CHARACTERIZATION OF ACTINOMYCETES FOR SOME INDUSTRIALLY IMPORTANT ENZYMES

Vyawahare S. S.¹, Kamble K. D.²*, Waghmare V.D.³ and Kamble L.H⁴.

¹-²Department of Microbiology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.
⁴ School of Life Sciences Swami Ramanand Teerth Marathwada University, Nanded.
(Email: kapilkamble@live.in)

ABSTRACT
Actinomycetes are well known for their ability to produce antibiotic. The role of actinomycetes in organic compound degradation is also considerable. However the potential of this group for enzyme production is not given much attention. In present study we have characterized actinomycetes from various soil samples. We collected soil samples from various areas of Amravati. Systematic screening methods were employed for the isolation of actinomycetes. A characteristic feature of actinomycetes on agarized media is formation of white powdered colonies. On this basis preliminary selection was done for actinomycetes. Predominance of Streptomyces species was observed. Though the genera are similar varied species are reported on the basis of biochemical characteristics. Around ten different species of actinomycetes are observed. A comparative study of the enzyme was carried with respect to three important enzymes viz. amylase, deoxyribonuclease (DNase) and L-asparaginase. Regarding enzyme production it was observed that most species could produce amylase. DNase and L-asparaginase were produced by very few species. The enzymes produced by these species are also abundant. As with the case of other prokaryotic cells, purification of enzymes from these group is also expected to be easier.

KEY WORDS: actinomycete, enzyme production , Soil samples,

INTRODUCTION
Actinomycetes are aerobic gram positive bacteria which predominate the soil. The variation of the species in the soil is because of the nature and conditions of the soil (Arifuzzaman et al, 2010). This group resembles the fungi being filamentous. The name ‘Actinomycetes’ was derived from Greek ‘aktis’ (a ray) and ‘mykes’ (fungus) and given to these organisms form initial observation of their morphology. These group is also helpful in degradation of organic compounds and synthesis of bioactive compound as well (Naikpatil and Rathod, 2011). Apart from antibiotic production the ability of actinomycetes in sludge digestion is taken quite seriously. These group causes the foaming of the sludge thereby enhancing the digestion of the sludge (Davenport et al, 2000). Similar studies were carried by Madoni et al, (2000) in Italy dealing with foaming and bulking. The foam production and stability was also carried by Heard et al, 2008.

Because of their ability for degradation of various organic compounds viz. carbohydrates, proteins and aromatic compounds they have been used quite efficiently in the treatment of waste matter (Lemmer and Kroppenstedt, 1984; Lemmer, 1986). The first antibiotic of actinomycetes origin was streptomycin produced by Streptomyces griseus. More than 12,000 antibiotics have been discovered in the last 55 years of which actinomycetes constitutes 70% and 30% is by fungi and other microorganisms (Nanjwade et al, 2010). Various microorganisms capable of producing antibiotics includes filamentous fungi and the prokaryotic actinomycetes e.g., Amycolatopsis, Nocardia and Streptomyces are reported (Wezel et al, 2006). Organo pesticides are the major problem these days because these are used frequently and carelessly. The bioaccumulation is causing various health hazards. However some actinomycetes are found to degrade these pesticides quite efficiently. The study was initiated in Argentina by Fuentes (et al,2010). The pesticides studied were chlordane, lindane or methoxychlor. Indeed actinomycetes has wide range of application many fields, however the ability of actinomycetes for various enzyme production is given less concern which is focus of our study. In this study we have characterized actinomycetes from various soil samples and studied the potential of the same for industrially important enzymes like DNase, L-asparaginase and amylase.

MATERIALS AND METHODS
Collection and preparation of soil sample
Top 4 cm soil is considered a good source of microorganisms as most activities takes place in this region. Samples were collected from garden soil in Amravati University area. Soil sample (approx. 10g) were collected using clean, dry and sterile polythene bags along with sterile spatula and were marked properly. Varied soil with regards to moisture, texture...
and content was selected. Samples were stored at 40°C until pretreatment. Microorganisms other than actinomycetes are degraded because of pretreatment (Arifuzzaman et al., 2010).

**Phenol treatment**

One gram soil was added in 10 ml sterile distilled water and was kept for 10 minutes. To this preparation an equal volume of 1.4% phenol solution was added. After 10 minute the supernatant was serially diluted and was used for further screening (El-Nakeeb and Lechevalier, 1963).

**Isolation of Actinomycetes**

The pretreated soil suspensions were spread over starch casein agar followed by incubation at 35°C up to 5 days. Dilution 10^{-7} gave well isolated white powdery growth on this agar surface a characteristic feature of actinomycetes (Reddy et al., 2011).

**Identification of actinomycetes**

Identification of isolates was based on cultural, morphological and biochemical characteristics. Motility has been performed according to the hanging drop method. The standard biochemical tests such as catalase, oxidase and fermentation various sugars, methyl red reaction, Voges Proskauer test and citrate utilization on Simmon’s citrate agar was performed. Further the enzymatic studies of these isolates have been studied.

**Enzymatic study of isolates**

The screened isolates were studied for the production of enzyme such as amylase, DNase and L-asparaginase respectively.

**L-asparaginase production**

A mineral base agar containing glucose as carbon source and L-asparagine as nitrogen source with phenol red as indicator system was used. The liberated ammonia after L-asparagine break down leads to change in color from yellow to pink around the colony. Pink coloration around the colony was noted (Prakasham et al., 2007).

