A Review on Isolation, Identification and Cellulolytic Potential of Cellulolytic Bacteria

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Abstract

Generally, plant cellulose accounts for 30% to 50% of the dry weight of plants. Cellulose is the main component of plant cell walls. Plant species, age, tissue type, and environmental factors like temperature, light, and water availability can affect a plant's cellulose content. The major component of plant cells is cellulose, and it is quite difficult to degrade. A wide range of cellulose-degrading microorganisms have been identified, mainly fungi and bacteria that play an important role in cellulose-degradation. The cellulase enzyme produced by different microbes differs in structure and action method. Bacteria that degrade cellulose were isolated from samples acquired from diverse sites. Cellulose-degrading bacteria were isolated from diverse samples using various media and cellulose and carboxymethyl cellulose as the only carbon sources for bacterial growth. Congo red and gram iodine were used to examine isolated bacteria, followed by the detection of hydrolytic zones around cellulose-degrading bacteria. These zones were used for determining the hydrolytic potential of various bacterial colonies, and morphological and biochemical tests were carried out to determine the bacterial colony's identification. According to morphological and biochemical characterization, the isolates were identified as *Bacillus subtilis, Bacillus thuringeinsis, Brevibacillus brevis, Brevibacillus parabrevis, Pseudomonas sp, Staphylococcus aureus, Staphylococcus epidermis, Salmonella sp, Klebsiella sp, Aeromonas sp, Pasteurella sp, Brucella sp, Micrococcus sp, Xanthomonas sp, Achromobacter sp,.*

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1. Introduction:

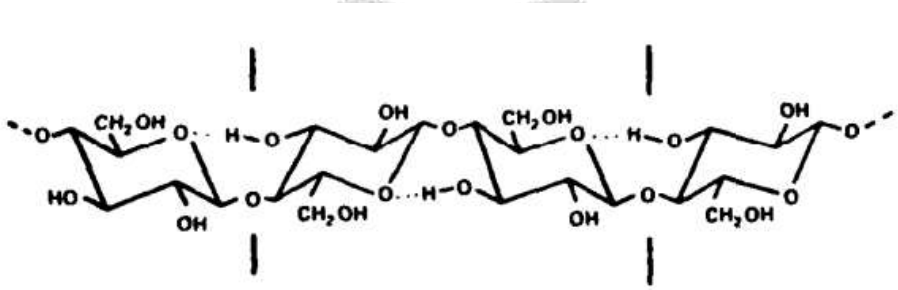
Cellulose is a complex carbohydrate and the most abundant organic compound on Earth, accounting for approximately 33% of all plant matter. Cellulose serves as a structural element of the cell walls of plants, algae, and some bacteria, giving them rigidity and strength. It also serves as a valuable source of dietary fiber for humans as well as animals, but only a selected group of microbes have the enzymes needed to break down and use it is used as an energy source. There are a number of industrial uses for cellulose, including the manufacturing of bioethanol, paper, and pharmaceuticals (Yamada *et.al.,* 2011). Cellulose is an important component of plant biomass and plays an important role in the environmental recycling of organic matter. The breakdown of cellulose in soil and water, which releases nutrients back into the ecosystem, is largely due to microorganisms like bacteria and fungi.

To improve soil organic matter decomposition and reduce fertilizer use, cellulose-degrading bacteria can be used as bioinoculants. This will increase soil fertility. They can be used to promote sustainable agriculture and lessen environmental pollution (Behera *et.al.,* 2013). By using either enzymatic hydrolysis or an acidic reaction, cellulolytic microorganisms can convert cellulose into multiple soluble sugars. Small amounts of cellulases are frequently produced by cellulose-degrading bacteria, and cellulose degradation appears to be carried out by a collection of multienzyme complexes. An enzyme called cellulase catalyzes the hydrolysis of cellulose, a complex carbohydrate that makes up the majority of plant cell walls. Numerous microorganisms, including bacteria, fungi, and protozoa, as well as some animals, including termites and ruminants, produce cellulase. Cellulase enzymes are extracted using specific procedures. Several cellulose-degrading bacteria isolates were grown on carboxymethyl cellulose (CMC) broth at 37°C for 72 hours to produce cellulase enzyme. The broth is centrifuged at 5000 rpm for 20 minutes after 3 days, and the obtained supernatant is used as the crude enzyme (Rawway *et.al*., 2018; Gupta *et.al.,* 2011; Shinde *et.al.,* 2020; Behera *et.al.,* 2013). The broth cultures were incubated in a shaker incubator at

37°C for up to 120 hours at 150 rpm, according to Sumita Biswas *et al*. The bacterial cultures were collected in 15 ml centrifuge tubes at intervals of every 24 hours and centrifuged for 10 minutes at 12000 rpm in a cooling centrifuge to collect the supernatant (Biswas *et. al.,* 2019).

