Zhang et al. [4])

Method		Sample (mL)	Reagent (mL)	Glucose amount (μg/sample)	Glucose concn. (mg/L)	References
Reducing Sugar Assay DNS	Micro	1- 3	3	20- 600	6.7- 600	[46]
DNS	Micro	0.5	3	100- 2500	200- 5000	[47]
Nelson-Somogyi	Micro	1- 5	2+2	1- 10	0.2- 10	[48]
Nelson-Somogyi	Micro	2	2+2	10- 600	5- 300	[48]
Nelson	Semi- Micro	2	2	5- 100	2.5- 50	[49]
Ferricyanide-1		1- 3	1+5	1- 9	0.3- 9	[50]
Ferricyanide-2		1	0.25	0.18- 1.8	0.18- 1.8	[51]
PAHBAH	Micro	0.5	1.5	0.5- 5	1- 10	[52]
PAHBAH	Micro	0.01	3	5- 50	500- 5000	[52]
BCA		0.5	0.5	0.2- 4.5	0.4 -9	[53]
Modified BCA		1	1	0.4 – 9	0.4 -9	[54]
Total Sugar Assay Phenol-H_2SO_4		1	1+5	5- 100	10- 100	[55,54]
Anthrone-H_2SO_4		1	1+5	5- 100	10- 100	[56,57]
Enzymatic Glucose Assay Glucose-HK/PGHD kit		0.01	1	2- 50	200- 5000	Sigma Kit
Glucose-HK/PGHD kit		0.2	0.5	2- 50	4 - 100	[58]

6. CELLULASE PRODUCING BACTERIA AND THEIR CHARACTERIZATION

Both fungi and bacteria have been exploited for their abilities to produce a wide variety of cellulases and hemicellulases. Most emphasis has been placed on the use of fungi because of their capability to produce copious amounts of cellulolytic enzymes and often less complex than bacterial cellulase and easy for extraction and purification. It can therefore be more readily cloned and produced via recombination in a rapidly growing bacterial host. However, the isolation and characterization of novel cellulase from bacteria are now becoming widely exploited. There are several reasons for these shifts viz. i) bacteria often have a higher growth rate than fungi allowing for higher recombinant production of enzymes, ii) bacterial cellulases are often more complex and are in multi-enzyme complexes providing increased function and synergy iii) bacteria inhabit a wide variety of environmental and industrial niches like thermophilic or psychrophilic, alkaliphilic or acidiophilic and halophilic strains, which produce cellulolytic strains that are extremely resistant to environmental stresses. These strains can survive and produce cellulolytic enzymes in the harsh conditions which are found to stable under extreme conditions and which may be used in the bioconversion process [59]. This may increase rates of enzymatic hydrolysis, fermentation, and, product recovery. Researchers are now focusing on utilizing, and improving these enzymes for use in the biofuel and bioproduct industries.

Many bacteria can grow on cellulose and many produce enzymes that catalyze the degradation of soluble derivatives of cellulose or the amorphous regions of crystalline cellulose. However few bacteria synthesize the complete enzyme system that can result in extensive hydrolysis of the crystalline material found in nature. These few bacteria should be called “true cellulolytic “bacteria and those bacteria that produce some endoglucanases and ß-glucosidases, but not the complete system, are called “pseudocellulolytic” [60]. Such pseudocellulolytic bacteria may have picked up the genes encoding these enzymes from true cellulolytic species by horizontal transfer.

There are different types of bacteria isolated from different environment produced cellulase. Some of the important bacteria and the characteristic features of their cellulase component are given below (Table 6)

Table 6. Properties of some Cellulase enzymes isolated from Anaerobic and Aerobic Cellulolytic bacteria (modified from Frank et al. 1992) [60]

Name of the bacteria	Enzyme	Mol. Wt.	Optimum temp.(ºC)	Optimum pH	References
Aerobic					
Bacillus licheniformis 1	Endoglucanase	-	55	6.1	[61]
Bacillus sp (alkalophilic) 1139	Endoglucanase	92	-	9.0	[62]
Bacillus sp (alkalophilic) (cloned in *E.coli*) N-4	Endoglucanase cel A Endoglucanase cel B Endoglucanase cel C	54 46 100	-	5.0-11.0 5.0-11.0 9.0	[63] [63]. [64]
Bacillus sp (neutrophilic) KSM-522	Endoglucanase	35	50	7.0-10.0	[65]
Bacillus subtilis	Endoglucanase	33	60	5.5	[66]

