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Physiological and Biochemical Characterization Cellulolytic Bacteria

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Abstract

Several efficient cellulase producing microorganisms were isolated. The purpose was to identify and characterize those isolates displaying the greatest cellulase activity for the possible use in the large scale biorefining. Cellulases are inducible enzymes that are synthesized by a large number of microorganisms during their growth on cellulosic materials. Cellulases have attracted much interest because of the diversity of their applications. Cellulases are used in the various industrial process, including textile and laundry, food, feed, leather, pulp and paper. The biochemical characterizations of the isolated Bacterial strains from termite gut. Isolated strains are efficient namely TG I and TG II. Indicate that they may play a role in cellulose digestion in termite gut.

***Key words*:** Cellulolytic bacteria, Cellulase, Termites, Biochemical study, Enzyme

Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Some bacterial species viz., *Cellulomonas species*, *Pseudomonas species*, *Bacillus species* and Micrococas have cellulolytic property [15]. Cellulose is a complex carbohydrate that forms the structural component of plant cell walls. To utilize cellulose for industrial purposes, it needs to be broken down into simpler sugars through a process known as cellulolysis, which is catalyzed by cellulase enzymes. Many bacteria are capable of producing cellulases, making them highly useful for bioconversion processes, such as producing biofuels, biodegradable materials, and various bioproducts from plant biomass [1].

By contrast, fungi, while slower in growth, are known for producing a large variety of extracellular enzymes and tend to generate higher levels of cellulase. Fungi such as *Trichoderma reesei* are widely used in commercial cellulase production. However, bacteria offer an alternative due to their faster growth and adaptability. Bacterial species like *Cellulomonas*, *Bacillus*, and *Pseudomonas* therefore present a viable option for sustainable, efficient cellulose degradation, with potential applications in fields such as biofuel production, waste management, and environmental remediation [4-5]. Large number of bacteria are capable of degrading cellulose, but only a few of them produce significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose in-vitro [16]. While many bacterial species possess the capability to degrade cellulose, only a small subset can produce significant amounts of cell-free bioactive compounds (primarily cellulases) that can completely hydrolyze crystalline cellulose in-vitro. This distinction is critical because breaking down crystalline cellulose, the highly ordered and resistant form of cellulose found in plant cell walls, requires a highly efficient and robust enzymatic system. However, even though many bacteria produce cellulases, few of them can produce sufficient quantities of cell-free bioactive enzymes to fully degrade crystalline cellulose. Most bacteria only degrade cellulose in direct contact with the substrate, and the enzymes they produce may be bound to their cell surface or function best in proximity to the cells. Producing cell-free cellulases in large quantities is more challenging [6-7].

Cellulolytic bacteria, which are bacteria capable of breaking down cellulose into simpler compounds, have been isolated from a wide variety of habitats. The presence of cellulose in diverse environments, primarily from plant material, has driven the evolution of these bacteria to thrive in different ecological niches [10]. The key habitats from which cellulolytic bacteria have been isolated include soil, compost, and water, each providing unique conditions for bacterial adaptation and cellulose degradation. Cellulolytic bacteria have been isolated from diverse habitats like soil, compost, and water [8]. cellulolytic bacteria are found in a variety of habitats, each offering distinct conditions for their growth and activity. Soil, compost, and water provide rich sources of cellulose, which these bacteria degrade, playing a critical role in decomposition and nutrient cycling across ecosystems [12-14]. These bacteria also have significant potential for industrial and environmental applications. Common classification separates different pretreatments into physical, chemical, physicochemical, and biological treatments [18]. The conversion of cellulosic materials into bioethanol is a multi-step process, with enzymatic hydrolysis being a critical phase. However, before enzymatic hydrolysis can effectively break down cellulose into glucose, pre-treatment of the cellulosic material is essential. This is because raw lignocellulosic biomass, such as agricultural residues, wood, and grasses, contains structural barriers that prevent enzymes from accessing the cellulose. Enzymatic hydrolysis, pre-treatment of cellulosic material is utmost importance to obtain glucose which can be further converted into bioethanol by microbes [2], [11], [17]. Pre-

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treatment of cellulosic material is essential for the efficient enzymatic hydrolysis of cellulose into glucose. Without proper pre-treatment, the dense and recalcitrant structure of lignocellulosic biomass would severely limit enzyme access, reducing the overall efficiency of the bioethanol production process. Pre-treatment makes cellulose accessible to cellulase enzymes, which hydrolyze it into glucose, providing the necessary substrate for microbial fermentation into bioethanol. Therefore, pre-treatment is the key step to unlocking the potential of cellulosic biomass as a renewable source of energy. Bacteria are now being widely explored for cellulase production because of their extremely high natural diversity and the capability to produce stable enzymes that can be applied in industries [3], [9]. Bacteria are increasingly being explored for cellulase production due to their unique advantages over other microorganisms, especially fungi. The natural diversity of bacteria allows for the discovery of species that produce cellulases with a wide range of properties, which are suitable for various industrial applications. Additionally, many bacteria can produce stable enzymes that are resilient to harsh industrial conditions such as high temperatures, extreme pH levels, and the presence of inhibitors. This has made bacteria a promising source of cellulases for biofuel production, waste management, and other industries.