1.1 Structure of cellulose and cellulases:

It is possible to extract cellulose from various plant parts. Wheat straw and sugarcane bagasse were used to extract cellulose by Anup Karn *et al*. Cellulose extraction is accomplished using a straightforward, practical method (Karn *et.al.,* 2022). A C4-OH group is present at one end of the D-glucose unit in cellulose, which is the non-reducing end, and a C1-OH group is present at the other end, which is the reducing end. The bond is made by removing the alcoholic OH group from carbon atom 4 of one -D-glucose molecule and the glycosidic OH group from carbon atom 1 of another -D-glucose molecule. The parallel alignment that results from the hydrogen bonding and van der Waals forces that hold together adjacent cellulose chains and sheets gives cellulose its overall structure. The D-glucopyranose units that make up cellulose are glucan polymers joined by b-1,4-glucosidic bonds (Gupta



et.al., 2013). Higher plant cell walls primarily consist of a class of polymers with ß-1, 4-linked monosaccharide backbones. Cellulose, which is the ß -1,4-homopolymer of anhydrous glucose, is the most prevalent one of them (Atalla, 2011).

Figure —Structures of cellulose (Atalla, 2011)

Cellulases are a class of enzymes that catalyze the breakdown of cellulose to produce cellobiose, glucose, and oligosaccharides. These enzymes are part of a group of cellulose-hydrolyzing enzymes that are made by bacteria and fungi. An important class of enzymes known as cellulose is crucial to both nature and industry. Cellulases are naturally occurring enzymes that convert insoluble cellulose into soluble forms, contributing to the global carbon cycle. Numerous industries, including pulp and paper, textiles, laundry, biofuel production, food and feed, brewing, and agriculture, have shown the potential use of microbial cellulases (Kuhad *et.al.,* 2022).

Three enzymes—exo- -1, 4-glucanases, endo-x -1, 4-glucanases, and -1, 4-glucosidases—make up the entire enzymatic system of cellulase. Exoglucanases, endoglucanases, -glucosidases, and cellobiohydrolases are the four main classes of cellulases according to how they work. Additionally, different organisms have different cellulases. For example, bacterial, fungal, and other cellulases have very different structures and roles. Contrary to bacterial cellulases, fungal cellulases have a short polylinker region connecting the carbohydrate-binding module (CBM) at the C-terminal to the catalytic domain at the N-terminal.

Cellulase production has been studied extensively, and bacteria that produce cellulose have been isolated from a variety of sources. For instance, Islam and Roy (2018) purified enzymes and isolated cellulase-producing bacteria from molasses (Farjana & Narayan, 2018).

2. Material and Methods:

2.1 Isolation:

Cellulose, a complex carbohydrate found within plant cell walls, can be broken down by cellulolytic bacteria into less complex sugars like glucose. These bacteria are essential to the cycling of carbon in ecosystems because they are in charge of decomposing plant matter and releasing carbon dioxide back into the atmosphere. Plant matter must be broken down by bacteria in order for it to decompose. Cellulolytic bacteria are frequently found in the digestive tracts of various animals, including cows and termites, as well as in soil and water. To make biofuels and other products, some cellulolytic bacteria can be utilized in industrial processes. To find the bacteria that can break down cellulose, samples from various sites were gathered for the current study. The collected samples contained a variety of bacterial strains, each with varying degrees of cellulolytic activity. Riad Mahmood *et al.* (2020) specifically selected sawdust, kitchen waste, and soil samples for cellulose degradation (Riad Mahmood *et al.*,2020). In the Assiut Governorate of Upper Egypt, samples were collected by Mohammed Rawway *et al.* (2017) from five distinct sources of natural cellulose degradation occurs, including compost, garden soil, agricultural soil, ruminant gut, and Nile River sediment. These samples were used to isolate bacteria that could break down the

