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Isolation of Cellulase Producing Microorganism and Characterization of Partially Purifed Enzyme

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Abstract: Cellulase enzymes can convert plant resources into simple sugars,cellulase enzymes are becoming more and more significant for industry. The goal of this study was to identify and investigate cellulase producing bacteria from local soli and fruit waste. We also tried to use liquid fermentation to enhance the environment so that these bacteria might produce more enzyme.To see if bacteria could degrade cellulose, they were tested on CMC agar plates. They were picked because they created distinct zones around them. Among these, two bacteria, CBM21 and CSG08, had a high capacity for cellulose degradation. It was determined that CSG08 was Pseudomonas and CBM21 was Bacillus.With an activity of 0.156 U/ml once the growing conditions were improved to include a pH of 7.0, a temperature of 40^{0}C, 5% glucose, and a 24-hour incubation period. The enzyme activity of CSG08 also increased, rising from 0.106 to 1.23U/ml.These results show that local bacteria can be useful for making enzymes for industries.Food processing, plant waste conversion, and biofuel production can all benefit from these enzymes. Changing the growing environment can also help the bacteria produce more enzyme, according to the study. (1)(2).

Keywords: Carboxymethyl Cellulose, *Bacillus Spp., Pseudomonas Spp., Carbon Source, Fermentation.*

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I. INTRODUCTION

Tomatoes (*Solanum lycopersicum*) are a common fruit rich in vitamins A and C. Cellulase enzyme break down the cell walls of tomatoes as they ripen, resulting in a soft, juicy fruit, Additionally, food processing, fruit juice production, enhancing juice clarity, and converting waste into valuable goods all require these enzymes. Bacteria that produce cellulase are frequently found in damaged fruits. *Aspergillus niger*, *Trichoderma reesei*, and *Penicillium* are fungus that generate cellulase, where as *Streptomyces*, *Bacillus subtilis*, and *cellulomonas* are some of these bacteria. The cellulase enzyme is made of different parts that work together middle sections of the cellulose chain are broken by endoglucanases. Small fragments known as cellobiose are broken off by exoglucanases and cellobiohydrolases. The simple sugar glucose is produced from cellobiose by beta-glucosidases. Actinomycetes, fungus, bacteria, plants, and animals (via beneficial microorganisms) all contain cellulase. How it works: Cellulase uses unique components known as carbohydrate-binding modules to adhere to cellulose after breaking the β-1,4 bonds in cellulose. Certain bacteria,such as *Clostridium*, produce large groupings of enzyme termed cellulosomes to aid in the breakdown of cellulose.In experiment,bacteria that make cellulase were taken form molasses.The endo-β- 1,4-glucanase enzyme exhibits promising industrial applications.Special groupings of enzymes known as cellulosomes are produced by certain bacteria,such as *Clostridium*.These big enzyme groups aid in the easy breakdown of cellulose by remaining on the bacteria surface.Cellulosomes consist of various components. Scientists studied the best conditions (like pH, temperature, and food sources) for making an enzyme. They cleaned and separated the enzyme using special methods. A test called SDS-PAGE showed the size of the enzyme. The enzyme turned out to be endo-β-1,4-glucanase, which is useful for breaking down plant materials. This makes it very promising for use in things like making biofuels and managing waste. Juice Extraction Breaks down cell walls to increase yield.Enhances clarity and decreases viscosity in juice.Waste Valorization tomato peels and leftovers into sugars that may be fermented to make biofuels or other items with added value.Food processing Increases productivity and product quality by utilizing additional enzymes, such as pectinase. Rotten fruits contain cellulose and pectin, making them perfect for cellulolytic microbes that release cellulase.(3)(4)

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II. MATERIALS AND METHODS

➢ *Sample Collection:*

Spoiled fruits were collected from local markets.These helped identify bacteria that produce cellulase, an enzyme that decomposes plant matter.In a refrigerator,the samples were kept in sterile containers to ensure their safety for testing ..(5)

➢ *Isolation of Cellulase Producing Bacteria:*

The samples gradually become weaker(diluted) by carefully mixing them with a sterile solvent.To cultivate bacteria,they were then spread out on CMC agar plates to aid in the growth of the bacteria, these plates were maintained at 37^0C for 24 to 48 hours. (6)

➢ *Screening of Cellulase Producer:*

Primary screening is the initial stage in identifying bacteria capable of producing cellulase.Bacteria from the samples are spread out on CMC(carboxymethyl cellulose) made special plates in this step.Iodine solution is introduced to the plates after they have been allowed to grow (incubate).The colonies of bacteria will have transparent rings or halos surrounding them if they manufacture cellulase.Cellulase activity can be observed by these transparent halos,which helps scientisits in selecting the most suitable bacteria for further studies.(7)

