Isolation and Identification of Cellulose producing Bacteria from Rotten Fruits

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Abstract:

Microbial cellulose, an exo polysaccharide produced by bacteria, has unique structural and mechanical properties and is highly pure compared to plant cellulose. Present study represents isolation, identification, and screening of cellulose producing bacteria and further process optimization. Isolation one bacterial cellulose producing isolates were selected out of 5 isolates was carried out from natural sources like rotten fruits. Among the one isolates the CBA 5 isolate has been identified as a potential bacterial cellulose producer. The bacterial isolates obtained from rotten apple, were identified as bacteria sp. CBA 5, through morphological and biochemical analysis. Optimization studies were conducted for process parameters like inoculum density, temperature, pH, agitation, and carbon and nitrogen sources using bacteria sp. CBA 5. The strain produced 0.25 g/ml after incubation time of 168 h with Hestrin and Schramm (HS) media in static culture of cellulose at optimum growth conditions of temperature (30∘ C), pH (6.0), sucrose (0.2%), peptone (0.05%), and inoculum density (0.5%). Characterization of microbial cellulose was done by scanning electron microscopy (SEM). (1)(2)

Key words: *Bacterial cellulose, Gluconoacetobacter, K- Xylinus, Carbon source, Fermentation.*

1. Introduction

Microbial cellulose is an extracellular polysaccharide produced by some bacterial genera such as Acetobacter, Agrobacterium, Gluconacetobacter, Rhizobium, Achromobacter, Aerobacter, Azotobacter, Rhizobium, Salmonella,Sarcina etc. It represents alternative to plant derived cellulose for some specialty applications in the medical field, food and other industries. Members of Gluconacetobacter genus like Gluconacetobacter xylinus and Gluconacetobacter hansenii are the most potential producers compared to other strains. The microbial cellulose (MC) produced by Acetobacter strain can be used as diet food and to produce new materials for high performance speaker diaphragms, medical pads, makeup pads, paint thickeners, and artificial skin because of the unique properties of this cellulose distinct from those of plant cellulose. The microbial cellulose has a large specific surface area, higher water retention value, mold ability, and high tensile strength compared with plant cellulose. Microbial cellulose can be produced by culturing a strain of bacteria, which is typically found on decaying fruits, vegetables, vinegar, fruit juices, and alcoholic beverages. The bacteria of the family convert ethanol to acetic acid. In the earlier studies, several attempts were made to isolate species. from rotten fruits. In the current study, we aimed to isolate cellulose producing bacteria from rotten fruits. The cellulose producing strains were identified by morphological and biochemical characterization. In fact, to achieve high yield cellulose production, the culture conditions have a crucial influence, in particular factors such as carbon source, nitrogen source, temperature, pH, and agitation. In this work, we have investigated the influence of the culture conditions on cellulose production by bacteria sp. CBA5 isolated from rotten Apple. The effects of carbon source, nitrogen source, pH, temperature, and inoculum density were investigated. The cellulose produced from bacteria sp. CBA5 was characterized by scanning electron microscopy (SEM).(3)(4)

2. Materials and Methods

Sample Collection: Collected the plant materials that are known to contain cellulose, such as leaves, stems or fruits. Ensure that the plants are healthy and free from visible diseases.

Isolation of cellulose producing bacteria: plant sample was taken separately and inoculated into 9mL saline and serially diluted up to 10-6 dilution, further streak plate method was done using standard Hestrin-Schramm Agar(D- glucose 20g/l, Yeast extract 5g/l, Peptones 5 g/l, disodium phosphate 2.7g/l, Citric acid 1.15g/l, Agar 15g/) and incubated for 48 hr at 37∘C . One loopful of each isolate was inoculated into 9 mL of Hestrin-Schramm media. These tubes were incubated statically at 37∘C for 7 -14 days. After incubation, the tubes with white pellicle covering the surface of liquid medium were selected. (5)

