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CELLULASE PRODUCTION: AN INSIGHT OF RESEARCH

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ABSTRACT

Cellulases have attracted the researcher's interest due to its varied applications in various fields including paper pulp industry, textile industry, Food Industry, animal feed industry, detergent industry, ethanol production. Major concern about cellulase production is to obtain highly active cellulases at a lower cost, current review gives an insight of major steps being taken for in order to get highly active cellulases.

Keywords: Cellulose, Cellulases, Fermentation, Strain Improvement

Celluloses

Lignocellulosic materials are cheap renewable resources available in large quantities (**Zhang and Cai, 2008**). Regardless of the source, lignocellulosic materials consist of three main polymers; **cellulose**, a homopolymer of glucose; **hemicellulose**, a heteropolymer of pentoses and **hexoses**; and **lignin**, an amorphous polymer of phenyl propanoid units (**Kuhad et al., 1997**). Every year plants produce about 180 billion tons of cellulose, making this polysaccharide a huge organic carbon reservoir on earth. Cellulosic biomass is an abundant global renewable resource and

includes a wide variety of materials, including various agricultural residues, fruit and vegetable wastes, woods, municipal solid wastes, wastes from the pulp and paper industry, as well as herbaceous energy crops. The degradation of cellulosic material is gaining increasing research attention due to its worldwide availability and the immense potential for its transformation into sugars, alternative fuels, and chemical feedstocks. Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes.

Cellulases

Cellulase is a family of O-glycoside enzymes that hydrolyse β -1,4-glycosidic bonds of native cellulose and other related cello-oligosaccharide derivatives. Cellulases are among the most extensively studied enzymes. They are distributed throughout the biosphere such as plants, animals and microorganisms. Cellulases are chiefly produced by microorganisms and they are distributed throughout the world. Cellulase comprise of at least three groups of enzymes: firstly endoglucanases (EC 3.2.1.4) which act randomly on soluble and insoluble cellulose chains; secondly exoglucanases (cellobiohydrolases EC 3.2.1.91) that act to liberate cellobiose from the reducing and non-reducing ends of cellulose chains and finally, β -glucosidases (EC 3.2.1.21) which liberate glucose from cellobiose (Li, *et al.*, 2010; Thongekkaew *et al.*, 2008).

Cellulases have attracted attention of the scientific world due to its varied industrial applications. It has got applications in industries including:

Pulp and Paper Industry

Cellulases have potential in the pulp and paper industry. The mechanical pulping processes (refining and grinding) of woody raw material leads to pulps with high content of fines, bulk and stiffness. Bio-mechanical pulping with cellulases results in substantial

energy savings (20-40%) during refining, and improvements in hand-sheet strength properties (Bhat, 2000).

Textile Industry

They have been successfully used for the biostoning of jeans and bio-polishing of cotton and other cellulosic fabrics. During the biostoning process, cellulases break off the small fiber ends on the yarn surface, thereby loosening the dye, which is easily removed by mechanical abrasion in the wash cycle (Sukumaran *et al.*, 2005, Uhlig, 1998).

Food Industry

In food industry cellulases are used for productions of fruit nectars and purees and isolation and separation of starch and gluten from wheat flour. Cellulases have been used for extraction, clarification and stabilization of fruit juices and vegetables. Also cellulases are applied for decreasing viscosity of pulp mush. (Bhat, 2000; De Carvalho *et al.*, 2008).

Animal Feed Industry

Cellulases have been used for treatment of animal feeds like silage and grain resulting in improvement of their nutritional values (Bhat, 2000; Dhiman *et al.*, 2002).

Detergent Industry

Cellulases acting under alkaline conditions have been used in detergents for selectively contacting the cellulose within the interior of

fibres and removing soil in the inter-fibril spaces. The cellulases are also applied to remove these rough protuberances for a smoother, glossier, and brighter-colored fabric (Bhat, 2000).

Ethanol production

Cellulases help in conversion of cellulosic materials like agricultural wastes to glucose and other fermentable sugars, which can be used as substrates for the production of products like ethanol.

Based on the varied applications cellulases have in the fields as mentioned above the present review gives an insight of cellulase production (Background and Current Status of Cellulase Production).

Cellulases research can be divided into three phases as has been shown in **Figure 1**. First phase started with research on production of cellulases from fungal sources. Later researchers focussed on cellulases production from bacterial sources, production of recombinants producing cellulases, and search for cheaper cellulosic substrates in order to reduce the cost of production. Current phase the third phase of cellulase research includes enhancement of cellulase production using mutations with radiations (ultra violet, IR, X rays) and chemicals (ethidium bromide, ethyl methane sulfonate etc.) (**Special focus has been on fungal source**), some researchers are focusing on preparation of metagenomic libraries.

