ISOLATION AND CHARACTERIZATION OF CELLULASE RODUCING BACTERIA FROM TERMITE GUT

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Abstract

Lignocelluloses are the most abundant organic compound in the nature. The cellulosic waste can be used as a potential source of Bio-energy. For the conversion of cellulosic waste to a fuel source, the complex structure of cellulose has to be broken and the glucose monomers obtained can be fermented to bio-ethanol the methods present today are chemicals and enzymatic method. Chemicals methods are expensive and their wastes are proving to be a potential hazard to the environment. On the other hand enzymatic processes are comparatively slow; therefore there commercial application is not feasible. The plants and tree have evolved over millions of years to perfect their structural components. They are so evolved that their degradation is difficult and this is attributed due to their cell wall. However several species of fungi and bacteria have developed a method to penetrate into this complex network of Lignin and hemicelluloses and consequently degrade the cellulose. In the present study soil samples from different areas of P.U and wood samples from degraded tree trunk and rotten wood furniture were picked up and were isolated and purified on a media having cellulose as a sole source of carbon. The morphologically different colonies were tested for the cellulytic activity using CMCase test and top 4 strains were selected for further tests.

Keywords: Isolation, Bacteria, Cellulose, Termite Gut

I. INTRODUCTION

Cellulase (EC 3.2.1.4) refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze Cellulolysis (i.e. the hydrolysis of cellulose). However, there are also Cellulases produced by a few other types of organisms, such as some termites and the microbial intestinal symbionts of other termites. Several different kinds of Cellulases are known, which differ structurally and mechanistically (Ertoldt et al, 1999). Other names for 'endoglucanases' are: endo-1,4-beta-glucanase, carboxy methyl Cellulase (CMCase), endo-1,4-beta-D-glucanase, beta-1,4-glucanase, beta-1,4-endoglucan hydrolase, and celludextrinase. The other types of Cellulases belong to exo Cellulases. Beta-glucosidase can also be considered as yet another group of Cellulases. The expression 'avicelase' refers to the total Cellulase activity of a given sample of the enzyme (Breznak et al, 1994). The Cellulase may be the result of the action of more than one type of enzymes (Brosius et al, 1981).

Cellulase in general enzymes that cleave lignin are occasionally classified as cellulase, but this is usually considered erroneous. Within the above types there are also progressive (also known as processive) and non progressive types. Progressive Cellulase will continue to interact with a single polysaccharide strand; no progressive Cellulase will interact once then disengage and engage another polysaccharide strand (Brune et al, 1995).

Most fungal Cellulases have a two-domain structure, with one catalytic domain and one cellulose binding domain that are connected by a flexible linker (Brune et al, 1995). This structure is adaption for
working on an insoluble substrate, and it allows the enzyme to diffuse two-dimensionally on a surface in a caterpillar way. However, there are also Cellulases (mostly endoglucanases) that lack cellulose binding domains. These enzymes might have a swelling function (Cleveland et al, 1924).

In many bacteria, Cellulases in-vivo is complex enzyme structures organized in supramolecular complexes, the cellulosomes (Cruden et al, 1979). They contain roughly five different enzymatic subunits representing namely endocellulase, exocellulase, cello biases, oxidative Cellulases and cellulose phosphorylases wherein only endocellulase and cello biases participate in the actual hydrolysis of the \( \beta (1 \rightarrow 4) \) linkage. Recent work on the molecular biology of cellulosomes had led to the discovery of numerous cellulosome-related “signature” sequences known as dockerins and cohesins. Depending on their amino acid sequence and tertiary structures, Cellulases are divided into clans and families (Devereux et al, 1984).

Cellulase is used for commercial food processing in coffee. It performs hydrolysis of cellulose during drying of beans. Furthermore, Cellulases are widely used in textile industry and in laundry detergents (Dickman et al, 1931). They have also been used in the pulp and paper industry for various purposes, and they are even used for pharmaceutical applications. Cellulase is used in the fermentation of biomass into bio fuels, although this process is relatively experimental at present. Cellulase is used as a treatment for phytobezoars, a form of cellulose bezoars found in the human stomach.

II. MATERIALS AND METHODS

Chemicals and Media:

All the chemicals used in this study were of analytical grade. Media constituents used in this study were procured from Hi-Media, and Merck.

Isolation of Bacteria from Termite Gut:

Termites were collected from different locations of Panjab University campus. Termites (4-5) were surfaced sterilized with 90% ethanol for 2 minutes and washed with sterile water 4-5 times. The alimentary canal of blot dried termites was dissected out and washed with sterile ringer’s solution. The gut was homogenized with Ringer’s solution in a glass homogenizer to disintegrate the gut wall and to disperse the contents. The homogenate was filtered. The filtrate of each sample was diluted up to 10⁻³ and plated on CMC agar medium. The plates were incubated at 30⁰C in incubator for 2–3 days.

Media preparation:

CMC (Carboxy methyl cellulose) agar medium is a selective media consist of media consist of:-

| Media Composition                   | g/L  |
|------------------------------------|------|
| KH\(_2\)PO\(_4\)                   | 1.0  |
| MgSO\(_4\).7H\(_2\)O               | 0.5  |
| NaCl                               | 0.5  |
| FeSO\(_4\).7H\(_2\)O               | 0.01 |
| MnSO\(_4\).H\(_2\)O                | 0.01 |
| NH\(_4\)NO\(_3\)                   | 0.3  |
| CMC (carboxy methyl cellulose)     | 10.0 |
| pH                                 | 70.  |
| Agar                               | 20.0 |
III. RESULTS AND DISCUSSION

Termites were collected from different locations of Panjab University campus; four bacterial strains were isolated and showing good result by zone of clearance. The following result were showing.

**Identification Summary**

| TEST                                  | ISOLATE 1 | ISOLATE 2 | ISOLATE 3 | ISOLATE 4 |
|--------------------------------------|-----------|-----------|-----------|-----------|
| Physiological Tests                  |           |           |           |           |
| Growth at Temp                       |           |           |           |           |
| 25 °C                                | +         | +         | +         | +         |
| 30 °C                                | +         | +         | +         | +         |
| 40 °C                                | +         | +         | +         | +         |
| 45 °C                                | -         | -         | -         | -         |
| Methyl Red Test                      | -         | +         | -         | -         |
| Voges Proskauer Test                 | -         | -         | -         | +         |
| Citrate Utilization                  | -         | -         | +         | -         |
| Urea Hydrolysis                      | -         | -         | -         | +         |
| H₂S Production                       | -         | -         | -         | -         |
| Indole Test                          | -         | -         | -         | -         |
| Catalase Test                        | -         | -         | -         | -         |
| Motility                             | +         | +         | +         | -         |
| Gram’s Staining Shape Arrangement    | Gram negative Coccus | Gram positive Bacillus Sinlge | Gram positive Coccus Sinlge | Gram positive Coccus Sinlge |

| ISOLATE | Result (slant/butt) | Interpretation                                      |
|---------|---------------------|-----------------------------------------------------|
| 3       | Red/Yellow          | Glucose fermentation only, peptone catabolized.     |
| 1       | Yellow/Yellow       | Glucose and lactose and/or sucrose fermentation.    |
| 2,4     | Red/Red             | No fermentation, Peptone catabolized.               |
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