Screening of cellulose producer

There are 2 methods

1. The flasks were observed for pellicle formation at air liquid interface. Those flasks with pellicle growth were selected and purified the culture by repeated streaking on HS agar plates to obtain isolated colonies. Each distinct isolate was inoculated on screening media, that is, HS agar with fluorescent brightener dye (0.02% w/v) and antifungal agent cycloheximide incubated at 37∘ C for 2-3 days. The fluorescent dye binds to the cellulose content in the

organism. Cellulose producing bacterial colonies fluoresces when observed under UV light. So the fluorescent colonies were selected as cellulose producers. (6)

2. Samples had been acquired and streak on GEY (Glucose, Ethanol and Yeast Extract) agar plates, which have been then incubated at 37°C for 48 hours or till colonies had been formed. The formation of a clear area across the colonies become selected for additional fermentation. (7)

Identification of Cellulose Producer.

Bacterial isolates were identified by performing gram staining, colony morphology, motility test, and biochemical characteristics followed by carbohydrate fermentation test.

Gram Staining: The Gram staining method is a fundamental technique used in microbiology to differentiate bacteria into two main groups: Gram-positive and Gram-negative. This classification is based on the structural differences in the bacterial cell walls, particularly their ability to retain a specific dye. (8)

Morphological characterization: In certain instances, colony form might be helpful in identifying microorganisms. Size, shape, texture, elevation, colour, consistency, opacity is impact on media are the factors used to represent colonies. (9)

Mortality test

Hanging drop method: Under a microscope, live cells or microbes may be observed the usage of the placing drop approach in a lab putting. To create a microenvironment that permits the statement of cellular activities, a droplet of way of life medium containing the cells is suspended from the underside of a coverslip. (10)

Semi soiled Agar: The consistency of the semi-strong agar should permit the migration of organisms at the same time as preserving structural integrity. Regularly take a look at the agar plates for indications of test organism mortality or boom inhibition. While mortality can be seen as the death of boom or apparent indicators of cell loss of life, boom inhibition can be indicated with the aid of decreased colony length or density. (11)

Biochemical Characterization:

Methyl red test: Methyl red test was performed by inoculating the bacterial isolate in MRVP broth and incubated at 37°C for 24-48 hrs.

Voges Proskauer test: The selected colony of bacterial isolate was inoculated into MRVP broth and incubated at 35°C for 24hrs.

Indole test: The tryptophan broth was inoculated by the isolate and incubated at 37°Cfor 24-48 hrs.

Citrate utilization test: The cultures of bacterial isolate were streaked on simmon citrate agar medium and plates were incubated at 37°C for 48 hrs.

Urease hydrolysis test: The test was performed by inoculating the bacterial culture into urease agar slants and incubated at 37 ºc for 24-48hr.

Catalase test: Catalase test was performed by adding H_2O_2 to the test sample. (12)

Carbohydrate Fermentation Test: Transfer a small amount of the bacterial culture into the carbohydrate broth. Incubate the inoculated broths at 35-37°C for 24-48 hours. This temperature range is optimal for most bacteria. After incubation, observe the color change in the medium. (13)

Detection of Cellulose Production and Quantification: Prepare HS medium. Inoculate and incubate (14 days at 37°C) the medium to allow cellulose production. After the incubation period, the pellicle that had shaped on the broth's air – liquid interface from the liquid. Wash the pellet with 1 NaOH or ethanol to purify the cellulose. The dry weight of cellulose became measured the use of the formely defined techniques. (14)

Optimization of cultural condition: It involves systematic experimentation with various parameters to enhance cellulose production. Using SEM, you can visualize the structural characteristics of cellulose and the morphology of microbial cells, which helps in correlating these structural features with different cultural conditions.