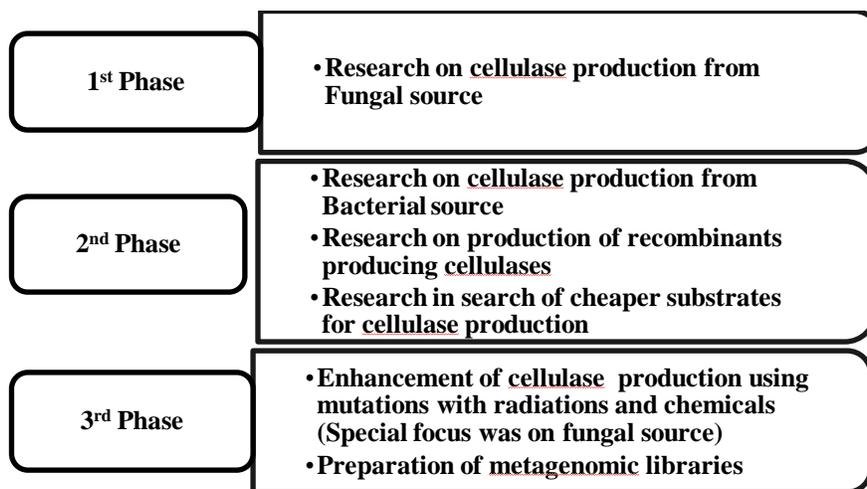


Figure 1.1: Background and Current Status

Microbial Sources of Cellulases

Bacterial

Deka *et al.*, 2011, worked on enhancement of cellulase activity of *Bacillus subtilis* AS3 by

optimizing the medium composition by statistical methods. The enzyme activity with unoptimised medium with carboxymethylcellulose (CMC) was

0.07U/mL and after optimization it was 0.43U/mL showing 6-fold increase as compared to unoptimised medium. **Maki, et al., 2011**, isolated efficient cellulase producing bacteria found in organic fertilizers and paper mill sludges which named tentatively as S1, S2, S3, S4, E2, and E4. Phylogenetic analysis of isolates revealed genera belonging to two major Phyla of Gram positive bacteria: Firmicutes and Actinobacteria. All isolates were tested for the visible degradation of filter paper; only isolates E2 and E4 (*Paenibacillus* species) were observed to completely break down filter paper within 72 and 96 h incubation, respectively, under limited oxygen condition. It was shown that 1% (w/v) CMC could induce total cellulase activities of 1652.2 ± 61.5 and 1456.5 ± 30.7 μM of glucose equivalents for E2 and E4, respectively. CMC could induce cellulase activities 8 and 5.6X greater than FP, therefore CMC represented a good inducing substrate for cellulase production. A cellulase-producing bacterial strain designated Z5 was isolated from the fecal matter of Zebra (*Equus zebra*). The strain was identified as *Microbacterium* sp. on the basis of 16S rDNA sequence analysis. Lactose as the sole carbon source was found to induce cellulase production and a positive synergistic effect of lactose and CMC was

also observed with enhancement of 3–4 times in cellulase activity. (**Sadhu et al., 2011**). Mango peel, a solid mango processing waste, comprising 15-20% of total fruit weight was used to isolate cellulase producing isolate identified as *Paenibacillus polymyxa*. Maximum CMCase production (7.814 U mg⁻¹) was observed in a medium containing 7% mango peel (w/v) with 1.5% ammonium sulphate (w/v) at 37°C and pH 5.5. Purification to an extent of 28.24 fold was achieved by affinity column chromatography. Bands corresponding to 26.5 and 34.0 kDa molecular sizes were observed on 12% denaturing Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) while of 72 kDa on 10% non-denaturing Native-PAGE, proving its heteromeric multienzyme nature. The enzyme was stable over a range of 20-60 degrees C and pH of 4.0-7.5. Michaelis-Menten equation constant (K_m and V_{max}) values of purified CMCase were 8.73 mg ml⁻¹ and 17.805 mM ml⁻¹ min⁻¹, respectively (**Kumar, et al., 2012**). 300 bacterial strains were isolated from various extreme environments for the presence of cellulase activity on CMC agar plates. Phylogenetic analysis of the positive strain, based on 16S rRNA gene sequences indicated that the isolates were clustered within Firmicutes and