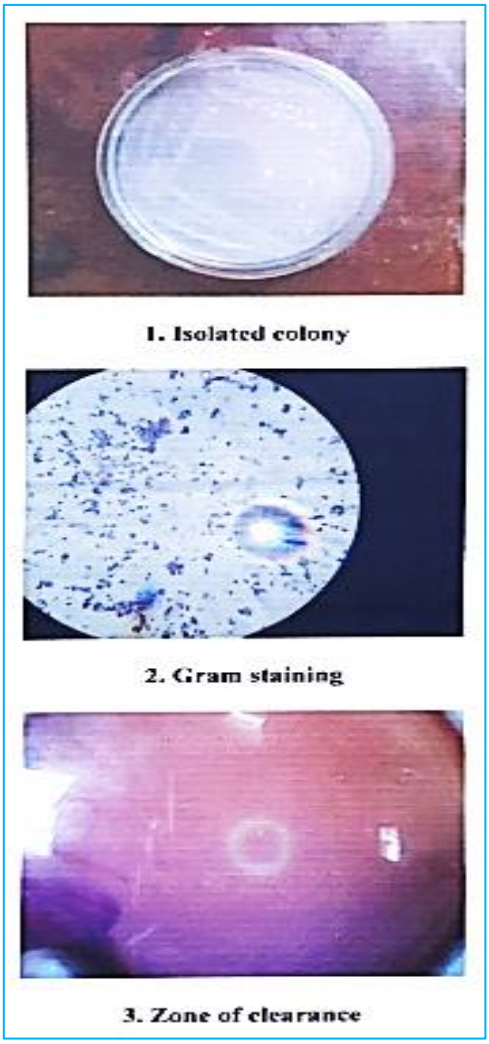

Fig 1 Isolated bacteria

MATERIALS AND METHODS

A. *Sample collection*

Termite Sample was collected from sites which includes cellulose feeding organisms, such as termite residing on woody western ghat region Maharashtra state. Sample dissect in 0.9% saline solution under sterile condition. 1.0 gram of each sample is placed in 9 ml of 0.9% saline, mixed it rapidly and Serial dilution techniques was followed and the dilutions selected for further studies.

B. *Isolation cellulolytic bacteria*

The mixture was mixed by vortex for 2-3 mins for removal of microorganisms. One ml of this sample was plated by serial dilution (up to 10^{4-} (technique amended with CMC agar and incubated at 37°c for 24 -48 hours. Bacterial cultures grown on CMC slants were cultured on basal mineral salt medium (BSM) as shown (Fig 1).

C. *Culture and biochemical characteristics*

I. *Morphology*

The morphological characteristics of the isolated bacteria from termite gut (TG I and TG II) were observed and recorded. Both bacterial strains TG I and TG II were Gram-positive, cocci-shaped, and motile, indicating their potential for active movement in their environment.

Test \ Sample	TG I	TG II
Shape	Cocci	Cocci
Gram strain	Positive	Positive
Motolity	Motile	Motile

II. *Colony characteristics*

The colony characteristics of the two isolates were studied in detail and documented. Both isolates exhibited dirty white colonies with a convex elevation and opaque density. They showed optimal growth at a temperature of 30°C and a pH of 7, indicating their preference for neutral pH conditions and moderate temperatures.

Test \ Sample	TG I	TG II
Colour	Dirty white	Dirty white
Elevation	Convex	Convex
Density	Opaque	Opaque
Optimum temperature	30°C	30°C
Optimum pH	7	7

III. *Biochemical test*

A range of biochemical tests were performed on the two bacterial isolates (TG I and TG II) to understand their metabolic properties and enzyme production capabilities.

Test \ Sample	TG I	TG II
I) Starch hudrolysis	+	-
II) Carbohydrate fermentation		
Arabinose	+	-
Maltose	+	+
Lactose	+	-
Mannitol	-	+
Starch	+	-
Cellulose	+	+
Glucose	-	+
III) H_2S production	- (Alkaline)	- (Acidic)
IV) Simmon citrate	+	+
V) Caseic hydrolysis	+	-
VI) Catalase	+	+

Starch hydrolysis: TG I showed positive starch hydrolysis activity, while TG II did not hydrolyze starch.

Carbohydrate fermentation:

- TG I fermented arabinose, maltose, lactose, and starch, but not mannitol or glucose.
- TG II fermented maltose, mannitol, and glucose, but not arabinose, lactose, or starch.

