SCREENING AND ISOLATION OF CELLULASE PRODUCING BACTERIA FROM DUMP YARDS OF VEGETABLE WASTES

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ABSTRACT

This work has been undertaken for the screening and isolation of cellulase producing strains of bacteria were carried out from ten soil samples, collected from dump yards of vegetable wastes various regions of Bangalore and used to screen for cellulase production by using Carboxy Methyl Cellulose (CMC) agar medium. In the present study, an attempt was made to isolate efficient cellulase producing bacteria from diverse regions of Bangalore. Different isolates were screened for possessing the ability to produce cellulase. About 6 bacterial isolates were found to be promising to produce cellulase. The organisms were tested for various biochemical tests, which leads to their identification as Bacillus licheniformis, Bacillus cereus, and Staphylococcus aureus.

Key words: Vegetable wastes, Cellulase, Carboxy Methyl Cellulose (CMC), Bacillus licheniformis, Bacillus cereus, Staphylococcus aureus.

INTRODUCTION

Enzymes are delicate protein molecules necessary for life. Cellulose is the most abundant biomass on the earth. Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extracellular during their growth on cellulosic materials [1]. Cellulose, a crystalline polymer of D-glucose residues connected by β-1, 4 glucosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature [2]. Therefore, it has become considerable economic interest to develop processes for effective treatment and utilization of cellulosic wastes as inexpensive carbon sources.
Complete enzymatic hydrolysis of cellulose requires synergistic action of 3 types of enzymes, namely exo-1,4-β-glucan cellobiohydrolase (EC 3.2.1.91), which cleave cellobiosyl units from the ends of cellulose chains; endo-1,4-β-glucanases (EC 3.2.1.4), which cleave internal glucosidic bonds; and β-D-glucosidase (EC 3.2.1.21), which cleave glucose units from cellulooligosaccharides \[3\]. Cellulose is commonly degraded by an enzyme called cellulase. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyse the cellulolysis (or hydrolysis) of cellulose \[4, 5\]. This enzyme is produced by several microorganisms, commonly by bacteria and fungi.

Cellulases have attracted much interest because of the diversity of their application. The major industrial applications of cellulases are in textile industry for ‘bio-polishing’ of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness \[6\]. Application of enzymes in textile, food, detergent, leather and paper industries demands identification of highly stable enzymes active at extreme pH and temperature \[7\]. Cellulase is used in the fermentation of biomass into biofuels, fibre modification and they are even used for pharmaceutical applications \[8\].

Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Cellulolytic property of some bacterial species viz., Cellulomonas species, Pseudomonas species, Bacillus species and Micrococcus \[9\]. Evidence showed that cellulase is inducible and they can produce from different carbon sources. In the course of time, numerous reports have appeared on the optimization of fermentation and microbiological parameters and different fermentation strategies for the production of cellulases. With the advent of molecular biology, vigorous research has been carried out on cloning and expression of cellulase genes in various hosts. Among the various cellulase, bacterial extracellular cellulases are the most significant, compared with protozoans, viruses and fungal extracellular cellulases \[10\]. Extracellular cellulase produced by Bacillus and Ccci species are of main interest from a biotechnological perspective and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as foods, pharmaceutical and paper industries.

These cellulases account for 10% of the total worldwide production of enzymes \[11\]. The genus Bacillus and Ccci contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the
srtains of Bacillus and Cocci. Cellulases are the single class of enzymes which play an important part in the metabolism of almost all organisms (Plants, Animals, Fungi, Bacteria and Viruses). Investigation of cellulases is a central issue in enzymology due to their wide applications in pharmaceutical, food, agricultural products and bioremediation process [12]. In this study an attempt was made for the screening and isolation of cellulases producing bacteria from dump yards of vegetable wastes in various regions of Bangalore.

**MATERIALS AND METHODS**

**Collection and isolation of sample**

Samples were collected from dump yards of vegetable wastes at Solddevanahalli, Chikkabanavara, Devasandra, K.R.puram, Tannary road and Yashwanthpur in and around Bangalore, Karnataka, India. The samples were labeled after collected. These were spread onto isolation media Carboxy Methyl Cellulose (CMC) agar medium and incubated at 37°C for 24 hours after serial dilution of 10⁻¹ to 10⁻⁶.

**Table 1: Tabulation for samples description**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>DESIGNATION OF SAMPLE</th>
<th>SAMPLE COLLECTED AREA</th>
<th>SAMPLE COLLECTED LAND MARK</th>
<th>SAMPLE NATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AP-1</td>
<td>Shivaji Nagar</td>
<td>Opposite to Maszid vegetable center</td>
<td>Semisolid sticky Seems to Brown in colour</td>
</tr>
<tr>
<td>2</td>
<td>AP -2</td>
<td>Tannery Road</td>
<td>Near to Bus stop vegetable center</td>
<td>Semisolid Seems to Black in colour</td>
</tr>
<tr>
<td>3</td>
<td>AP -3</td>
<td>Tannery Road</td>
<td>Opposite to Bus stop vegetable Center</td>
<td>Semisolid Seems to Brown in colour</td>
</tr>
<tr>
<td>4</td>
<td>AP-4</td>
<td>Tannery Road</td>
<td>Slaughter house opposite vegetable center</td>
<td>Hard consist clay seems to Brown in colour</td>
</tr>
<tr>
<td>5</td>
<td>AP -5</td>
<td>Solddevanahalli</td>
<td>Near to Bus stop vegetable Center</td>
<td>Semisolid Seems to Brown in colour</td>
</tr>
<tr>
<td>6</td>
<td>AP -6</td>
<td>Chikka Banavara</td>
<td>Near to Bus stop vegetable Center</td>
<td>Sticky consist clay seems to Brick red in colour</td>
</tr>
<tr>
<td>7</td>
<td>AP -7</td>
<td>K.R.Puram</td>
<td>Devasandra vegetable dump</td>
<td>Semisolid Seems to red in colour</td>
</tr>
<tr>
<td>8</td>
<td>AP -8</td>
<td>Tin Factory</td>
<td>Opposite to</td>
<td>Semisolid Seems to</td>
</tr>
</tbody>
</table>
Screening of Cellulase production by plate assay