cellulose (Rawway *et.al*., 2018). To isolate potential cellulolytic bacteria from the gut flora of two Lepidopteran pest larvae: the black cutworm (*Agrotis ypsilon*) and the *Colocasia esculenta* leaf roller (*Cnaphalocrocis sp*.) (Mohammed Rawway *et al.* 2017). Cellulose-degrading bacteria have been isolated from sawdust and coffee residue compost (Eida *et.al.,* 2012). In addition, cellulose-feeding organisms such as termites, caterpillars, bookworms, and snails were collected from woody habitats for the isolation of cellulose-degrading bacteria as also reported (Gupta *et.al.,* 2011). Cellulose-degrading microbial strains were isolated from compost samples taken at the different composting stages (Zhang and Dong, 2022). Moreover, Ashok A. Shinde *et al.* (2020) collected saliva samples from Jaffrabadi breed buffaloes to isolate cellulolytic bacteria (Shinde *et.al.,* 2020). The samples were collected from various locations to isolate cellulose-degrading bacteria. B. C. Behera *et al.* (2014), has collected soil samples from multiple locations within the mangrove forest, including Jumbo, Kharnasi, Triveni, Nuagda, Atharabanki, and the Indian Farmer Fertilizers Corporation (IFFCO) (Behera *et.al.,* 2013). Bhagat and Kokitkar, (2021), were collected soil samples from dump yards, undisturbed garden soil, and undisturbed forest soil (Bhagat and Kokitkar, 2021). Additionally, S. Keerthana *et al.* (2019) isolated cellulose-degrading bacteria from samples of termite gut and decaying leaves (Keerthana *et. al.,* 2019).

The isolation, screening, and identification of cellulolytic bacteria are important for studying their ecology and potential applications in various fields. Cellulose-degrading bacteria can break down cellulose into simpler molecules, such as glucose, which other microorganisms can use as a carbon and energy source. Cellulose-degrading bacteria are therefore important for the recycling of plant material and for the decomposition of plant litter in natural ecosystems. In previous studies, samples were collected from various locations for the isolation of cellulose-degrading bacteria. Serial dilutions of the collected samples were performed, and the spread plate technique was used for bacterial isolation in several studies, as reported in references (Mahmood *et al.*,2020; Rawway *et. al*. 2018; Eida *et.al*., 2012; Ahmad *et. al.,* 2013; Behera *et. al,* 2014; Bhagat and Kokitkar, 2021; Magotra 2020). In the research conducted by Ashok Shinde *et al*. (2020), saliva samples from buffalo were serially diluted in 0.85% NaCl before bacterial isolation. In some studies, cellulose-degrading bacteria were isolated from the gut of herbivorous pest larvae, termites, caterpillars, and bookworms by macerating the gut and performing serial dilutions, as described in references (Biswas *et. al.,* 2019; Gupta *et.al.*, 2011; Keerthana *et. al.,* 2019).

2.2 Growth medium:

Many scientists have grown cellulose-degrading bacteria on CMC agar medium. In this growth medium, cellulose is the only carbon source utilized. Composition of CMC agar medium: Peptone, 10.0 g, CMC (carboxymethyl cellulose), 10.0 g, K_2HPO_4, 2.0 g, Mg $SO_4.7H_2O$, 0.3 g, (NH4) $2SO_4$: 2.5 g were added to prepare the CMC -Agra medium. Hail, 0.5 mm, was mixed with 1 liter of water, and a medium pH of 6.8 to 7.2 was maintained (Mahmood *et al.*,2020; Rawway *et.al*., 2018; Biswas *et. al.,* 2019; Shinde *et.al.,* 2020; Ahmad *et. al.,* 2013; Behera *et.al.,* 2013; Bhagat and Kokitkar, 2021; Magotra, 2020; Motwali *et.al.,* 2016). Researchers have isolated cellulose-degrading bacteria from termites, caterpillars, bookworms, and snails. They were initially given a basal salt medium for growth. Composition of basal salt medium: To prepare the basal salt medium, 1 liter of water was mixed with 2.5 g of $NaNO_3$, 2 g of KH_2PO_4, 0.2 g of $MgSO_4$, 0.2 g of NaCl, and 0.1 g of $CaCl_2.6H_2O$ and the medium was then filtered. And then cellulose-degrading bacteria were isolated on cellulose agar (Gupta *et.al.,* 2011; Keerthana *et. al.,* 2019). Eida *et al.* (2012), used Dubos mineral salt medium for bacterial isolation.

2.3 Screening:

For the screening of cellulolytic bacteria, many methods were used by various researchers-

2.3.1 Using Congored

The incubated plates were flooded with 0.1% colored solution for 20 minutes. After those plates were rinsed with 1M NaCl solution. A clear zone indicates cellulose degradation (Mahmood *et al.*,2020; Rawway *et.al*., 2018; Shinde *et.al.*, 2020; Ahmad *et. al.,* 2013; Behera *et.al.,* 2013; Bhagat and Kokitkar, 2021; Keerthana *et. al.,* 2019; Zhang and Dong, 2022).

