

Review Article: Cellulose degrading microorganism and their Application



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ABSTRACT

Cellulose is the most abundant polysaccharide on the earth, it is a large organic polymer compound made up of several hundred to many thousands of β (1 \rightarrow 4) linked D-glucose unit. It is important component in the plants cell wall, found in algae and the oomycetes, some species of bacteria secrete it to form a biofilms, Cotton fibers and wood contains approximately 90 % and 40-50% of cellulose respectively. It can be use for paper making and apply in small quantities for cellophane and rayon manufacturing. Utilize as cellulosic ethanol as fuel source. Some animals (ruminants and termites) can digest cellulose due to symbiotic microorganisms that live in their guts and they degrade cellulose by producing

cellulose enzyme. Cellulase enzyme is produced by many fungi, bacteria and protozoa; they decompose cellulose and convert into monosaccharide. It can be used for commercial food processing in coffee, performs hydrolysis of cellulose during drying of beans. It can widely apply in textile industry and in laundry detergents. In review paper involves microorganisms which are capable for producing Cellulase enzyme and their source of isolation with application.

Keywords: cellulose, Cellulose producing bacteria and fungi, Cellulase enzyme

Pritam *et al* (2012) suggested that Municipal Solid Waste (MSW) is a rich source of ligno-cellulosic materials so it can provide a powerful environment for the growth of cellulolytic bacteria. They identify cellulose degrading bacteria from MSW dumped in different localities of Peshawar and their screening for potential antimicrobial activity. The cellulolytic bacteria isolated from the collected samples by serial dilution method on modified Czapeck (CMC) agar medium and subsequent Congo red assay. They isolated total 108 isolates obtained were further analyzed for cellulose degrading activity qualitatively through Congo red assay. Only 15 isolates were selected on the basis of cellulose hydrolyzing activity (zone ratio 2.5 and above) and performed antimicrobial activity of those isolates against different bacterial and fungal human pathogens. Identified isolates on the basis of standard biochemical tests in Bergey's manual. Among the 15 isolates one belonged to *Pseudomonas* spp., one to *Aeromonas* spp., one to *Pasteurella* spp., two belonged to *Staphylococcus* spp. and ten to *Bacillus* genus. Among them, *Bacillus* spp. SD F has shown a remarkable ability of cellulose hydrolysis in terms of both Congo red assay by giving a zone ratio of 3.4 and enzyme activity of 0.2514 IU/mL. High activity against *A. niger* by giving a zone of inhibition of 17mm while isolate 36 F (*Aeromonas salmonicida*) showed high antifungal activity against *C. albicans* by giving a clear zone of 16 mm. The results may provide the basis for the utilization of cellulose as an energy source for such bacteria having the ability to produce therapeutic agents by utilizing a less expensive carbon source.

Y.W.Han and v. R. Srinivasan (1968) was conducted to isolate cellulose degrading bacteria from mangrove soil of Mahanadi river delta, Odisha, India and also to evaluate their cellulase production ability. Total fifteen cellulose degrading bacteria were isolated based on their halo zone formation on Congo red agar medium. Their maximum carboxymethylcellulose hydrolysis capacities (HC value) were ranged from 1.18 to 2.5 cm.

A CMCase test of these fifteen isolates showed that their enzyme activity ranged from 2.471 to 98.253 U/ml/min. From their morphological, and biochemical characterization, the isolates were identified as *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp. and *Brucella* spp.

Gurdeep Rastogi *et al* (2009) reported that cellulose-degrading bacteria (CDB) were isolated from four different invertebrates (termite, snail, caterpillar, and bookworm) by enriching the basal culture medium with filter paper as substrate for cellulose degradation. Isolates CDB 8 and CDB 10 exhibited the maximum zone of clearance around the colony with diameter of 45 and 50 mm with the hydrolytic value of 9 and 9.8, respectively. The enzyme assays for two enzymes, filter paper cellulase (FPC), and cellulase (endoglucanase), were examined by methods recommended by the International Union of Pure and Applied Chemistry (IUPAC). The extracellular cellulase activities ranged from 0.012 to 0.196 IU/mL for FPC and 0.162 to 0.400 IU/mL for endoglucanase assay. Cultures were also further tested for their capacity to degrade filter paper by gravimetric method. The maximum filter paper degradation percentage was estimated to be 65.7 for CDB 8. Selected bacterial isolates CDB 2, 7, 8, and 10 were co-cultured with *Saccharomyces cerevisiae* for simultaneous saccharification and fermentation. Ethanol production was positively tested after five days of incubation with acidified potassium dichromate.