The isolates were screened for cellulase activity. This was done by inoculating the organisms on the Carboxy Methyl Cellulose (CMC) agar medium plates containing 1gm Carboxy Methyl Cellulose, 0.02gmFeSO₄, 0.3gm K₂HPO₄, 0.01 gm MgSO₄.7H₂O, 0.04gmCaCl₂ and 2.5 gm agar in 100 ml, the initial pH of medium was adjusted to 7 and incubated at 37°C for 24 h. The plates were flooded with 0.1% Congo red for 15 to 20 min, washed with 1 M NaCl for 15-20 min, and incubated for 15 min at 37°C. A clear zone around the growth of the bacteria was indicated to cellulase activity \(^{[13-20]}\).

Table 2: Tabulation for results of colony characteristics which shows cellulase activity

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Colony Surface</th>
<th>Colony Colour</th>
<th>Visual Characteristics</th>
<th>Shape Of The Colony</th>
<th>Height Of The Colony</th>
<th>Cellulase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>Smooth</td>
<td>Brown</td>
<td>Opaque</td>
<td>Irregular</td>
<td>Raised</td>
<td>Positive</td>
</tr>
<tr>
<td>G-2</td>
<td>Smooth</td>
<td>Off white</td>
<td>Translucent</td>
<td>Circular</td>
<td>Raised</td>
<td>Positive</td>
</tr>
<tr>
<td>G-3</td>
<td>Smooth</td>
<td>Brown</td>
<td>Translucent</td>
<td>Irregular</td>
<td>Flat</td>
<td>Positive</td>
</tr>
<tr>
<td>G-4</td>
<td>Smooth</td>
<td>Off white</td>
<td>Opaque</td>
<td>Irregular</td>
<td>Raised</td>
<td>Positive</td>
</tr>
<tr>
<td>G-5</td>
<td>Smooth</td>
<td>Brown</td>
<td>Opaque</td>
<td>Irregular</td>
<td>Raised</td>
<td>Positive</td>
</tr>
<tr>
<td>G-6</td>
<td>Smooth</td>
<td>Brown</td>
<td>Translucent</td>
<td>Irregular</td>
<td>Flat</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, grams staining, endospore staining, capsule staining and biochemical tests \[21\].

Table 3: Tabulation for results of staining techniques

<table>
<thead>
<tr>
<th>STRAIN NO.</th>
<th>GRAM STAINING</th>
<th>MORPHOLOGY (BACILUS/COC CI)</th>
<th>ENDOSPORE STAINING</th>
<th>CAPSULE STAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>Positive</td>
<td>Rods</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>G-2</td>
<td>Positive</td>
<td>Rods</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>G-3</td>
<td>Positive</td>
<td>CoccI</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>G-4</td>
<td>Positive</td>
<td>Rods</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>G-5</td>
<td>Positive</td>
<td>Rods</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>G-6</td>
<td>Positive</td>
<td>CoccI</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 4: Tabulation for results of Various Biochemical tests

<table>
<thead>
<tr>
<th>S.No.</th>
<th>SAMPLES</th>
<th>INDOLE</th>
<th>MR</th>
<th>VP</th>
<th>AMYLASE</th>
<th>NITRATE</th>
<th>OXIDASE</th>
<th>CATALASE</th>
<th>UREASE</th>
<th>GELATINAS</th>
<th>CELLULASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G-1</td>
<td>+Ve</td>
<td>-Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
<tr>
<td>2</td>
<td>G-2</td>
<td>-Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
<tr>
<td>3</td>
<td>G-3</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
<tr>
<td>4</td>
<td>G-4</td>
<td>-Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
<tr>
<td>5</td>
<td>G-5</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
<tr>
<td>6</td>
<td>G-6</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
<td>+Ve</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION
Six bacterial isolates were obtained (Table: 3) from soil samples of AP 1 to AP 10 (Table: 1), identified morphologically and biochemically as *Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus*. The colonies were subjected to grams staining, capsule staining and endospore staining. The colonies which were positive and negative for grams staining, capsule and endospore staining were considered for further studies (Table 3&4). The selected colonies were streaked on Carboxy Methyl Cellulose (CMC) agar medium plates. The plates were subjected to incubation for a period of 24 hours at 37° C. The plates which showed clear zone around the streaked area of test organisms were selected as cellulase producing strains. The organisms named (Table2) showed the inhibition zone and were subjected to various biochemical tests (Table4). G isolates (Table2) showed the following results for the biochemical tests. These were positive for Methyl red test, Starch hydrolysis, Citrate utilization test, oxidase test, gelatin hydrolysis test, urease test and nitrate reduction test, and few isolates were shows negative for Voges Paskauer test, Indole test and Catalase test. After biochemical tests these organisms were confirmed to belong to the *Bacillus* and *Cocci* species (*Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus*) producing cellulase.

CONCLUSION
The search for promising strains of cellulase producers is a continuous process. The isolates which show higher cellulase activity were selected for biochemical characterization and identification. The organisms were identified as *Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus*. On the basis of data obtained in the present work it can be concluded that *Bacillus* and *cocci* species isolates can be employed in the production of cellulase.

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REFERENCES