Actinobacteria. A majority (17) of the isolates were identified as *Bacillus*, *Paenibacillus*, and *Lysinibacillus* sp., and the remaining three were identified as *Arthobacter*, *Rhodococcus*, and *Bhargavaea cecembensis*. Among the 20 positive isolates, 6 were evaluated for the production of cellulases on five different cellulosic substrates. Two isolates, *B. cecembensis* and *Bacillus* sp., based on maximum enzyme production on all cellulosic substrates, especially CMC and rice straw, were evaluated in terms of enzyme properties and kinetics (Pandey, et al., 2013). Five new bacterial isolates of *Bacillus licheniformis* were discovered from indigenous sources and characterized on the basis of phylogeny using 16S rDNA gene analysis. All the five strains showed significant capability of producing some of the major industrially important extracellular hydrolytic enzymes including α -amylase, glucoamylase, protease, pectinase and cellulase in varying titers. Morphological and physiological characteristics showed that these isolates can easily be cultivated at different temperatures ranging from 30°C to 55°C with a wide pH values from 3.0 to 11.0. All these 05 isolates were salt tolerant and could grow even in the presences of high salt concentration ranging from 7.0 to 12.0% (Ghani, et al., 2013). Mohite, et al., 2013, investigated the optimal fermentation

conditions for enhanced BC (bacterial cellulase) production by *Gluconacetobacter hansenii* NCIM 2529 under shaking conditions. The investigation on media components and culture parameters revealed that 2 % (w/v) sucrose as carbon source, 0.5 % (w/v) potassium nitrate as nitrogen source, 0.4 % (w/v) disodium phosphate as phosphate source, 0.04 % (w/v) magnesium sulfate, and 0.8 % (w/v) calcium chloride as trace elements, pH 5.0, temperature 25 °C, and agitation speed 170 rpm with 6 days of fermentation period are optimal for maximum BC production. Production of BC using optimized media components and culture parameters was 1.66 times higher (5.0 g/l) than initial non optimized media (3.0 g/l). Amore, et al., 2013, isolated ninety bacteria from raw composting materials for their cellulolytic activity on solid medium containing carboxymethylcellulose. The bacteria producing the highest cellulolytic activity levels were identified by 16S rRNA sequencing as *Bacillus licheniformis* strain 1, *Bacillus subtilis* subsp. *subtilis* strain B7B, *Bacillus subtilis* subsp. *spizizenii* strain 6, and *Bacillus amyloliquefaciens* strain B31C. Cellulase activity production by the most productive strain *B. amyloliquefaciens* B31C was optimized in liquid culture varying the carbon source. A research by Plecha, et al.,

2013, established a search for novel microorganisms capable of using and degrading switchgrass to produce sugars and ethanol.

Fungal

Various fungal strains have been used for cellulases production namely *Trichoderma reesei* ZU-02 was used for cellulase production using corn cob residue from xylose manufacture as substrate by **Liming and Xueliang, 2004**. The produced cellulase could effectively hydrolyze the corn cob residue, and the yield of enzymatic hydrolysis reached 90.4% on 10% corn cob residue (w/v) when the cellulase dosage was 20 IU/g substrate. **Latifian, M. et al., 2007**, used response surface methodology (RSM) to evaluate the effects of fermentation parameters for cellulase production by *Trichoderma reesei* QM9414 and *T. reesei* MCG77 in solid-state fermentation using rice bran as substrate. Cellulase production by *T. reesei* was also carried out by **Ahamed and Vermette, 2008**, using high concentration of cellulose to substitute glucose with the aim to improve cellulase production while trying to reduce production costs. A mixture of lactose and lactobionic acid was added into the bioreactor as cellulase inducers. The use of a cellulose–yeast extract culture medium yielded the highest enzyme and cell

production with a volumetric enzyme activity of $69.8 \text{ UL}^{-1} \text{ h}^{-1}$. **Baldrian & Valaskova, 2008**, reported that basidiomycetous fungi are most potent degraders of cellulases because many species grow on dead wood or litter, in environment rich in cellulose. Fungal cellulolytic systems differ from the complex cellulolytic systems of bacteria. For the degradation of cellulose, basidiomycetes utilize a set of hydrolytic enzymes typically composed of endoglucanase, cellobiohydrolase and β -glucosidase. In some species, the absence of cellobiohydrolase is substituted by the production of processive endoglucanases combining the properties of both of these enzymes. A white rot fungus, *Trametes hirsuta* was isolated by **Jeya, 2009**, which was found to contain efficient cellulose degrading enzymes. The strain showed maximum endoglucanase (EG), cellobiohydrolase (CBH) and β -glucosidase (BGL) activities of 55, 0.28 and 5.0 U/mg-protein, respectively. Rice straw was found to be a potentially good substrate for growth of *T. hirsuta* for cellulase production. **Singhania, et al., 2010**, worked for developments in bioprocess technologies, solid-state and submerged fermentation as well as on the strategies adopted for improving cellulase production or properties, including engineering the genes or designing

