IJBPAS, April, Special Issue, 2021, 10(4): 62-74

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MICROBIAL PRODUCTION OF CELLULASE

MODI P[1], UPADHAYAY D[2], MARCHAWALA F[2], BHATTACHARYA I[2] AND ANDHARE P[2*]

1: Student, MSc Microbiology, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia, Gujarat, 391760

2: Assistant Professor, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia, Gujarat, 391760

***Corresponding Author: E Mail: Dr. Prasad Andhare: prasad.andhare82145@paruluniversity.ac.in; Tel: +918200614350**

Received 19th Jan. 2021; Revised 20th Feb. 2021; Accepted 19th March 2021; Available online 1st April 2021

https://doi.org/10.31032/IJBPAS/2021/10.4.1007

ABSTRACT

Cellulases are enzymes that hydrolyze cellulosic biomass and are formed by microorganisms that grow on these materials. Cellulase is a valuable enzyme that can be made from low-cost agrowastes and cellulose as substrates by submerged and solid-state fermentation. Cellulase enzyme formed by cellulolytic bacteria and fungi can degrade cellulose protein. This enzyme has a wide range of industrial uses and is part of a large community of industrial enzymes. *Pseudomonas fluorescens, Bacillus subtilis, E. coli,* and *Serratia marcescens* were isolated from soil and classified as cellulase-producing bacteria. It was decided to optimise the fermentation medium for maximum cellulase output. pH, temperature, carbon sources, and nitrogen sources were all optimised in the culture. The optimum conditions for cellulase production were found to be 40 degrees Celsius at pH 10, with glucose as the carbon source and ammonium sulphate as the nitrogen source, and coconut cake stimulates cellulase production. Pseudomonas fluorescens produces the most cellulase of the four bacteria, followed by *Bacillus subtilis, E. coli*, and *Serratia marscens*. The aim of this review paper is to discuss different Microbial sources of cellulase, classification of cellulase and & quot; cellulase processing & quot; by submerged and solid-state fermentation with various bacteria and fungi. The application of this same scenario for industrial purposes is identified as an

emerging area of research. Biofuel production, textile polishing and finishing, paper and pulp industry, and lifestyle agriculture are among the key areas where cellulase enzyme shows a broader potential. The objective of this chapter is to discuss the structure, function, possible applications, as well as novel biotechnological trends of cellulase enzymes. Furthermore, possible low-cost, enzymatic pretreatment methods of lignocellulosic material in order to use it as an efficient raw material for biofuel production will be discussed.

Keywords: **Cellulases**, ***Pseudomonas fluorescens, Bacillus subtilis, E. coli,* and *Serratia marcescens***

INTRODUCTION

Cellulose is the most abundant biomass on the planet. It is the most abundant renewable bioresource generated in the biosphere and the primary result of photosynthesis in terrestrial environments **[1]**. Cellulase is a common enzyme that breaks down cellulose. Several microorganisms, most notably bacteria and fungi, generate this enzyme **[2]**. Many terrestrial plants & cell walls are made up primarily of cellulose. Plants are the source of cellulose, which is found as microfibrils with diameters of 2–20 nm and lengths of 100–40,000 nm. These provide the cell walls with a structurally sound structure. Despite widespread and extensive use of natural cellulosic sources, there are still vast amounts of cellulosic sources and cellulose-containing raw materials and waste products that are underutilised or could be used more effectively. However, developing processes that are commercially profitable is a challenge in this regard. Cellobiohydrolase, endoglucanase or carboxymethylcellulase (CMCase), and beta-glucosidases, in combination, are required for complete hydrolysis of the enzyme **[3]**. Every year, about 200 gigatons of CO_2 are fixed to the earth, and the equivalent amount of organic material must be depleted by plants and animals to 70% by microorganisms **[4]**. Cellulose makes up 50% of the dry weight of plant biomass on average. The only foreseeable renewable source of fuels and resources available to mankind is plant biomass. Agricultural residues are a great source of sustainable, mostly untapped, and low- cost lignocellulosic biomass. Leaves, stems, and stalks from corn fibre, corn stover, sugarcane bagasse, rice straw, rice hulls, woody crops, and forest residues are examples of renewable resources. Citrus peel waste, coconut biomass, sawdust, paper pulp, industrial waste, urban cellulosic solid waste, and paper mill sludge are only a few examples of lignocellulosic waste from industrial and agricultural processes. In addition, perennial grasses such as Switch grass and