2.3.2 Using Gram Iodine Solution:

The incubated plates were flooded with Gram's Iodine or Iodine solution. A clear zone indicates cellulose degradation (Rawway *et.al*., 2018; Bhagat and Kokitkar, 2021).

2.3.3 Using Congored Agar:

Bacterial colonies are streaked on congored agar media for confirmation of cellulose-degrading bacteria. Discoloration of congored were taken as positive result (Gupta *et.al.*, 2011; Magotra, 2020).

2.4 Identification:

For the identification of cellulose-degrading bacteria, the morphological characteristics and biochemical tests of the bacterial isolates are done. Bacterial identification is done with the help of Bergey's manual of determinative bacteriology based on biochemical tests and morphological characteristics. Riad Mahmood *et al.* (2020). utilized selective and differential media, including MacConkey, EMB, BGA, King's B, Bouillon agar, and MSA, for the

identification of bacteria. Morphological characteristics such as size, color, margin, elevation, opacity, Gram stain, and motility were observed in the bacterial isolates (Biswas *et. al.,* 2019; Behera *et.al.,* 2013). For the identification of strains of interest cultural characteristics, morphological characteristics and biochemical tests were conducted and identified on the basis of characteristics as given in Bergey's manual of systematic bacteriology (Reddy *et.al,* 2017). Some biochemical tests are done for bacterial identification including the Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test, Oxidase test, Gelatin test, Motility test, Amylase test, Nitrate reduction test, Carbohydrate fermentation test by standard methods (Mahmood *et al.,*2020; Biswas *et. al.,* 2019; Shinde *et.al.,* 2020; Behera *et.al.,* 2013; Reddy *et.al,* 2017).

3. Cellulolytic activity:

The cellulolytic activity was measured as a diameter of the clear zone after the CMC plate was poured by 1% Congored. The cellulolytic index was calculated using the formula as follows (Mahmood *et al.,*2020; Biswas *et. al.,* 2019; Shinde *et.al.,* 2020; Reddy *et.al,* 2017).

Cellulolytic index = (Diameter of zone -Diameter of Bacterial colony)

A clear zone indicates cellulose degradation.

4. Enzyme production:

Pure culture of cellulose-degrading bacteria was cultured in Carboxymethyl Cellulose (CMC) broth. The culture was incubated at 37^{o}C for 72 hours with an agitation speed of 160 rpm. Cells were harvested by centrifugation at 5000 rpm for 20 minutes at 4^{o}C. The supernatant was used as a crude enzyme (Rawway *et.al*., 2018; Gupta *et.al.,* 2011; Shinde *et.al.,* 2020; Bhagat and Kokitkar, 2021).

5. Results and Discussion:

Riad Mahmood et al. used sawdust, food scraps, and soil to isolate cellulose-degrading bacteria. *Bacillus sp, Pseudomonas sp, Staphylococcus aureus, Staphylococcus epidermidis*, and *Salmonella sp* were among the bacterial strains they discovered. Cellulose-degrading bacteria have been found in compost, garden soil, agricultural soil, ruminant guts, and river Nile sediment, among other places. *Brevibacillus brevis, Brevibacillus parabrevis*, Bacillus subtilis, and other strains were among those identified (Rawway *et.al*., 2018). Several samples, including the guts of herbivorous pest larvae, termites, caterpillars, bookworms, snails, and the saliva of the 'Jaffrabadi' breed of buffalo, were grown on a carboxymethyl cellulose medium in an effort to isolate cellulose-degrading bacteria. *Achromobacter sp, Pseudomonas sp, Bacillus subtilis, Klebsiella sp*, and other bacterial strains were isolated as a result of bacterial identification using biochemical tests and morphological characteristics (Biswas *et. al.,* 2019; Gupta *et.al.,* 2011; Shinde *et.al.,* 2020; Keerthana *et. al.,* 2019). Several researchers have isolated bacteria that break down cellulose from various samples using the carboxymethyl cellulose medium. Some strains of isolated bacteria, including *Aeromonas sp, Pasteurella sp, Brucella sp, Micrococcus sp, Xanthomonas sp*, and others, were discovered to be capable of degrading cellulose, despite the fact that the majority of the isolated bacteria exhibited cellulolytic ability.

6. Conclusion:

Cellulose-degrading bacteria were isolated by collecting samples from various sources. The Carboxymethyl cellulose medium was found to be the most suitable medium for the growth of cellulolytic bacteria. Bacterial identification was carried out successfully by performing biochemical tests on the zones that were formed around bacteria due to the application of Gram's iodine solution or Congo red stain.

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