enzyme cocktails. A newly isolated brown rot fungus, *Fomitopsis* sp. RCK2010 was used for enhanced cellulase production by **Deswal, et al., 2011**, an initial pH of 5.5 and moisture ratio of 1:3.5 (solid: liquid) were found to be optimal for maximum enzyme production. Saccharification of pretreated rice straw and wheat straw by crude enzyme extract from *Fomitopsis* sp. RCK2010 resulted in release of 157.160 and 214.044 mg/g of reducing sugar, respectively. *Acremonium cellulolyticus* a filamentous fungus with high cellulase production was isolated by **Kanna et al., 2011**, 2.4 Kb b-xylosidase gene was identified in the *A. cellulolyticus* genome sequence information and it encoded 798 amino acids without introns. **Hu et al., 2011**, used Polyurethane Foam (PUF) as inert supports in solid state fermentation (SSF) for cellulase production. When compared with the control without PUF, the maximal enzyme yields in batch fermentation were improved by 70.6% and 60.4% for Filter Paper Activity (FPA) and CMCase, respectively. A thermophilic fungi *Thermoascus aurantiacus* RBB1 was isolated from composting soil and optimised for production of cellulolytic enzymes (endoglucanase, filter paper cellulase by SSF). Initial experiments showed that culture medium containing wheat bran as carbon source, prepared in a synthetic basal

medium, supported maximum enzyme production at 50°C (**Dave, et al., 2013**), Under optimized conditions 420.8 and 22.73 units/g substrate of endo-b-1,4-glucanase and filter paper cellulase were produced, respectively.

Strain Improvement

Fungal

Kovacs et al., 2008, developed mutants of *Trichoderma atroviride* by using *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG) treatment and UV-light followed by a semiquantitative plate clearing assay on Walseth-cellulose/agar plates. In an another research **Adsul, 2007**, subjected *Penicillium janthinellum* NCIM 1171 to mutation involving treatment of Ethyl Methyl Sulfonate (EMS) for 24 h followed by UV-irradiation for 3 min. Successive mutants showed enhanced cellulase production (EMS-UV-8), clearance zone on Avicel containing plate (SM2) and rapid growth on Walseth cellulose agar plates containing 0.2% 2-deoxy-D-glucose (SM3). This is the first report on the isolation and selection of mutants based on hydrolysis of Avicel, which is the most crystalline substrate. A new mutant strain of fungus *Trichoderma viride* T 100-14 was developed (**Zhou, et al., 2008**), on 1% microcrystalline cellulose (Avicel) for 120 h. To identify the predominant catalytic components, cellulases were separated by an

adapted two-dimensional electrophoresis technique. The apparent major spots were identified by high performance liquid chromatography electrospray ionization mass (HPLC–ESI-MS). Seven of the components were previously known, i.e., the endoglucanases Cel7B (EG I), Cel12A (EG III), Cel61A (EG IV), the cellobiohydrolases Cel7A (CBH I), Cel6A (CBH II), Cel6B (CBH IIb) and the b-glucosidase. Three isolates had abilities of hydrolyzing both carboxymethyl-cellulose (CMC) and Avicel indicate their endoglucanase activities. Another mutant strain of *Trichoderma citrinoviride* was developed by **Chandra, et al., 2009**, which was capable of increased cellulases production and insensitive to catabolite repression for industrial use by multiple exposures to EMS and ethidium bromide. The mutant produced 0.63, 3.12, 8.22 and 1.94 IU ml⁻¹ FPase, endoglucanase, b-glucosidase and cellobiase, respectively. These levels were, respectively, 2.14, 2.10, 4.09 and 1.73 fold higher than those in parent strain. Genetic distinction of the mutant was revealed by the presence of two unique amplicans in comparative DNA fingerprinting performed using 20 random primers. **Li, et al., 2009**, used microwave and ultraviolet radiations to develop seven mutant strains (M-B1–M-B7) of *Trichoderma viride* a