other forage feed stocks such as Miscanthus, Elephant grass, Bermuda grass, and others may be used as dedicated energy crops for biofuels **[5]**. Plant biomass is locked up in 5- and 6-carbon sugars to thetune of 70%. These sugars are contained in lignocellulosic biomass, which is primarily made up of cellulose (a homologous polymer of glucose connected by 1.4 glycosidic bonds) and is hydrolyzed by the cellulase enzyme system (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). Hemicellulose contains in hardwoods mainly xylans, but in softwood, glucomannans mainly are present. Xylan degradation requires endo-1-4,-β-xylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, as well as acetylxylanesterases. In glucomannan degradation β-mannosidase and β-mannanase are required to break the polymer backbone **[6]**. Enzymes are one of the most essential items derived from microbial sources for human needs **[7]**. Enzymes are used in a wide range of manufacturing processes in the fields of industrial, environmental, and food Biotechnology **[8]**. Enzymes are finding new uses thanks to recent advances in biotechnology **[9]**. Energy procurement is one of the big issues that humanity faces in the new techno-economic period. All waste cellulose is a food source as well as a potential energy source **[10]**. As a chemical feed stock, cellulose contained in renewable lignocellulosic material is thought to be the most abundant organic substrate on the planet **[11]**. Cellulose is a branched glucose polymer made up of 1,4 glucose units connected by a – 1, 4- D-glycosidic bond **[12, 13]**. Acid hydrolysis and enzymatic hydrolysis can also be used to break down cellulose into sugar. However, according to Mandels *et al*., enzymatic hydrolysis is favoured because it produces less by-products and continues under milder conditions. If cellulosic biomass is used, cellulase, a group of enzymes that catalyse the hydrolysis of cellulose and related oligosaccharide derivatives, may be a useful tool for industrial saccharification and the efficient use of cellulosic materials is thought to require a cost-effective manufacturing process **[14]**. In simultaneous saccharification and fermentation systems (SSFS), the cellulase complex typically includes C1 ((EC 3.2.1.9), (exo-1, 4-β-D-glucanase, cotton lyase), C x (EC 3.2.1.4), (endo- 1, 4 - β-D- glucanase, carboxymethyl cellulase, or CMC ase), C b (EC 3.2.1.21), (β- glucosidase)), and pectinase. Cellulases are the most

commercially important of all the enzyme families, having been used and researched for most of the twentieth century. When SSF enzymes were generated instead of traditional SmF enzymes, the enzyme activities increased by about 30% to 80%. Multifaceted approaches to lowering cellulase production costs include the use of low-cost lignocellulosic substrates and cost-effective fermentation methods such as solid-state fermentation. Most fungi, such as Aspergillus and Trichoderma Sp., produce a lot of enzymes **[15]**. One of the world's most polluting sectors is the pulp industry. One of the reasons for the contamination is that woody and nonwoody lignocellulosic compounds are used as raw materials by the industry. Huge amounts of inorganic compounds are used during pulp preparation, which react with lignin such as lignocellulose compounds and form toxic organic compounds in pulp and paper mill effluents **[16]**. To extract lignin, different chemicals are used during the cooking process. Delignification of wood pulp is a necessary step to improve the quality of paper because lignin causes paper to turn yellow. Due to this operation, effluent emerging from pulp and paper mill has large amount of lignin and its derivatives. Black liquor is distinguished by its dark brown colour, high temperature, heavy odour, high alkaline pH, remarkably high demand for chemical oxygen (COD; 200,000 mg/l) and biochemical demand for oxygen (BOD; 40,000– 50,000 mg/l) **[17]**.

Microbial Sources of Cellulases

Cellulase is a very useful enzyme in the industry. Fungi, bacteria, and actinomycetes may all produce cellulase, but fungi are the most popular producers **[18]**.