(cloned in *B.megaterium*)					
Bacillus subtilis DLG	Endoglucanase	35	55	4.8	[67]
Cellulomonas uda	Exocellobiohydrolase	81	45-50	5.5-6.5	[68]
Cellvibrio gilvus ATCC13127	Cellobiose phosphorylase	280	<40	7.6	[69]
Microbispora bispora	Endoglucanase I	44	-	5.5-7.2	[70]
	Endoglucanase II	57	-	5.5-7.2	[71]
	Exoglucanase I	75	-	5.9-7.2	
	Exoglucanase II	95	-	5.9-7.2	
	ß-Glucosidase	-	-	6.0	[70]
Thermomonospora fusca YX	Endoglucanase 1	94	74	6.0	[72]
	Endoglucanase 2	46	58	6.0	[72]
Bacillus M-9	Endoglucanase	54	60	5.0	[73]
Bacillus amyoliguefaciens DL3	Endoglucanase	54	50	7.0	[74]
Bacillus sp. HSH-810	Endoglucanase	80	40-70	10.0	[75]
Thermomonospora sp.	Endoglucanase	38	50	5.0	[76]
Cellulomonas sp. YJ5	Endoglucanase	43.7	60	7.0	[77]
Pseudomanas flurescens	Endoglucanase	36	35	7.0	[78]
Nocardiopsis sp. KNU	Endoglucanase	-	40	5.0	[79]
Bacillus subtilis YJ1	Cellulase	32.5	60	7.0	[80]
Bacillus sp (cloned in E.coli) AC-1	Endoglucanase (*Ba* - EGA)	74.87	-	-	[81]
Cellulomonas sp. ASN2.	Endoglucanase	-	60	7.5	[82]
Bacillus coagulans Co4	Endoglucanase	-	60	7.5	[83]
Anaerobic					
Acetivibrio cellulolyticus ATCC33288	Exoglucanase C1	38	-	-	[84]
	Endoglucanase C2	33	-	-	
	Endoglucanase C3	10.4	-	-	
	ß-Glucosidase B1	81.0	-	-	
Alcaligenes faecalis	ß-Glucosidase	100	-	-	[85]
Bacteroides cellulosolvens S-85	Endoglucanase EG1	65	39	6.4	[86]
Bacteroides succinogenes	Endoglucanase EG2	118	39	5.8	[86]
Clostridium josui	Endoglucanase	45	60	6.8	[87]
Clostridium thermocopriae	Endoglucanase	46	-	6.5	[88]

JT3-3					
Clostridium thermocellum LQRI	Endoglucanase I	94	62	5.2	[89]
Ruminococcus albus SY3	Endoglucanase	30	-	-	[90]

7. MODE OF ACTION OF CELLULASE IN BACTERIAL SYSTEM

Researchers have focused on four structures believed to be important in specific adhesion to cellulose viz. 1) large multicomponent complexes called cellulosomes [91]; 2) fimbriae or pili adhesions [91]; 3) Carbohydrate epitopes of bacterial glycocalyx layer [92]; and 4) enzyme binding domains [93].

7.1 Adhesion via Cellulosome like Complexes

Cellulosomes are large, stable, multi-enzyme complexes specialized in the adhesion to and degradation of cellulose that reside with protuberances visible on the cell surface. The cellulosome complex is composed of a central non-catalytic subunit (termed scaffoldin) which contains a cellulose binding domain (CBD) and a number of attachment sites (called cohesins), which serve to bind the enzymatic submits. The enzymatic submits contain a catalytic domain and a docking domain (called dockerin), the latter interacting with due of the cohesions on scaffoldin [94]. The most complex and best investigated cellulosome is that of the thermophilic bacterium *Clostridium thermocellum.*