Sumit kumar dubey *et al* (2014) reported that cellulose-decomposing aerobic and mesophilic bacterium from soils of sugar cane fields. The terminal dilution method was applied to isolate a single clone of cellulolytic organism from closely related contaminants. The cultural and physiological characteristics of the isolate were studied and identified as a member of the genus *Cellulomonas*. The isolate excreted cellulase into the menstruum, and it hydrolyzed various cellulosic materials producing cellobiose as the final breakdown product in the menstruum. The organism could be decomposed up to 90% of the initial substrate within 5 days. Amino acid analysis of the cell crop revealed a high content of lysine, and the essential amino acid pattern compared favorably with that of Food and Agricultural Organization reference protein.

Gomashe AV *et al*,(2013) investigated that the cultivable mesophilic (37°C) and thermophilic (60°C) cellulose degrading bacterial diversity in a weathered soil-like sample collected from the deep subsurface (1.5 km depth) of the Homestake gold mine in Lead, South Dakota, USA. Chemical characterization of the sample by X-ray fluorescence spectroscopy revealed a high amount of toxic heavy metals such as Cu, Cr, Pb, Ni, and Zn.

All phylotypes retrieved from enrichment cultures were affiliated to Firmicutes. Cellulose degrading mesophilic and thermophilic pure cultures belonging to the genera *Brevibacillus*, *Paenibacillus*, *Bacillus*, and *Geobacillus* were isolated from enrichment cultures and selected cultures were studied for enzyme activities. For a mesophilic isolate (DUSELG12), the optimum pH and temperature for carboxymethyl cellulase (CMCase) were 5.5 and 55°C, while for a thermophilic isolate (DUSELR7) they were 5.0 and 75°C, respectively. Furthermore, DUSELG12 retained about 40% CMCase activity after incubation at 60°C for 8 h. Most remarkably, thermophilic isolate, DUSELR7 retained 26% CMCase activity at 60°C up to a period of 300 h. Isolates have strong implications for biological conversion of cellulosic agricultural and forestry wastes to commodity chemicals including sugars.

Ayyappa Kumar (2018) studied that cellulose degrading bacteria from biogas slurry and assessment of their cellulolytic activity. Two efficient strains of cellulolytic microorganisms namely Sc and Sw were isolated from biogas slurry showed the remarkable ability to degrade Carboxy-methyl Cellulose in the media. Optimum temperature was recorded 35°C, pH 7.0 and Salt concentration 1% for both strain of bacteria (namely Sc and Sw). They also showed their abilities to degrade amylase. Both the strain showed 20% similarities to each other. These bacterial isolates can be used as inoculants for enhancing the degradation process of cellulose present in agricultural waste.

Puspita Lisdiyanti et al (2012) isolated cellulose degrading microbes from soil samples from different regions and to identify cellulose degrading microbes including bacteria and fungi. Two different types of cellulose-degrading bacteria and two types of cellulose degrading fungi were isolated from six different soil samples for cellulose degradation. A total of two isolates each of *Thermo actinomycetes* spp. and *Pseudomonas* spp. were isolated as well as two isolates of *Aspergillus* spp. and one isolate of *Penicillium* spp. were also isolated. Clear zone around the colony was the indication of the cellulose degradation activity of the microorganisms.

R. P. Maruthamalai Rasi (2012) studied Peat soil composes of organic compounds derived from plant debris with organic carbon content higher than 25%. The organic fraction of peat soil generally contains lignin, cellulose, hemicellulose, proteins, waxes, tannins, resins, and suberin. the cellulolytic bacteria isolated from peat soils from Ogan Komering Ilir, South Sumatera, Indonesia, tested for their cellulase activities, and the potential isolates were identified into genus or species level. The degradation ability of cellulose was screened using 1% CMC (carboxy methyl cellulose) as a substrate and the cellulase activities of crude

enzyme were determined using DNS (3,5-dinitro salicylic acid) method. Bacterial isolates (S3B40, S3B32, S3B37, and AB16) that have high cellulase activities. Among 4 isolates, *Paenibacillus elgii* S3B40 has the highest cellulase activity recorded. Optimum activity of isolate S3B40 at pH 8 and temperature 60 °C was 14.5 U/mL, S3B32 at pH 8 and temperature 80 °C was 0.506 U/mL, AB16 at pH 5 and temperature 50 °C was 0.361 U/mL, and S3B37 at pH 5 and temperature 30 °C was 1.167 U/mL, respectively