Bacteria

Bacterial systems have also been studied for biomass saccharification and may have a benefit due to bacteria & rapid growth rate. It has also been documented that cellulolytic bacteria enzyme preparations can effectively saccharify a variety of cellulosic substrates. For an organism that can attack crystalline cellulose, *Cytophaga hutchinsonii* showed an unusual set of genes. On various substrates, the location, development, and biosynthetic control of cellulases in *hutchinsonii* were demonstrated.

Many researchers have looked into how endo-glucanase and exo-glucanase can be controlled by induction and catabolite repression in *Acetivibrrrio cellulolytieus*. It varies from other cellulolytic species in that it can develop solely on cellulose, cellobiose, or salicin.

The high basic activities for endo- and exo-glucanase in this organism have piqued interest **[19]**. The release of reducing sugars from CMC was measured to establish the endocellulase activity of the culture broth

during the growth of *Acinetobacter anitratus* and *Branhamella* species. *A. cellulolyticus* enzyme preparations have the ability to saccharify cellulose, the conditions for sustaining a high level of specific activity have also been identified. The cellulase system of *Pseudomonas sp*. has been studied extensively. Yamane *et al*., investigated the biogenesis of multiple cellulose components in P. fluorescens var. Cellulose, with a focus on the impact of culture conditions on the organism & ability to generate a wide range of cellulose titres. Tewari *et al*. described the synthesis, purification, and properties of *Pseudomonas Sp*. cellulase (extracellular).

The location of cellulase components in *Pseudomanas Sp*., which was isolated from activated sludge, was investigated.

The main components of cellulase complexes are endo-glucanases, three of which come from *P. fluorescens* var. and more than four by *Pseudomomas Sp*. In *E. coli*, the D-galactose dehydrogenase gene has been cloned **[20]**. *Pseudomonas fluorescens, Bacillus subtilis, E. coli*and *Serratia marcescens*, Cellulase-producing bacteria were isolated from soil, and the fermentation medium was optimised for optimum cellulase production.

Pseudomonas fluorescens was found to be the best cellulase maker. Many people have been interested in Bacillus Species cellulolytic enzymes because of their possible use in converting agricultural wastes into useful items. *Bacillus subtilis* CBTK 106 has been shown to produce a significant amount of cellulase activity **[21]**.

Discovered that an aqueous extract of the woods significantly inhibited the growth and development of cellulase in *B. subtilis* strains. The use of *B. subtilis* in the saccharification of wheat straw, rice straw, and bagasse was investigated the development of cellulase by *B. pumilus* EB3 using carboxy methyl cellulose as a substrate investigated the development of cellulose from cow dung using Bacillus subtilis CEL PTK 1.

The CMC zone and dinitro salicyclic acid to investigate cellulose productivity in Bacillus subtilis KO strains. Bacillus licheniformis MVS1 and Bacillus Sp. MNS3, both isolated from an Indian hot spring, were used to investigate the effect of certain nutritional and environmental factors on cellulase development **[22]**. The Plackett – Burman design to screen and select significant variables for enhancing alkaline cellulase production from Bacillus subtilis AS3, and the most important variables for increasing alkaline cellulase development were screened and chosen. *Clostridium thermocellum*, a thermophilic anaerobic bacterium, is gaining popularity as an LC biomass converter.

Since thermophilic organisms generate enzymes with improved thermostability and substantially higher specific activity, they have many advantages over mesophilic organisms **[23]**. Furthermore, moderate amounts of glucose or cellobiose do not inhibit Clostridia & cellulases. Exo- and endo-glucanase multi forms are generated by the organism and work together during saccharification. The organism shapes aggregates with a high molecular weight. Isolated a thermophilic bacterium *Aneurini bacillus thermoaerophilus* WBS2 which produces extracellular thermophilic cellulases from a hot spring in India and also optimised various fermentation parameters in order to increase cellulase output **[24]**.

Isolated and described the thermophilic bacteria strain Geobacillus pallidus from empty fruit bunches (EFB) and palm oil mill effluent compost. Cellulolytic bacteria in the rumen have been extensively studied. *Ruminococcus albus* cellulase can hydrolyze CMC and acid swollen cellulose, causing a rapid decrease in degree of polymerization while only affecting a minor degree of hydrolysis.