7.2 Adhesion via Fimbriae or Pili

Fimbriae or pili, which have been implicated in bacterial adhesion which are surface appendages. It is 5 to 7 nm in width and 100 to 200 nm in length found in gram-negative bacteria [95]. As more has been learned about the role of fimbriae in adhesion, it has become clear that structural subunits of fimbriae are responsible for the adhesions. Some subunits in the gram-positive bacteria *Actinomyces viscosus* [96] and *Streptococcus sanguis* [97] associated with the fimbriae have been identified. The carbohydrate binding sites of *E.coli* has three types of fimbriae which are in small (28 to 35 Kda) repeated subunits and most are in the lips of the fimbriae with a few additional sites along their length [98].In *Ruminococcus albus*, a novel forms of cellulose-binding protein (cbpC 17.7 KDa) has been recognized that belongs to the pil protein and most similar to the type 4 fimbrial proteins of gram-negative, pathogenic bacteria [99].

7.3 Adhesion via Carbohydrates Epitopes of Bacterial Glycocalyx

Most of the evidence about adhesion via carbohydrate epitopes has been found from electron microscopy observation [100]. Several studies revealed that the slime layer surrounding *Ruminococcus albus* and *Ruminococcus flavefaciens* has composed of glycoprotein (Carbohydrate residues) were involved in adhesions of the bacteria [100]. If glycocalyx carbohydrate was removed by periodate oxidation with the protease and dextranase treatment, the adhesion of *R.albus* and *Fibrobacter succinogenes* to cellulose has been decreased significantly [95]. More direct evidence for the role of carbohydrate in adhesion was given in *Fibrobacter* sp. [92].

7.4 Adhesion via Cellulose- Binding Domains of Cellulolytic Enzymes

It has been revealed that two functional domains are found in cellulase structure viz. the active catalytic domain responsible for the hydrolytic cleavage of the glycosidic bonds and the binding domain that binds the bacterial enzymes to its substrate. The cellulose binding domain (CBD) is linked to the catalytic core by linkers rich in hydroxyl amino acids found in many cases and most of the CBD has four conserved tryptophan and two additional cysteine residues. Because of the conserved aromatic residues, it was thought that CBD attached to cellulose either by hydrogen bonding or hydrophobic interaction. It has been shown that bacteria lacking these domains were less adherent and in some cases, less able to digest crystalloid cellulose [101]. Distinct binding domains have been identified in *Fibrobacter succinogenes*, including the CBD of endoglucanase 2 (EG2) and EGF [102]. Karita et al [103] cloned a gene *egVI* from *R.albus* F-40 and found that the enzyme contained a distinct CBD.

8. CO-CULTURE

Bacterial co-cultures are applicable to improve hydrolysis of cellulose and enhance product utilization to obtain increase desirable fermentation products. *Clostridium thermocellum* has advantage for co-culture with organisms capable of fermenting pentose sugars to ethanol because *C. thermocellum* can only ferment hexose sugars. So, *C. thermocellum* has been co-cultivated with other anaerobic thermophilic clostridia such as *Clostridium thermosacccharolyicum* (now known as *Thermoanaerobacterium saccharolyticum*) [104], *Clostridium thermohydrosulfuricum* [105], *Thermoanaerobacter ethanolicus* [106] and *Thermoanaerobium brockii* [107]. These organisms have ability to share with syntrophic relationship with *C.thermocellum* which can be exploited for hydrolysis of cellulose and hemicelluloses and the ultimately converters to ethanol (Fig. 2). The advantage of co-culture is the increased production but the challenge is that of by-products production such as acetate and lactate which decrease ethanol production by showing the growth rate of cells [108].

Developing bacterial co-cultures is a difficult task. Media and growth requirements, such as temperature, atmosphere and carbon source must be synchronized to permit equal growth of each strain) are necessary for establishment of co-culture. Stable co-cultures may also be controlled more specifically by metabolic interactions (i.e. syntrophic relationships or alternatively competition for substrates) and other interactions (i.e. growth promoting or growth inhibiting such as antibiotics) [109].