- Adjust temperature, pH, carbon sources, nitrogen sources, inoculum size, oxygen levels, and incubation time.
- Analyze cellulose production quantitatively and qualitatively.
- Use SEM to visually assess and confirm the structural characteristics of cellulose under optimized conditions.
- By integrating both optimization techniques and SEM analysis, we can comprehensively improve and validate cellulose production processes. (15)

3. Result

Isolation and screening of cellulose producing bacteria

In the present study, five bacterial isolates were obtained from natural sources which are found to produce cellulose. Those isolates showed fluorescence when observing their growth in screening medium under UV light. In screening media fluorescent dye binds to the cellulose content in the organism. Thus, cellulose producing bacterial colonies fluoresces when observed under UV light. So the fluorescent colonies were selected as cellulose producers. The isolates which obtained bacteria sp. CBA 5 (rotten apple), showed better cellulose production compared to other isolates.

Samples had been acquired and streak on GEY (Glucose, Ethanol and Yeast Extract) agar plates, colonies had been formed, The Figure 2 shows formation of a clear area across the colonies become selected for additional fermentation.

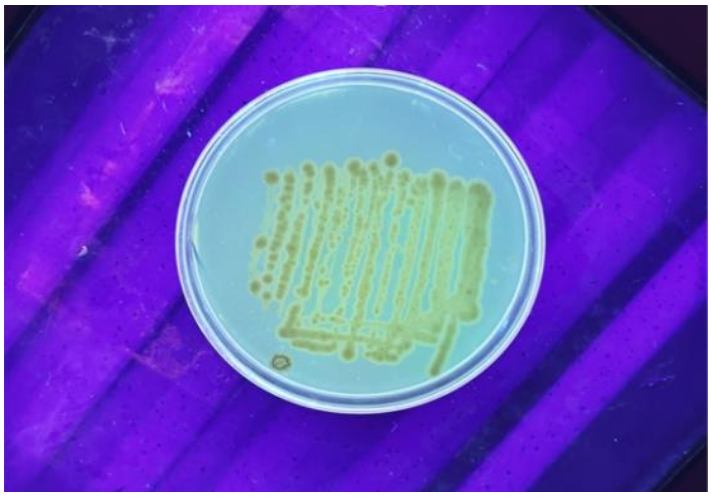

Figure 1: Fluorescent colony of bacteria sp

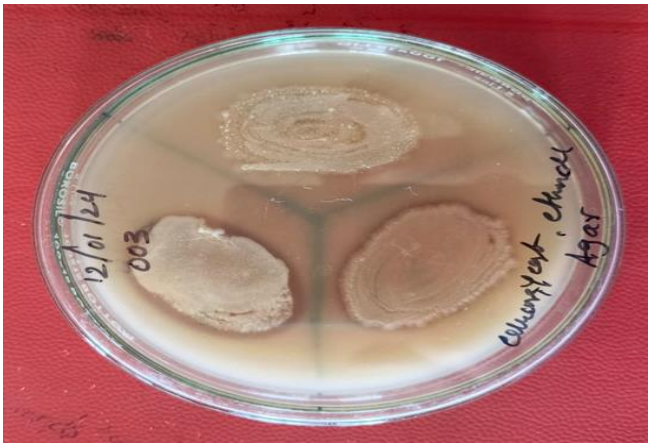

Figure 2: Formation of clear zone Under UV light across the colonies

Isolated so many bacteria from rotten apple like CBA1, CBA 2,CBA 3,CBA 4, CBA 5.
So CBA 5 microorganism is a cellulose producer.

Table 1: Number of bacteria isolates and their respective codes.

Rotten fruits sample	Total number of isolates	Codes
Apple	5	CBA1,CBA2,CBA3,CBA4,CBA5

Identification of cellulose producer

Identification of the strain was based on cultural characterization, biochemical characterization, and carbohydrate fermentation tests and results were tabulated (Tables 2, 3, and 4) (Figure 3). On the basis of biochemical characteristics, bacterial strains were identified as bacteria sp. CBA5,