Bacterium Succinogens, an anaerobic rumen cellulolytic bacterium with high CMC-ase and cellobiase activity, **[25]**.

Fungi

These organisms grow in suitable conditions to produce cellulase; for example, when grown in solid submerged culture, *Trichoderma viride* and *Trichoderma reesei* produce active cellulase.

When grown in liquid media by surface and submerged culture methods, as well as solid state fermentation, *Aspergillus niger* produces highly active cellulase **[26].** *Trichoderma reesei* was used to conduct a thorough investigation into the development of cellulase.*T. reesei* has also been reported to manufacture cellulase using substrates such as cellulose, xylose, and lactose. Investigated the synthesis of cellulose in solid state fermentation using *Trichoderma reesei*.

When grown under different conditions, some Penicillium species, such as *Penicillium iriensis* and *P. citriviride*, produce substantial amounts of cellulase. *Penicillium funiculosum* produces enough cellulase to fully dissolve cotton. Cellulase is produced by fungi such as Stachy, Botsysatra, Pesalotia, Merulius, Lnerymans, Polyspores, Palmarum, and Neuraspora **[27]**. For the development of cellulase, *Chaetomium* sp.NIOCC 36 was found to be superior. Using submerged fermentation, evaluated cellulases, xylanases, and beta-glucosidases formed by two fungi, the thermotolerant *Acrophialo phoranainiana* and *Ceratocystis parodoxa* documented cellulase production in solid state fermentation using brown rot fungus

[28]. Investigated the development of cellulase by certain fungi and discovered that microorganisms can degrade native cellulose. Since the first observation of the action of converting cellulosic biomass to fermentable sugar, a cost-effective cellulase production method has been needed. A large number of microorganisms such as bacteria, actinomycetes, algae, and fungi, which make up a large part of soil, inhibit it. Despite the fact that microorganisms make up a relatively small portion of the soil, they are responsible for many of the chemical and even physical changes that occur in it. Fungi, in particular, play a unique role in these changes, which result in proper soil fertility maintenance**.**

Classification of Cellulase

Extracellular cellulases, either free or cell related, were developed by microorganisms to hydrolyze and metabolise insoluble cellulose. During the last three decades, the biochemical study of cellulose systems from aerobic and anaerobic bacteria and fungi has been thoroughly examined **[29]**. Based on their mode of catalytic operation, the following components of cellulase systems were classified.

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

Endoglucanases sever internal amorphous sites in the cellulose polysaccharide chain at random, producing oligosaccharides of different lengths and, as a result, new chain ends. Its mostly effective against acid-swollen amorphous cellulose, soluble cellulose derivatives like CMC, and cellooligosaccharides **[30]**.

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

Exoglucanases function on the reducing and non-reducing ends of cellulose polysaccharide chains in a possessive manner, liberating glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as main products. These enzymes are active against crystalline substrates like Avicel, amorphous celluloses, and cellooligosaccharides, as well as amorphous celluloses. They are, however, ineffective against cellobiose and substituted soluble celluloses like CMC **[31]**. Exoglucanases or 1, 4-β-D-Oligoglucan Cellobiohydrolases (Also Known as Cellodextrinases) (EC 3.2.1.74) It removes cellobiose from cellooligosaccharides or p-nitrophenyl - -D-cellobioside, but it is inactive against amorphous cellulose or CMC.

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

From the non-reducing end, glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose. It has no effect on cellulose that is crystalline or amorphous.

Cellobiose Phosphorylase or Cellobiose: Orthophosphate Alfa–D-Glucosyl Transferase

(EC 2.4.1.20)

It catalyses the phosphorolytic cleavage of cellobiose in a reversible manner **[32]**. The first to discover it in *Ruminococcus flavefacience* cells.