cellulase-producing fungi. Five of the strains namely (M-B1, M-B2, M-B3, M-B5 and M-B7) had significantly stronger ability to produce enzymes than the normal wild type, and they were also very stable for a long period up to 9 generations to produce cellulase. Molecular studies showed that there were some base mutations in endoglucanase I (EG I) genes of mutants M-B1, M-B2, M-B3 and MB5, but no change in M-B7, suggesting that some amino mutations in EG I proteins caused by base mutations could lead to enhanced cellulase production. **Fang, et al., 2009**, subjected *Acremonium cellulolyticus* strain C-1 to mutagenesis using UV-irradiation and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and strain CF-2612 was obtained. Strain CF-2612 exhibited higher filter paperase (FPase) activities (17.8 U/ml) than the parent strain C-1 (12.3 U/ml). *Trichoderma reesei* Rut C-30 was subjected to mutation after treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NG) for 6 h followed by UV irradiation for 15 min. Successive mutants showed enhanced cellulase production, clear hydrolysis zone and rapid growth on Avicel-containing plate. Particularly, the mutant NU-6 showed approximately two-fold increases in activity of both FPA and CMCase in shake flask culture when grown on basal medium

containing peptone (1%) and wheat bran (1%) (Jun, *et al.*, 2009). Gamma radiations have been used to enhance cellulase production by *Chaetomium cellulolyticum* NRRL 18756 to various doses of gamma irradiation to enhance the production of the industrially important enzyme carboxymethyl cellulase (CMCase). Among all the mutants tested, M-7 obtained through 0.5 KGy irradiation showed highest extracellular CMCase production which is 1.6-fold higher than that of the wild type. Optimal conditions for the production of CMCase by the mutant fungal strain using solid-state fermentation were examined. The optimized medium consisted of, sugarcane bagasse supplemented with 1% (w/w) peptone, 2.5mM MgSO₄, and 0.05% (v/w) Tween 80. Optimal moisture content and initial pH was 40% (v/w) and 5.0-6.5, respectively. The medium was fermented at 40° C for 4 days. The resulting CMCase yield was 4.0-fold more than that of the wild type strain grown on the basal wheat bran medium (Fawzi and Hamdy, 2011). Mutants of *Trichoderma viride* have been developed by using ethyl methyl sulfonate (EMS) treatment and UV-irradiation followed by a semi-quantitative plate clearing assay on phosphoric-acid-swollen cellulose plates. Mutant EU2-77 proved to be the most promising extracellular cellulase producer

among 20 mutants in a screening program performed in shake flask fermentation after plate screening. Soluble protein content, filter paper cellulase (FPase) activity, β -glucosidase activity and endoglucanase (CMCase) activity of the fermentation broths of the mutant strain were increased to 1.67, 2.49, 2.16, and 2.61 folds, respectively, compared with the wild strain (Giang and Geng, 2011). Low intensity ultrasonic radiation have been studied for the effect of on the cellulase activity (Subhedar and Gogate, 2014), The effect on the kinetic and thermodynamic parameters as well as the molecular structure of cellulase enzyme has been evaluated with the help of the chemical reaction kinetics model, Arrhenius equation, Eyring transition state theory, Michaelis–Menten equation, fluorescence spectroscopy and circular dichroism (CD) spectroscopy. It has been established that ultrasound had a positive effect on the activity of cellulase enzyme, though the selection of operating conditions played a crucial role in deciding the intensification. The maximum cellulase activity was observed at 17.33 W/cm² intensity and ultrasonic treatment time of 30 min, under which the enzyme activity was increased by about 25% over the untreated enzyme (Subhedar and Gogate, 2014). *Trichoderma asperellum* RCK2011 has been

mutated through UV-irradiation for enhanced cellulase production and lower catabolite repression. The production of FPase, CMCase and β -glucosidase was optimized under solid state fermentation; up to 20 mM of glucose did not inhibit cellulase production. The mutant strain *T. asperellum* SR1-7 produced FPase (2.2 IU/gds), CMCase (13.2 IU/gds), and β -glucosidase (9.2 IU/gds) under optimized conditions, which is, 1.4, 1.3, 1.5-fold higher than the wild type (Raghuvanshi *et al.*, 2014).

Bacterial

Bakare, *et al.*, 2005, used ethylmethanesulphonate (EMS) to mutagenize

the wild-type *Pseudomonas fluorescens*, to produce mutants. The isolated mutants were screened for the isolation of catabolite repression resistant mutants in the presence of 1% (w/v) glucose as carbon source. A total of fifty mutants were isolated. All the mutants produced cellulase in the presence of CMC as an inducer with specific activity of 0.057, 0.088 and 0.074 units/mg protein for the wild-type, catabolite repression resistant mutant⁴ (CRRmt⁴) and catabolite repression resistant mutant²⁴ (CRRmt²⁴), respectively.