Scientists use a second test known as secondary screening to determine the amount of cellulase produced by the selected bacteria.The bacteria are grown in a liquid with CMC (a plant-based material).The liquid that has been left uncontaminated after a while is collected this is known as the culture supernatant.It is then mixed with DNS solution.Cellulase breaks down CMC releasing sugars that react with the DNS.Sugar content indicates the cellulase enzymes activity more sugar level indicates higher enzyme activity.(8)

➢ *Identification of Cellulase 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 cellwalls, particularly their ability to retain a specific dye. (9)

➢ *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. (10)

➢ *Cellulase Activity Assay (DNS Methods)*

Crude enzyme extract was incubated with 1% CMC in citrate buffer. After 30 minutes at 50°C, DNS reagent was added, and then reaction mixture was boiled for 10 minutes. Absorbance was measured at 575nm. Reducing sugar concentration was determined using a glucose standard curve. One unit of enzyme activity was defined as the amount of enzyme releasing 1µmol of glucose per minute. (11)

➢ *Biochemical Characterization:*

Using a number of biochemical assays, such as citrate utilization, methyl red, and fermentation tests, the bacterial isolates were described in accordance with accepted identification procedures. (12)

➢ *Fermentation Test:*

An inoculum from a pure culture was transferred using a sterile loop into a tube filled with sterile phenol red sucrose broth for the fermentation test. The tube was then incubated at 35–37°C for 24 hours, and the results were evaluated based on color change and gas production. (13)

➢ *Citrate Utilization Test:*

The names of the two test tubes were appropriately labeled with the test medium type, bacterial isolate name, and group name. The entire slant surface of the citrate agar in both tubes was covered with a small amount of bacterial culture from a well- isolated colony using a sterile inoculating loop. After that, the tubes were incubated aerobically for up to four days at 35–37°C. To assess citrate usage, a color shift was monitored. (14)

➢ *Methyl Red Test:*

The bacterial culture was put to a new MR-VP broth tube, which was then incubated for 24 hours at 35°C. Five drops of methyl red reagent were then added to the tube to see if the color changed, signifying the generation of stable acids. (15)

➢ *Optimization of Cultural Condition:*

Cellulase enzyme synthesis is enhanced by changing several environmental and environmental parameters to achieve optimal enzyme activity and quantity. The efficiency and cost-effectiveness of enzyme synthesis for research or industry can be increased by modifying variables including temperature, pH, and substrate (food supply).(16)

➢ *Temperature:*

The effects of different temperatures (e.g., 25°C to 50°C) are analyzed. The temperature where cellulase activity is maximum is the optimal one.(17)

pH. The effects of varying pH values, such as pH 4 to pH 9, on the synthesis of enzymes are studied. The pH at which the enzyme produces the most product is the ideal pH.(18)

➢ *Substrate:*

The effect of utilizing varying substrate concentrations (0.1 to 1.0, for example) is studied. The quantity where the cellulase enzyme is most active is the optimum value. (19)

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III. RESULT

Fig1 This Image Shows Rotten Tomato

Table 1 Sample Collection

Sample collection	10 –20g
pH	3–4
Temperature	4°C

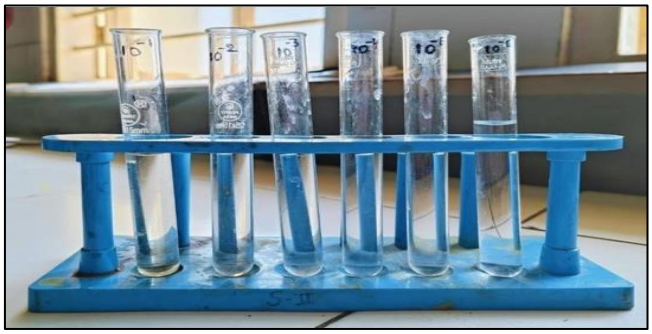

Fig 2 Serial Dilution from Rottten Tomato

Table 2 Serial Dilution Identification of Cellulase Producer

Method	Dilution factor
Number of dilution	6
Dilution factor	6:1
Starting solution concentration	1ml
Final solution concentration	0.00001ml

Identification of the strain was based on cultural characterization, biochemical characterization, and results were tabulated (Tables,3,) (Figure3). On the basis of biochemical characteristics, bacterial strains were identified as bacteria sp.