**Amylase Production Test**

Starch Agar Medium containing soluble starch as carbon source was prepared and by the method of Marasabessy et al., (2011). The activity of amylase was studied by flooding the plates with iodine.

**DNase Production Test**

The isolates were spot inoculated on media supplemented with 0.2% DNA and indicator system as toluidine blue. The decolorization of DNA from blue to colorless around the colony was noted (Schreier, 1969).

**RESULTS AND DISCUSSION**

**Isolation of Actinomycetes**

Tedious screening procedures were adopted for large number of soil samples. Finally obtained cultures were designated Act-1 to Act-10.

Most colonies were either white or off white and size ranged from 3 to 4.5mm. Colonies on starch casein agar were irregular with filamentous margin rarely margin was wavy. Elevation was raised, in few cases it was convex and flat (Table 1; Figure 1).

**Microscopic studies and carbon sources utilization:**

The peculiar properties of actinomycetes is that these bacteria are filamentous and gram positive. All bacteria were non-motile and spore forming. All strains were found to be oxidase and catalase positive. All strains utilized arabinose and citrate as carbon source. Cultures were negative for indole and VP test. Lactose and sorbitol was not fermented. Culture designated Act-8 was found to utilize maltose and raffinose but rest did not. Dextrose and trehalose was hydrolyzed by Act-6 and Act-8 only. Methyl red test was found to be positive except Act-4 and Act-8. Salicin was fermented by Act-2, Act-6 and Act-10 whereas mannitol was fermented by Act-8 only (Table 1).
Enzymatic studies of Actinomycetes:

Figure - 1. Photographic plate1 white powdery colony of actinomycetes

Figure - 2, 3 and 4 showing enzymatic activity of DNase, amylase and L-asparaginase respectively
Cultures further were studied for production of three important enzymes viz. L-asparaginase, DNase and amylase. Both L-asparaginase and DNase are therapeutic enzymes. DNases are employed in genetic engineering processes also along with some industrial applications as well. Amylases are universally popular for use in textile and other industries. Therefore these enzymes were selected. Cultures designated Act-1, Act-2 and Act-3 were found to produce amylase only and other enzymes could not. Act-4, Act-7 and Act-8 were able to produce all three enzymes selected. Act-5 and Act-6 could produce amylase and DNase but could not produce L-asparaginase. Act-9 could only produce amylase whereas Act-10 could only produce DNase. The test was performed by spot inoculation though it was qualitative zones of enzymatic hydrolysis were measured which showed that largest amylase activity was found in case of Act-6. Act-10 was most efficient for DNase activity whereas largest L-asparaginase activity was found in case of Act-8 (Table 2 and photographic plate 2, 3 and 4).

<table>
<thead>
<tr>
<th>Culture designation</th>
<th>Cultural characteristics</th>
<th>Morphologic al properties</th>
<th>Biochemical characteristics</th>
<th>Identified Bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Act-1</td>
<td>White</td>
<td>3 mm</td>
<td>Filamentous</td>
<td>Irregular</td>
</tr>
<tr>
<td>Act-2</td>
<td>White</td>
<td>4.5 mm</td>
<td>Irregular</td>
<td>Wavy</td>
</tr>
<tr>
<td>Act-3</td>
<td>White</td>
<td>4 mm</td>
<td>Irregular</td>
<td>Wavy</td>
</tr>
<tr>
<td>Act-4</td>
<td>Off-white</td>
<td>3.5 mm</td>
<td>Filamentous</td>
<td>Filamentous</td>
</tr>
<tr>
<td>Act-5</td>
<td>Off-white</td>
<td>3 mm</td>
<td>Filamentous</td>
<td>Filamentous</td>
</tr>
<tr>
<td>Act-6</td>
<td>Off-white</td>
<td>2.5 mm</td>
<td>Filamentous</td>
<td>Filamentous</td>
</tr>
<tr>
<td>Act-7</td>
<td>White</td>
<td>3 mm</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Act-8</td>
<td>White</td>
<td>3 mm</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Act-9</td>
<td>White</td>
<td>3 mm</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Act-10</td>
<td>White</td>
<td>3 mm</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
</tbody>
</table>

Table 1: Characterization of Actinomycetes isolated from the soil.
Table 2. Comparative study of enzymatic activities.

<table>
<thead>
<tr>
<th>Culture designated</th>
<th>Zone of Enzymatic Hydrolysis (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amylase</td>
</tr>
<tr>
<td>Act-1</td>
<td>14</td>
</tr>
<tr>
<td>Act-2</td>
<td>16</td>
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<td>Act-3</td>
<td>20</td>
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<td>Act-4</td>
<td>25</td>
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<td>Act-5</td>
<td>15</td>
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<td>Act-6</td>
<td>28</td>
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<td>Act-7</td>
<td>22</td>
</tr>
<tr>
<td>Act-8</td>
<td>22</td>
</tr>
<tr>
<td>Act-9</td>
<td>18</td>
</tr>
<tr>
<td>Act-10</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION

In conclusion actinomycetes are well known for antibiotic productions but other applications are given less concern. In the present study we have isolated ten strains of actinomycetes from Amravati city in which \textit{Streptomyces} species are predominantly present in the soil. These strains are definitely preferable source for DNase, amylase and L-asparaginase which are quite important. Thus, these isolated strains of actinomycetes can be an economical source of industrial enzymes.

REFERENCES