Above given literature has been summarized in Table 1.1 below

Table1.1: Literature Survey

Year	Research	Researchers	Source
1959	Phosphorolysis and synthesis of cellobiose by cell extracts from <i>Ruminococcus flavefaciens</i>	Ayers WA	Biochem Biophys Res Comm. 140: 219-229.
1967	Epimerization of disaccharides by enzyme preparations from <i>Ruminococcus albus</i> .	Tyler, T. R. and Leatherwood, J. M.	Arch Biochem Biophys. 119: 363-367.
1969	Purification and properties of β -1, 4-oligoglucan: orthophosphate glucosyl transferase from <i>Clostridium thermocellum</i> .	Sheth K. and Alexander, J. K.	J. Biol. Chem. 244: 457-464.
1981	Lignin biodegradation and transformation	Crawford, R. L.	John Wiley and Sons., New York ISBN: 0-471-05743-6.
1989	Mechanisms of cellulose degradation by enzymes from aerobic and anaerobic fungi, p. 17-35. In: MP Coughlan (ed.), Enzyme systems for lignocelluloses degradation	Wood, T. M.	Elsevier Appl, Sci.
1997	Microorganisms and enzymes involved in the degradation of plant fiber cell walls.	Kuhad, R.C., Singh, A., Eriksson, K.E.L.,	Adv. Biochem. Eng. Biotechnol. 57, 46-125.
1998	Industrial Enzymes and Their Applications,	Uhlig H	John Wiley & Sons, New York, NY, USA,
2000	Cellulases and related enzymes in biotechnology	Bhat, M. K.	Biotechnol. Advances, 18: 355-383.
2002	Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding,	Dhiman T. R., M. S. Zaman, R. R. Gimenez, J. L. Walters, and Treacher, R.	Animal Feed Sci. and Technol. 101 (1-4): 115-125.
2002	Crystal Structure and Hydrogen-Bonding System in Cellulose I β from Synchrotron X-ray and Neutron Fiber Diffraction.	Nishiyama, Y., Langan, P. and Chanzy, H.	J. Am. Chem. Soc. 124 (31): 9074-82. Doi;10.1021/ja0257319
2004	High-yield cellulase production by <i>Trichoderma reesei</i> ZU-02 on corn cob residue.	Liming X and Xueliang S.	Biores. Technol. 91: 259-262. PMID: 14607485
2004	Enhancing the activity of cellulase enzyme using ultrasonic irradiations.	Subheddar, P.B. and Gogate, P. R.	101, 108-114