Muhammad Irfan¹ (2012) reported that Cellulose may be hydrolyzed using enzymes to produce glucose; it may be used for production of ethanol, organic acids and other chemicals. Cellulases are a group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e. cellulose to smaller sugar components including glucose subunits. Cellulolytic fungi were isolated from different areas of Himachal. Total 21 fungal isolates were isolated from these soil samples; isolates were showing the cellulase activity. The fungal isolate designated as PISS-3 isolated from Paper Industry soil sample from HRA Mill Village Tibhi, Indora, Kangra H.P was noticed to show maximum zone of hydrolysis of carboxy-methyl cellulose. The cellulase activity was assayed by Carboxymethyl cellulase “CMCase” (endoglucanase) assay.

Sami Flimban .,(2019) Electricity can be directly biogenerated by bacteria in a microbial fuel cell (MFC) using many different biodegradable wastes as substrate. When cellulose is used as a substrate, the cellulolytic and electrogenic activities require a microbial consortium for energy generation. cellulose-degrading bacteria were isolated from an MFC using CMC (carboxymethylcellulose) agar medium and their cellulolytic activity was assessed. Cellulolytic bacteria isolated from the MFC were characterized and identified based on their phenotypic characteristics, thirty-two isolates, only ten cellulolytic bacterial strains were successfully isolated from the MFC reactor under aerobic conditions. In the MFC, the genus *Staphylococcus* was found to be the most dominant group of cellulose-degrading bacteria which used as biofuel producer

Riad Mahmood et al (2014) reported that isolation and identification of bacteria with cellulase activity from cellulose containing samples. Samples were collected from soil, domestic kitchen waste, and sawdust. 42 bacterial isolates were isolated through serial dilutions and spread plate method in carboxymethyl cellulose (CMC) agar media and screened using Congo red staining on CMC agar plates for cellulolytic activity. Among the 42 isolates, only 24 (57%) isolates showed cellulolytic activity. Morphological and biochemical assays suggested that the cellulose-degrading bacterial isolates were members

of the genus *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus aureus*, *S. epidermidis*, and *Salmonella* sp. these bacterial species can be utilized for effective biodegradation of cellulose-containing substrates.

Yan-Ling Liang (2014) investigated that cellulose degrading bacteria present in kitchen waste for highest cellulase activity and growth of bacteria at optimum temperature and pH. Sample was collected from domestic kitchen waste and bacterial strains were isolated using nutrient agar media. Enrichment technique was used for isolating CDB (cellulose degrading bacteria) strains. The objective of research was to utilize effective bacteria for degrading complex polymer cellulose into simpler sugars like glucose under optimum working conditions. 21 isolated bacterial strains, only four were having effective cellulose degrading bacteria. These four isolates were screened for qualitative estimation through Congo red dilution assay and quantitatively tested by DNS method (Miller, 1959). The optimum pH and temperature for most potent isolate was recorded as 7 and 35°C respectively. These optimum working conditions were recommended for biomass utilization.

Sandrasekaran Naresh (2019) reported that the biodiversity of thermophilic cellulolytic bacterial strains that present in the north Malaysian mangrove ecosystem. Soil samples were collected at the four most northern state of Malaysia (Perak, Pulau Pinang, Kedah and Perlis). The samples obtained were first enriched in nutrient broth at 45°C and 55°C prior culturing in the carboxymethylcellulose (CMC) agar medium. Repeated streaking was performed on the CMC agar to obtain a pure culture of each isolate prior subjecting it to hydrolysis capacity testing. Total seven isolates (two from Perak, three from Kedah, another two were from Perlis and Penang each) showed halozone. The isolate (KFX-40) from Kedah exhibited highest halozone of 3.42 ± 0.58 , meanwhile, the one obtained from Perak (AFZ0) showed the lowest hydrolysis capacity (2.61 ± 0.10). Based on 16S rRNA sequencing results, 5 isolates (AFY-40, AFZ-0, KFX-40, RFY-20, and PFX-40) were determined to be *Anoxybacillus* sp. The other two isolates were identified as *Bacillus subtilis* (KFY-40) and *Paenibacillus dendritiformis* (KFX-0). Based on growth curve, doubling time of *Anoxybacillus* sp. UniMAP-KB06 was calculated to be 32.3 min. Optimal cellulose hydrolysis temperature and pH of this strain were determined to be 55°C and 6.0 respectively. Addition of Mg^{2+} and Ca^{2+} were found to enhance the cellulase activity while Fe^{3+} acted as an enzyme inhibitor.