Fig 3 Colony of Morphology of Species

➢ *Colony Morphology of Bacteria Sp*

Growth on nutrient agar plates supplemented with 1% carboxymethyl cellulose (CMC) was used to characterize the morphology of the isolated cellulase-producing bacterial colonies. Following 24 to 48 hours of incubation at 35°C, the colonies were inspected for the following traits:

Table 3 Culture Characterization

Colony Morphology	Bacteria sp
Configaration	Round
Margin	Entire
Elevation	Rasied
Surface	Smooth, mucoid
Colour	Pink
Cell Shape	Rod
Spore Formation	Negative

Table 4 Biochemical Characterization

Isolation	CBA-05
Citrate utilization Test	-
Methyl redTest	-
Fermention Test	-

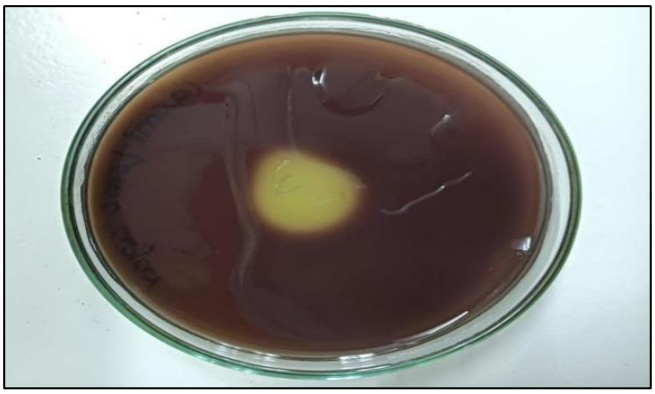

Fig 4 Zone of Inhibition

➢ *Effect of Temperature on Cellulase Activity*

To determine the optimal temperature, fermentation was conducted at various temperatures (30°C–50°C). The enzyme showed peak activity at 40°C for CBM21 and 35°C for CSG8, indicating mesophilic behavior typical of Bacillus and Pseudomonas spp.(fig.5)

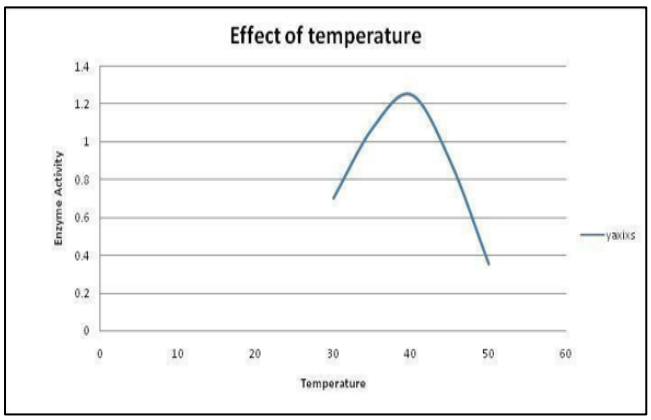

Fig 5 Effect of Temperature on Cellulase Activity (Optimal Temperature: 40^0C(1.24µ/mL) Effect of pH on Enzyme Activity

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The activity of cellulase was evaluated across pH 6–10. The enzyme exhibited maximum activity at pH7.0, aligning with neutral ophilic microbial preferences. (fig.6)

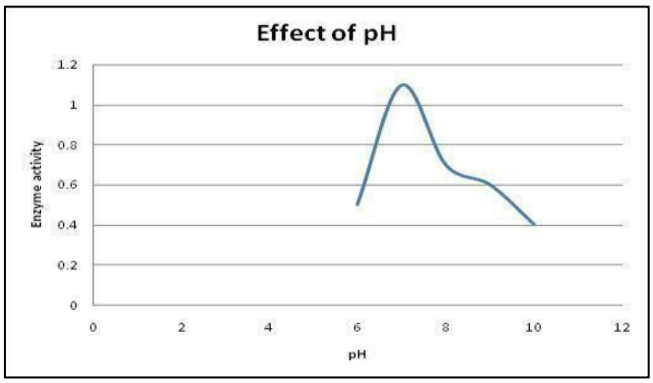

Fig 6 Effect of pH on Cellulase Activity (Optimal pH:7.0(1.11µ/mL)

➢ *Effect of Substrate (Glucose) Concentration*

The influence of glucose concentration (1%to5%) on enzyme activity wastested. The highest cellulase activity was observed at 5%glucose concentration (fig.7 and 8)