2005	Regulatory mutations affecting the synthesis of cellulase in <i>Pseudomonas fluorescens</i>	Bakare M. K., Adewale I. O., Ajayi A. O., Okoh A.I., Shonukan, O. O.	Af. J. Biotechnol. 4 (8): 838-843.
2005	Microbial cellulases—production, applications and challenges	Sukumaran, R. K., Singhanian, R. R. and Pandey, A.	<i>J. Scientific and Industrial Res.</i> 64 (11) 832–844.
2007	Strain improvement of <i>Penicillium janthinellum</i> NCIM 1171 for increased cellulase production.	Adsul, M.G.,	<i>Biores. Technol.</i> 98: 1467-1473.
2007	Food and fuel for all: realistic or foolish?	Cassman, K.G and Liska, A.J.	<i>Biofuels. Bioprod. Biorefin.</i> 1: 18–23.
2008	Culture-based strategies to enhance cellulase enzyme production from <i>Trichoderma reesei</i> RUT-C30 in bioreactor culture conditions.	Ahamed, A. and Vermette, P.	<i>Biochemical Engg. J.</i> 40: 399-407.
2008	Degradation of cellulose by basidiomycetous fungi.,	Baldrian, P and Valaskova V.	<i>FEMS Microbiol Rev.</i> 32: 501–521.
2008	A study of retention of sugars in the process of clarification of pineapple juice (<i>Ananas comosus</i> , L. Merrill) by micro- and ultra-filtration	De Carvalho L. M. J., I. M. de Castro, and Da Silva, C. A. B.	<i>J. Food Engg.</i> 87 (4): 447–454.
2008	<i>Trichoderma atroviride</i> mutants with enhanced production of cellulase and -glucosidase on pretreated willow.	Kovacs, K., Megyeri, L., Szakacs, G., Kubicek, C. P., Galbe, M. and Zacchi, G.	<i>Enzyme and Microbial Technology</i> 43: 48–55.
2008	An acidic and thermostable carboxymethyl cellulase from the yeast <i>Cryptococcus</i> sp. S-2: purification, characterization and improvement of its recombinant enzyme production by high cell-density fermentation of <i>Pichia pastoris</i> .	Thongekkaew, J., Hiroko, I. H., Masaki, K. and Iefuji H.	<i>Protein Expression Purif.</i> 60 (2): 140-6. PMID: 18479937
2008	Enzymatic hydrolysis of alkali pretreated rice straw by <i>Trichoderma reesei</i> ZM4-F3	Zhang, Q. Z., Cai, W. M.	<i>Biomass Bioenergy</i> , 32: 1130-1135.
2008	Identification and purification of the main components of cellulases from a mutant strain of <i>Trichoderma viride</i> T 100-14.	Zhou, J., Wang, Y. H., Chu, J., Zhuang, Y. P., Zhang, S. L., Yin, P.	<i>Biores. Technol.</i> 99, 6826–6833
2009	Development of a mutant of <i>Trichoderma citrinoviride</i> for enhanced production of cellulases Mutated by EMS and EtBr	Chandra <i>et al</i>	<i>Bioresource Technology</i> 100 (2009) 1659–1662, doi:10.1016/j.biortech.2008.09.011
2009	Strain improvement of <i>Acremonium cellulolyticus</i> for cellulase production by mutation	Fang, X., Yano, S., Inoue, H. and Sawayama, S.	<i>J. Biosci. and Bioengg.</i> 107 (3): 256–261,
2009	Enhanced saccharification of alkali-treated rice straw by cellulase from <i>Trametes hirsuta</i> and statistical optimization of hydrolysis conditions by RSM.	Jeya, M., Zhang, Y. W., Kim, In-Won, Lee, J.K.	<i>Bioresource Technol.</i> 100: 5155–5161. PMID: 19540109
2009	Strain improvement of <i>Trichoderma reesei</i> Rut C-30 for increased cellulase production	Jun, H., Bing, Y., Keying, Z., Xuemei, D., Daiwen, C.	<i>Indian J. Microbiol.</i> 49: 188-195.
2010	Enhanced cellulase production of the <i>Trichoderma viride</i> mutated by microwave and ultraviolet	Li, <i>et al</i>	<i>Microbiological Research</i> 165 (2010) 190–198. doi:10.1016/j.micres.2009.04.001
2010	Enhanced Production of Cellulase(S) By <i>Aspergillus</i> spp. Isolated From Agriculture Wastes by Solid State Fermentation	Abo-State, M. A. M <i>et al</i>	<i>American-Eurasian J. Agric. & Environ. Sci.</i> 8 (4): 402-410.
2010	Cellulase production on glucose-based media by the UV-irradiated mutants of <i>Trichoderma reesei</i> .	Ike, M., Park, J. Y., Tabuse, M. and Tokuyasu K.	<i>Appl. Microbiol. and Biotechnol.</i> 87 (6), 2059-2066.
2010	Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases.	Singhanian, R. R., Sukumarana, R. K. Patelb, A. K. and Pandey, A	<i>Enzyme and Microbial Technol.</i> 46: 541–549.
2011	Enhancement of Cellulase Activity from a New Strain of <i>Bacillus subtilis</i> by Medium Optimization and Analysis with Various Cellulosic Substrates.	Deka, D., Bhargavi, P., Sharma, A., Goyal, D., Jawed, M. and Goyal, A.	<i>Enzyme Res.</i> Article ID 151656, 8 pages. doi:10.4061/2011/151656.
2011	Optimization of cellulase production by a brown rot fungus <i>Fomitopsis</i> sp. RCK2010 under solid state fermentation	Deswal D, Yogender Pal Khasa, Ramesh Chander Kuhad.	<i>Bioresource Technol.</i> 102: 6065–6072.
2011	Improvement of carboxymethyl cellulase production from <i>Chaetomium cellulolyticum</i> NRRL 18756 by mutation and Optimization of solid state fermentation	Fawzi, E. M. and Hamdy, H. S.	<i>Bangladesh J. Bot.</i> 40 (2): 139-147