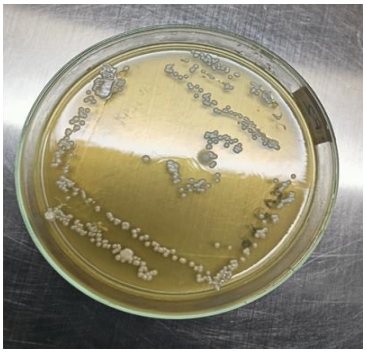

Figure 3: Colony morphology of bacteria sp, CBA

Gram staining: Gram-negative bacteria CBA5.sp cells are indicated through their pink or red look beneath a microscope. The normal characteristics of Gram-negative bacteria are supported through their rod-shaped morphology and the dearth of a thick peptidoglycan coating, as shown through Gram staining.

Mortality Test: Semisolid Agar: While mortality can be seen as the death of boom or apparent indicators of cell loss of life, boom inhibition can be indicated with the aid of decreased colony length or density. In this test bacteria are motile.

Table 2 : Culture Characterization

Colony Morphology	**CBA-05 K.Xylinus sp**
Configaration	Round
Margin	Entire
Elevation	Rasied
Surface	Smooth, mucoid
Colour	Pink
Opacity	Translucent
Motility	Motile
Cell Shape	Rod
Spore Formation	Negative

Table 3: Biochemical characterization of CBA-5 bacteria sp

Isolation	**CBA-05**
Catalase Test	-
Methyl red Test	-
Indole Test	-
Urease Test	-
Voges – ProskauerTest	-

Table 4: Carbohydrate fermentation test

Carbon source	**CBA-05 K.Xylinus**
Sucrose	+
Glucose	+
Lactose	+
Maltose	+
Cellulose	-
Starch	-

Detection of Cellulose Production and Quantification.

The strain bacteria sp. CBA 5, were observed to form pellicle at air liquid interphase (Figure 4). The pellicle was treated with alkali at 37∘ C followed by washing with 1 NaOH. The cellulose is resistant to this treatment and remains un dissolved and is accepted as pure cellulose. Yield is quantified for each isolate and presented in Table 5

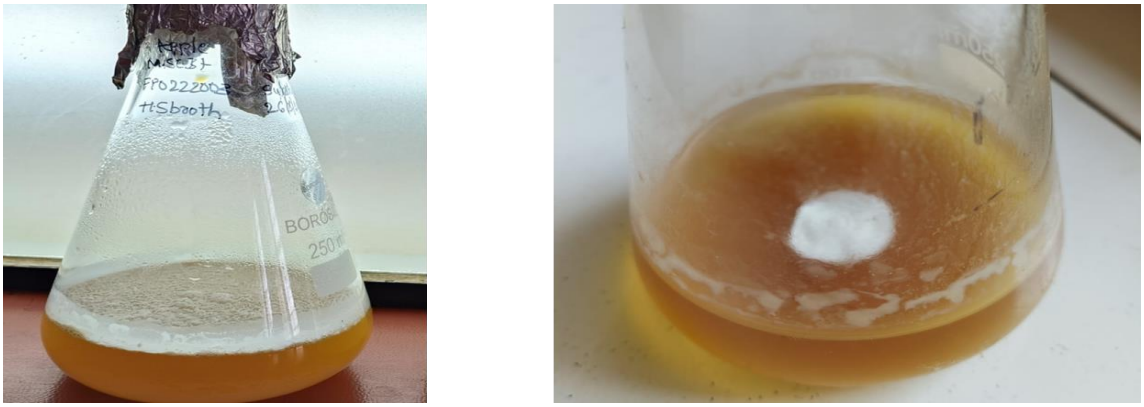

Figure 4: Pellicle formed at air liquid interface by the isolates bacteria sp CBA5

Table 5: Screening of isolation for cellulose production

Sample	Source	Isolated Code	Gram Reaction	Yield g/mL
1	Apple	CBA 1	Negative	0.23 g/mL
2	Apple	CBA 2	Negative	0.27 g/mL
3	Apple	CBA 3	Negative	0.20 g/mL
4	Apple	CBA 4	Negative	0.29 g/mL
5	Apple	CBA 5	Negative	0.25 g/mL

Optimization of cultural condition:

Scanning Electron Microscope. The ultrafine structure of bacteria cellulose constituted by cellulose nanofibre structure magnified at 5000 at 15.0 kV. Cellulose microfibrils and nanofibres were evidenced through SEM studies (Figure 5).we can use scanning electron microscopy (SEM) to verify if there may be cellulose present inside the pellicle. It should be possible to peer cellulose fibers inside the pellicle matri.