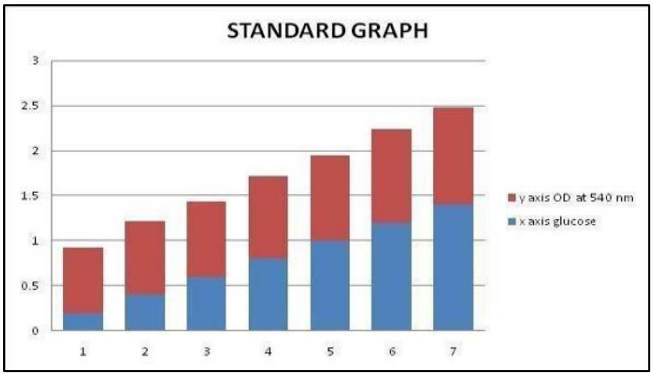

Fig 7 Effect of Glucose Concentration on Cellulase Activity

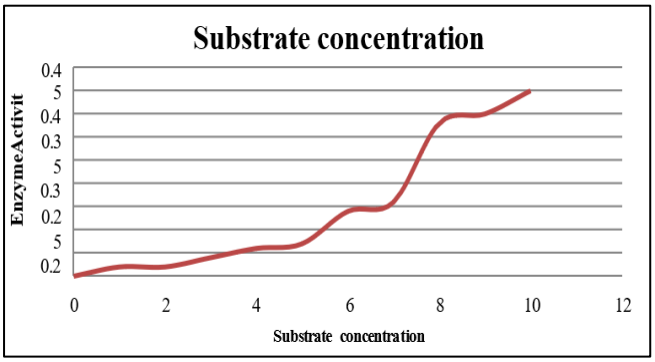

Fig 8 This Image Shows the Graphs of Substrate Concentration

IV. DISCUSSION

The results of this study show a clear relationship between the quantity of substrate and cellulase activity, as determined by optical density (OD) at 540 nm. The spectrophotometer's data shows that, particularly at increasing substrate levels, enzyme activity increases in parallel with substrate levels.

A simple equation derived from the substrate data regression analysis can be utilized for projecting enzyme activity at various substrate levels. The R2 result indicates that there is a strong positive correlation between the amount of substrate and cellulase activity within the measured range of 0 to 1.0 concentration. This result shows what is known about enzyme kinetics, which states that as substrate concentration rises, the reaction speed increases until it hits a saturation point, at which time it can no longer proceed faster. Similar patterns appear when we compare these findings with those of other studies that are referenced in the text. Previous research on cellulase production optimization also emphasize the importance it is to understand the interaction between enzymes and substrates in order to increase enzyme output. Deka et al.'s study, for example, demonstrated that increasing enzyme production requires an understanding of enzyme-substrate interactions. (2017) on *Bacillus subtilis* demonstrated CM Case activity of 0.43 units per milliliter after optimization, while our data shows comparable activity levels depending on the substrate concentration used. The enzyme's actions with different substrate concentrations is made easier to understand by the graph that displays substrate concentration and optical density (OD). The information points indicate that the OD values increase in line with the substrate concentration, with a particular more rapidly rise in OD occurring when the substrate concentration goes above 0.5. Higher substrate levels exhibit a non-linear pattern, which indicates that the enzyme's behavior may vary based on the amount of available substrate. Cooperative binding or different enzyme types that react differentially to the substrate could be the reason of this. According to the literature evaluation in this publication, it is essential to optimize the conditions for enzyme production for industrial usage. Our findings on the relationship between substrate concentration and enzyme activity contribute valuable knowledge to this field of research. The observed pattern in our sata suggests that for optimal cellulase activity measurement, Since they provide the most accurate and dependable values in the test, the pattern in our results indicates that substrate concentrations between 0.7 and 1.0 are ideal for assessing cellulase activity. It's also essential to keep in mind that cellulase activity can be impacted by additional variables such as pH, temperature, and the presence of helpers (co-factors) or blockers (inhibitors). This paper contains pH and temperature optimization experiments that confirm our findings about substrate concentration and provide a comprehensive picture of the enzyme's behavior under various circumstances. According to the protocol part of this paper, a popular and reliable technique in cellulase research is the enzymatic test that uses the DNS (3,5-dinitrosalicylic acid) reagent to measure the reducing sugars generated during cellulose breakdown. Our study's use of this technique produced accurate and reliable results, demonstrating its usefulness to assess cellulase activity. The data from this study can be used as a starting point for more detailed research on the enzyme's behavior, such as finding out its Km and Vmax values. These values help us understand how strongly the enzyme attaches to the substrate and how fast it can work at its maximum speed, which is very important for using cellulase in industries.

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- *Conflict of Interest*

  Authors declare no conflict of interests.

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