2011	Enhanced cellulase production by solid state fermentation with polyurethane foam as inert supports	Hu, T., Zhou, Y., Dai, L., Wang, Y., Liu, D., Zhang, J. and Liu, H.	Procedia Engg. 18: 335-340. DOI: 10.1016/j.proeng.2011.11.053
2011	New isolate of <i>Trichoderma viride</i> strain for enhanced cellulolytic enzyme complex production.	Jiang, X. and Geng, A.	J. Biosci. and Bioengg. 111 (2): 121–127.
2011	Enhancement of b-xylosidase productivity in cellulase producing fungus <i>Acremonium cellulolyticus</i>	Kanna, M., Yano, S., Inoue, H., Fujii, T. and Sawayama, S	AMB Express. 1: 15.
2011	Characterization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizers	Maki, M. L., Broere, M, Leung K.T. and Qin, W.	Int. J. Biochem. Mol Biol., 2 (2): 146-154. PMID: 21969070
2011	Lactose-Enhanced Cellulase Production by <i>Microbacterium</i> sp. Isolated from Fecal Matter of Zebra (<i>Equus zebra</i>).	Sadhu, S., Saha, P., Mayilraj, S., Maiti, T. K.	Current Microbiol. 62 (3), 1050-1055
2011	Strain Improvement for Enhanced Production of Cellulase in <i>Trichoderma viride</i> .	Xu, F., Wang, J., Chen, S., Qin, W., Yu, Z., Zhao, H., Xing, X. and Li, H.	Appl. Biochem. and Microbiol. 47 (1): 53-58.
2012	Production and characterization of carboxymethyl cellulase from <i>Paenibacillus polymyxa</i> using mango peel as substrate.	Kumar D, Ashfaque M, Muthukumar M, Singh M, Garg N.	J Environ Biol. 33(1):81-4. PMID: 23033647
2013	Industrial waste based compost as a source of novel cellulolytic strains and enzymes.	Amore, A., Pepe, O., Ventrino, V., Birolo, L., Giangrande, C., Faraco, V.	FEMS Microbiol Lett. 339 (2): 93-101. doi: 10.1111/1574-6968.12057.
2013	Enhancement of cellulase activity by a new strain of <i>Thermoascus aurantiacus</i> : Optimisation by statistical design response surface methodology.	Dave, B. R., Sudhir, A. P., Parmar, P., Pathak, S., Raykundaliya, D. P., Subramanian, R. B.	Biocatalysis and Agricultural Biotechnol. 2: 108–115.
2013	Isolation and characterization of different strains of <i>Bacillus licheniformis</i> for the production of commercially significant enzymes	Ghani M, Ansari A, Aman A, Zohra RR, Siddiqui NN, Qader SA.	Pak. J. Pharm. Sci. 26 (4): 691-7. PMID: 23811444
2013	Enhanced production of bacterial cellulose by using <i>Gluconacetobacter hansenii</i> NCIM 2529 strain under shaking conditions.	Mohite, B. V, Salunke, B. K. and Patil, S. V	Appl. Biochem. Biotechnol. 169 (5): 1497-511. doi: 10.1007/s12010-013-0092-7
2013	Phylogenetic diversity and characterization of novel and efficient cellulase producing bacterial isolates from various extreme environments.	Pandey, S., Singh, S., Yadav, A. N., Nain, L. and Saxena, A. K.	Biosci. Biotechnol. Biochem. 77 (7), 1474-80. PMID: 23832366
2013	Screening for novel bacteria from the bioenergy feedstock switchgrass (<i>Panicum virgatum</i> L.).	Plecha, S., Hall, D. and Tiquia-Arashi, S. M.	Environ. Technol. 34 (13-16):1895-904.
2014	Bioprocessing of enhanced cellulase production from a mutant of <i>Trichoderma asperellum</i> RCK2011 and its application in hydrolysis of cellulose. 124: 183–189	Raghuwanshi, S., Deswal, D., Karp, M. and Kuhad, R. C.	Guildford., Elsevier Sci.B. V.,Amsterdam, 124:183-189.

CONCLUSION

The main concern about cellulase production is the enzyme activity and cost of production, researchers are focusing on the ways to reduce the cost of production, some are searching for cheaper cellulosic substrates, others are trying to use recombinant DNA technology in order to reduce time and cost of production, some other are trying to use the

genes present in the uncultured microflora through metagenomic libraries preparation. In order to enhance the enzyme activity lots of methods are being followed including media optimization, strain improvement using physical (UV, IR, Ultrasonic radiations) or chemical mutagens (EMS, Ethidium bromide, NTG).

However the literature shows that lots of strain improvement work has been applied to fungal strains especially *Trichoderma sp.* and *Aspergillus sp.* has been explored a lot.

It can be concluded that higher metabolic rate of bacteria can be explored and if strain improvement is applied to a good extent, it will automatically lead to cost cutting as well as highly active cellulases can be produced.

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