Both TG I and TG II could degrade cellulose, indicating their cellulolytic capability.

Simmons citrate test: Both isolates were positive, indicating they could utilize citrate as a carbon source.

Casein hydrolysis: TG I hydrolyzed casein, while TG II did not.

Both isolates showed catalase activity, meaning they could decompose hydrogen peroxide into water and oxygen.

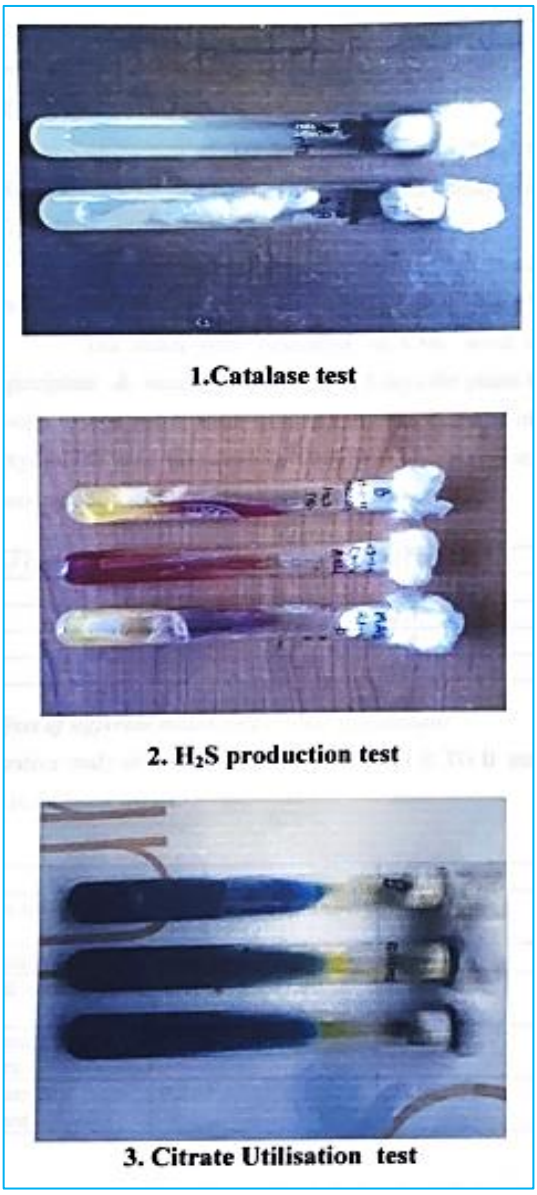

Fig 2 Biochemical test

RESULTS AND DISCUSSION

Gram stain was an empirical method of distinguishing bacterial species into two large groups (Gram-positive and Gram-negative) based on the presence of chemicals, primarily the presence of high levels of peptidoglycan and physical properties of their cell walls. A number of cellulolytic bacterial colonies were isolated from Termite gut. The colony morphology was studied in detail and the results are presented in Tabulation with heading also followed by biochemical tests performed for the isolated microorganisms as shown (Fig 2). Clear zone producing bacterial isolates were then subjected to various biochemical test.

The Gram stain confirmed that both bacterial isolates (TG I and TG II) were Gram-positive cocci. A variety of cellulolytic bacterial colonies were successfully isolated from the termite gut samples. Their colony morphology was studied in detail, with both isolates exhibiting similar characteristics (dirty white color, convex elevation, opaque density).

The isolates were subjected to biochemical tests to identify their metabolic pathways and enzyme activities. The results confirmed that both isolates had cellulolytic activity, as evidenced by their ability to degrade cellulose, and exhibited distinct biochemical profiles [19-20].

The biochemical characteristics such as starch hydrolysis, carbohydrate fermentation, H_2S production, citrate utilization, and catalase activity provided insights into the potential industrial applications of these bacteria. Their clear zone formation on CMC agar and their ability to produce cellulases make them promising candidates for further studies in cellulase production for industries like biofuel production and waste management [21-23].

CONCLUSION

The Gram staining process is a valuable empirical method for classifying bacterial species based on their cell wall characteristics, especially in studies involving the isolation of cellulolytic bacteria. In this context, bacteria isolated from the termite gut are screened for their ability to degrade cellulose by examining their colony morphology and testing for clear zone production. These cellulolytic bacteria are further analyzed using a range of biochemical tests to determine their enzymatic activities and metabolic properties. The combination of Gram staining, clear zone production, and biochemical tests allows for a comprehensive characterization of cellulolytic bacterial species, which are of great interest in industrial applications such as biofuel production, waste management, and enzyme production. With the help of biochemical tests, we could conclude that bacterial isolate belongs to genus belongs to genus Bacillus. A potential cellulose degrading enzyme from B. subtilis was characterized and studied for its possible hydrolyzing capability for disintegrating the cellulosic biomass residues.

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Conflict of interest – None

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