The alternative of bacterial co-culture would be the genetically engineered microorganism that has ability to complete the entire task from start to finish itself. This would mean that metabolically engineered *C. thermocellum* strain ferment pentose and hexose sugars, but, it is a difficult task as far as molecular engineering goes in clostridia due the recalcitrance of clostridia to genetic manipulation. Therefore, co-cultivation has advantage as it can reduce the number of exogenous elements produced by a single bacterial population and therefore reduces the chance of metabolic imbalance for host cells. Additionally, division of labour will simplify the optimization of each reaction path way [110]. Although bacterial co-culture is not an uncommon concept for bioconversion of lignocellulosic biomass, but still it is premature stage and offers great potential.

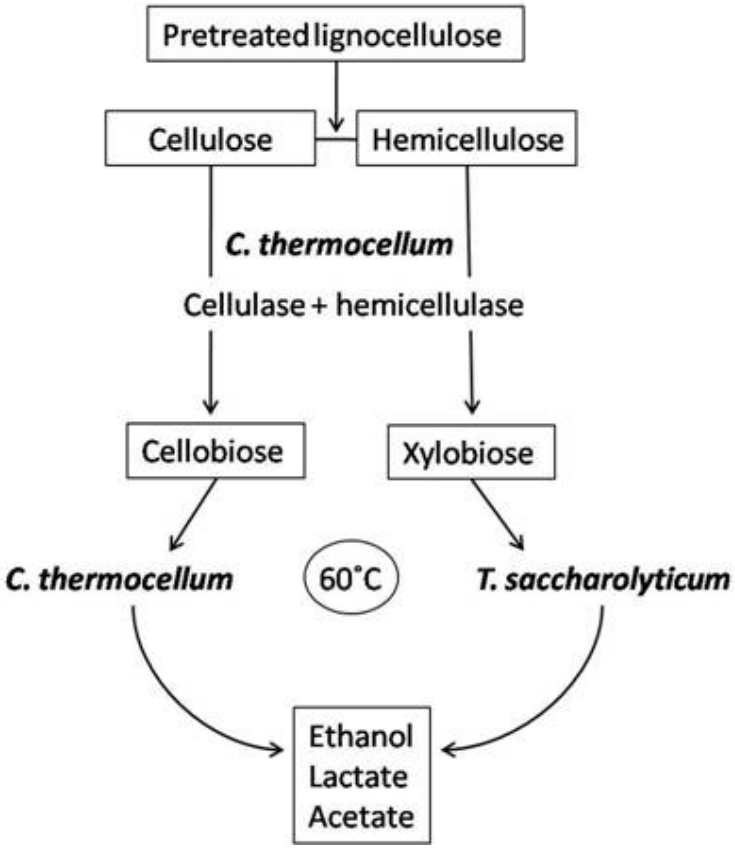

Fig. 2. Simplified bio-conversion process of *C. thermocellum* and *T. saccharolyticum* used in co-culture for ethanol production

C. thermocellum produces the cellulases and hemicellulases for hydrolysis of lignocelluloses to cellobiose and xylobiose. C. thermocellum can also additionally utilize hexose sugars derived from celluloses to produce ethanol. While, the hemicelluloses derived pentose can be utilized by T. saccharolyticum. T. saccharolyticum also contributes to cellobiose reduction and ethanol production (modified from Demain et al. 2005, [111]).

9. CLONING AND EXPRESSION OF CELLULASE GENES IN HETEROLOGOUS HOSTS

Cellulase genes cloning and expressed in bacterial hosts have been reviewed by Pasternak and Glick [112]. Forsberg et al. [113] have reviewed the characteristics and cloning of bacterial cellulases, particularly from the rumen anaerobe *F. succinogenes.* The most important of these are i) The strategies of cloning cellulase genes from eukaryotic fungal hosts cannot rely on direct expression in a prokaryotic cell because of the differences in the translation mechanism in the two groups, (ii) since the eukaryotic genomes are much larger than those of prokaryotes, a genomic clone bank from a eukaryotic cell needs to be constructed with piece of DNA which are 20-40 kb long. A vector like pBR 322 which does not replicate well with an insert greater than 10-15 Kb fails to give satisfactory results.

The recombinant cellulolytic strategy for organism's development for cellulose conversion via with non cellulolytic microorganisms, involves heterologous expression of a functional cellulase system. Such heterologous expression has been undertaken for a variety of purpose with a variety of microorganisms.