Cellobiose + H 3 PO 4 = alfa – D –glucose 1-P +glucose

Cellodextrin Phosphorylase Or 1,4-B-D-Oligoglucan Orthophosphate Alfa – D-Glucosyl

Transferase (EC 2.4.1.49)

Clostridium thermocellulam cells were found to contain. It catalyses the reversible phosphorylytic cleavage of cellodextrins ranging from cellotriose to cellohexose, rather than cellobiose **[33]**.

(1,4 – β-D-glucosyl) n + H 3 PO 4 = (1,4 – β-D-glucosyl)n-1 + alfa –D-Glucose-1-P

Cellobiose Epimerase (EC 5.1.3.11)

It was discovered in *Ruminococcus albus* cells for the first time. According, it catalyses the following reaction: Cellobiose = 4-O- β-D-glucosylmannose

Cellulase Production Using the Submerged

Fermentation (Smf) And Solid-State Fermentation

(SSF) Or Cultivation (SSC)

Fermentation is the biological process of microorganisms transforming complex substrates into basic compounds. It has been commonly used in the manufacture of cellulase, which has a wide variety of applications in industry. Fermentation techniques have grown in popularity over time as a result of their economic and environmental benefits. As a result of this rapid growth, two broad fermentation techniques have emerged: Submerged Fermentation (SmF) and Solid-State Fermentation (SSF), Solid-State Fermentation (SSF) / Solid-State Cultivation (SSC) **[34]**. SSF uses solid substrates such as bran, bagasse, paddy straw, other agricultural waste, and paper pulp. The key benefit of using these substrates is that nutrient-rich waste materials can be quickly recycled and used as less costly substrates.SSF is ideally suited for low-moisture fermentation techniques involving fungi and microorganisms. It cannot, however, be used in fermentation processes involving species that need a lot of water, including bacteria **[35]**.

Submerged Fermentation (SmF)/Liquid Fermentation (LF)

SmF uses liquid substrates that flow freely, such as molasses and broth **[36]**. This fermentation method is best for microorganisms that require a lot of moisture, such as bacteria. Another benefit of this method is that product purification is simplified.

CONCLUSION

Microorganisms converting cellulosic biomass is a potentially safe way to create new bioprocesses and products. Several factories around the world are now commercially processing microbial cellulases, which are widely used in fruit, animal feed, fuel, paper, textile, and chemical industries. While most cellulase research has centred on fungi, there is growing interest in bacteria producing cellulase due to their faster growth rate and thermo and alkali stability. The creation of rapid and reliable methods for screening cellulases from microorganisms in inhospitable environments will enable for the isolation of a greater number of novel bacterial cellulases for industrial use. Our current understanding of the enzymes' synthesis, purification, characterization, biochemistry, and molecular biology, as well as the bacteria that generate them, is extensive. These novel enzymes, on the other hand, can be rationally designed using existing knowledge of enzyme structure and function. Alternatively, they can be enhanced by guided evolution using random mutagenesis techniques with an emphasis on selection of preferably augmented traits. Furthermore, improving the activities of bacterial cellulases or imparting desired enzyme characteristics through protein engineering may be another field of cellulase study. Despite the progress made so far in the field of bacterial cellulases, further work is needed to ensure that cellulases and bacteria have a major industrial effect. The current progress in applications of cellulases is truly remarkable and attracting worldwide attention. It has already conquered the global market in an unbeatable way. Microbes are an attractive topic of interest for the production of cellulases due to their immense potential for cellulase production. However, it is apparent that more efficient species are still out there in the environment unnoticed by researchers. Further exploration and understanding of hidden mechanisms behind the activity of these enzymes are much more important. Microbial cellulases are preferred for their potential applications in a broad range of industries. Their ventures are expanding day by day. More and more researchare required to produce scientific knowledge to meet the growing demands for microbial cellulase. The advances in the emerging fields such as biotechnology, microbiology, and molecular biology will open up novel strategies to magnify the still-unlocked potentials of these enzymes. Eventually, it will be able to fine-tune the areas which still are dragging on the way to their utmost success.

Acknowledgement

It is our privilege and honour to express our sincerest gratitude to the Parul University,

Vadodara, Gujarat for providing me all the necessary support and facilities including state of the art infrastructural facilities with advanced technological scientific laboratories and everything else that was required to carry out this work.

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