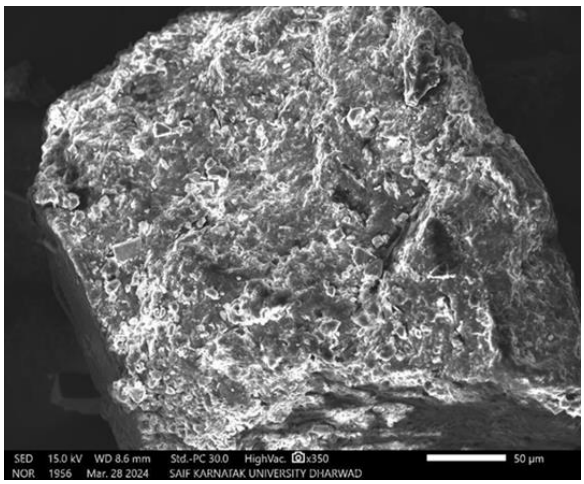

Figure 5: SEM image of cellulose produed at bacteria CBA5 sp

4. Discussion

Microorganisms which could produce cellulose are notably admired due to their potentialmakes use of in a whole lot of industries, consisting of environmental remediation and biotechnology. Of them, Komagataeibacter xylium (formerly Gluconacetobacter xylinus)is one of the most important and adaptable cellulose producers. A thorough summary of bacteria CBA-5sp isolation, characterisation, and biotechnology uses derived fromrotten apples.

The first step within the system of isolating CBA-05bacteria. sp from rotting apples isto acquire and prepare rotten apple samples. After surface sterilization, these samples areinoculated into HS media which have been especially designed to support the increase of CBA-5 bacteria sp. Following isolation, colonies are purified with the aid of streakingand recognized as CBA-5 bacteria sp strains using morphological, biochemical, and molecular strategies. To enhance cellulose production, CBA-5 bacteria sp strains are cultured below carefully regulated conditions after being remoted and described. The synthesis of cellulose is advanced with the aid of optimizing some of lifestyle conditions, together with as temperature, pH, carbon deliver, and oxygenation. To increase cellulose synthesiseven as retaining product exceptional and yield. Evaluating the physical, chemical, and structural characteristics of the cellulose generated by means of CBA-5 bacteria sp is known as characterization. The morphology, crystallinity, chemical content, and mophological behavior of CBA-5 bacteria sp- derived cellulose are defined by using techniques inclusive of scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and rheological evaluation. The cellulose generated from CBA-5 K bacteria sp has a wide range of biotechnologicalprograms. Applications for CBA-5 bacteria sp cellulose in the food enterprise encompass thickening, stabilizing, and gelling sellers in more than a few food objects. It functions as a scaffold for tissue engineering, wound healing, drug transport, and encapsulation within the biomedical and pharmaceutical regions. Furthermore, the abilityof CBA-5 bacteria sp cellulose in sustainable substances like bio plastics, paper items, and biofilms is being investigated.

CBA-5 bacteria sp strains remoted from rotten apples were characterized, remoted, and biotechnologically exploited. This represents a feasible avenue closer to furthering our know-how of cellulose manufacturing and using it for loads of commercial makes useof. This observe seeks to stimulate more research tasks targeted at understanding the overall ability of this adaptable microbe by way of clarifying the complex mechanisms atthe back of CBA-5 bacteria sp cellulose production and investigating its wide range of makes use.

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Conflict of Interest

Authors declare no conflict of interests.

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