9.1 Heterologous Cellulase Expression in Bacteria

9.1.1 *Zymomonas mobilis*

Several cellulase encoding genes have been cloned and expressed in *Z. mobilis* with various degrees of success. The endoglucanase gene (*egl*X) from *Pseudomonas fluorescens* sub sp *cellulosa* was introduced into *Z.mobilis* by mobilizable plasmid vector [114]. This recombinant strain, however, produced the heterologous endoglucanase intracellularly throughout the growth phase independent of the glucose concentration in the medium [114]. Similarly, introduction of the *Bacillus subtilis* endoglucanase into *Z.mobilis* also resulted in poor expression and again no activity was obtained in the culture supernatant of the transformants [115].

In contrast to above said *P. fluorescens* and *B. subtilis* genes, the endoglucanase gene (*celZ*) of *Erwinia chrysanthemi* was efficiently expressed in *Z. mobilis* [116]. The specific activity of the *Z. Mobilis* enzyme was comparable to that of the parent strain of *E. chrysanthemi.* Biosynthesis of Cel Z was reported to occur during the exponential growth phase of *Z.mobilis and about 3*5% of the enzyme was released into the medium in the absence of detectable cell lysis. Another cellulase gene has been successfully cloned from *Acetobactor and* expressed in *Z. mobilis xylinum* [117]. The CMCase gene from *A. xylinium* was efficiently expressed in *Z. mobilis* and about 75% of the enzyme activity was detected in the periplasmic space.

9.1.2 Enteric bacteria

Two *E. chrysanthemi* endoglucanases, encoded by celY and celZ and the cellulase gene of *A. xylinum* have been expressed in both *E. coli* as well as the related enteric bacterium *K. oxytoca* [118]. Initially the expression of *celY* in *E. coli* was poor was due to promoter construction [119]. However, by using a surrogate promoter from *Z. mobilis*, the expression of *celZ* in *E. coli* was increased six fold.

10. CELLULASE BIOTECHNOLOGY: THE FUTURE

The use of lignocellulosic materials for the production of ethanol or other chemical feedstock is one of the most difficult tasks encountered in the history of biotechnology. The study of microbial cellulose utilization is by quantification of enzymes in the cultures, purification, characterization and application of such enzyme is one of the important aspects of microbial biotechnology. Quantitative description of cellulose hydrolysis is addressed with respect to adsorption of cellulase enzymes, rates of enzymatic hydrolysis, bioenergetics of microbial cellulose utilization and contrasting features compared to soluble substrate kinetics. A biological perspective on processing cellulosic biomass is presented, including features of pretreated substrates and alternative process configurations. Organism development is considered for "Consolidated bioprocessing" (CBP) , in which the production of cellulolytic enzymes, hydrolysis of biomass and fermentation of resulting sugars to desired products occur in one step. Two organism developmental strategies for CBP are examined: 1)

improve product yield and tolerance in microorganisms able to utilize cellulose or (ii) express a heterologous system for cellulose hydrolysis and utilization in microorganisms that exhibit high product yield and tolerance.

11. CONCLUSION

The conversion of cellulosic biomass by microorganisms is a potential sustainable approach to develop novel bioprocesses and products. Microbial cellulases are now commercially producing by several industries globally and are being widely used in food, animal feed, fuel, paper industry, textile industry and also various chemical industries. Cellulase research has been concentrated mostly in fungi but there is increasing interest in cellulase production by bacteria due to their higher growth rate and thermo stable and alkali stable properties. The development of rapid and reliable methods for the screening of cellulases from microorganisms within inhospitable environments will allow a greater number of novel bacterial cellulases to be isolated with purpose for industrial use. Our current knowledge of the production, purification, characterization, biochemistry, molecular biology of these enzymes and of the producer bacteria is considerable. However, these novel enzymes can be further engineered using available knowledge of enzyme structure and function through rational design. Or, they can be improved using random mutagenesis techniques with focus on selection of ideally augmented traits through directed evolution. Moreover, improvement of bacterial cellulase activities or imparting desired characters of enzyme by protein engineering is may be another area of cellulase research. Despite the progress achieved so far for bacterial cellulases, more effort is also needed for cellulases and bacteria to have important industrial impact.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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