L.Sujatha and K.P.J. Hemalatha (2020) Fifty one isolates of cellulolytic bacteria were isolated from cow dung and two soil samples were obtained from Kambalakonda Wildlife

Sanctuary and RCD Biodiversity Park in Visakhapatnam, Andhra Pradesh by Enrichment method in basal salt medium with cellulose as substrate for degradation. The cellulolytic activity of the isolated bacteria was determined by the diameter of the zone of hydrolysis by Gram's iodine dye staining method. After primary screening, a total of fifty one isolates showed cellulolytic activity. Out of fifty one strains of cellulolytic bacteria, twenty three isolates from Kambalakonda Wildlife Sanctuary, seventeen isolates from RCD Biodiversity Park and Eleven isolates from cow dung sample obtained from cattle ranch, Visakhapatnam showed cellulase activity. Seven strains showed maximum hydrolytic value greater than 4.0 cm, nineteen strains showed average hydrolytic value between 3.0 and 3.9 cm and twenty one strains showed minimum hydrolytic value between 1.5 and 2.9 cm. The potential isolates were obtained from Kambalakonda Wildlife Sanctuary and RCD Biodiversity Park than cow dung sample. The 13 C strain exhibited maximum hydrolytic value of 5.6 cm which was designated as KKV1. The strain KKV1 was identified as *Streptomyces corchorusii* (MN244066) by morphological, cultural, biochemical and 16S rRNA sequence. The CMCase and FPase activity of the crude sample were examined by DNS method and found to be 0.21 U/ml and 0.041 U/ml respectively and the specific activity was 4.38 U/mg proteins and 0.86 U/mg proteins respectively. The *Streptomyces corchorusii* have a higher cellulase activity and the soils of bio reserves have a lot of scope for isolating high cellulolytic bacteria which can be exploited for different industrial purposes

Farjana Islam., 2019 studied that 371 bacteria were isolated from different ecological niches. Cellulolytic potential of the isolates was evaluated by qualitative as well as quantitative screening methods. 124 bacterial cultures showed production of zone of hydrolysis in the plate assay method. The hydrolytic potential of the isolates measured in terms of cellulolytic index indicated that 84 isolates showed cellulolytic index (CI) values between 1 and 4, 22 isolates between 4 to 6 and 18 isolates in the range of 6 to 13. The positive isolates with CI values ≥ 4.0 were screened quantitatively for the production of cellulases, determined as carboxy methyl cellulase (CMCase) activity and filter paper activity (FPase). Comparison of the activities shown by the isolates with that recorded in the standard isolate of *Cellulomonas fimi* indicated that large number of bacteria produced considerably high amounts of cellulases. The isolate NAB37 showed highest levels of CMCase (0.948 ± 0.011 U/ml) as well as FPase (0.125 ± 0.005 FPU/ml) activities. The cellulolytic potential of the

bacteria can be exploited in various cellulases based applications including detergents, textile, paper and pulp, food and bioethanol industries.

CONCLUSION

Cellulose is world's most abundant organic polymer substance and comprises a major storage form of glucose. Cellulose occurs exclusively in plants and is the most abundant organic substance in plant kingdom. Microbial cellulose utilization is responsible for one of the largest material flow in the biosphere. Increased interest in developing cellulose-based ethanol over the last few years was the main reason behind inflated research to find cellulose-degrading microorganisms. Biogas from agriculture waste is considered a valuable source of renewable energy. Cellulase is expensive and contributes only 50% to the overall cost of hydrolysis due to the low specific activity. This enzyme has enormous potential in industries and is used in food, beverages, textile, laundry, paper and pulp industries etc. Therefore, there has been much research aimed at obtaining new microorganisms producing cellulose enzymes with higher specific activities and greater efficiency.

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