ISOLATION AND ENZYMATIC CHARACTERIZATION OF STREPTOMYCES ISOLATES FROM WESTERN RAJASTHAN


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Abstract
Twenty-one Streptomyces isolates were isolated and purified from soils of Jodhpur district. These molecularly well characterized isolates were tested for their biocatalytic potential of industrial important enzymes i.e. amylase, protease and lipase. The Qualitative and qualitative screening revealed that 8 isolates for amylase, 3 isolates for protease and 6 isolates for lipase are hitherto new producers of these enzymes. S. albogriseolus ITD-12 exhibited the maximum amylase enzyme potential while S. rochei ITD-5 exhibited the maximum protease and lipase production potential. S. rochei ITD-5 is recorded the best candidate for industrial application having the maximum potential of producing all the three enzymes tested. These Streptomyces isolates can be potentially used in fermentation, textile and paper industries.

Keywords: Enzyme, isolation, Streptomyces, Amylase, Protease, Lipase

Introduction
The Streptomyces is an important genus of Actinobacteria and have been extensively exploited for the production of secondary metabolites and enzymes of commercial significance (Narayana and Vijayalakshmi, 2009; Maleki et al. 2013). It represents one of the largest taxonomic units of identified Actinomycetes with more than 550 species (Euzeyb, 2008; Kampfer, 2012) and characterized by aerobic, gram positive, chemo-organotrophic, non-acid fast, with of aerial, substrate mycelia bearing short chains of spores (Willey et al. 2008; Kekuda et al. 2010; Naine et al. 2011) with higher GC content (>70%) in their DNA (Ventura et al. 2007). Streptomyces are widely distributed in different nutritionally, biologically and physically complex soils and perform a broad range of metabolic processes and to produce an immense diversity of bioactive secondary metabolites including antimicrobial, anticancer, immunosuppressant and industrially important enzymes (Willey et al. 2008; Kekuda et al. 2010; Naine et al. 2011; Sajid et al. 2011).

Industrial enzymes are used in various detergents, textile, pulp and paper industries. Microbial enzymes are getting preference over the chemical catalysts in manufacturing chemicals, food, leather and pharmaceuticals in recent years. Microbial fermentation may encounter the market demand for industrial enzyme due to quick doubling potential of microbes (Kumar and Takagi, 1999). Amylase and protease play a vital role in various industrial applications and the demand of these enzymes is increasing day by day (Uyar and Baysal, 2004).

Lipases have wide range of enzymatic properties and are used in the processing of fat and oils, additives, detergents, cosmetics, paper manufacturing and pharmaceuticals (Hasan et al. 2006). Amylase is the most significant industrial enzymes (Cowan, 1996; Kirk et al. 2002) and has been used in many industries including food and beverages, textile, pharmaceutical, detergent and also in waste management (Aiyer, 2005). Protease is used in baking bread and manufacturing of crackers (Godfrey and West, 1996; Gupta et al. 2002; Norus, 2006; Binod et al. 2008). Cereal foods are treated with proteolytic enzymes to modify their proteins for better
processing, handling and increasing drying capacity (Leisola et al. 2002; Schafer et al. 2006; Poulsen and Buchholz, 2003). Similarly, lipase has emerged as the most significant industrial enzyme with vast array of applications in food and dairy, detergents, oleo-chemicals, agro-chemicals, pharmaceuticals, textiles, leather, tea processing, pulp, paper, polymer synthesis, biosensors, waste management and many others industries (Saxena et al. 1999, Saxena et al. 2005, Meghwanshi and Vashishtha, 2012).

Amylase, protease and lipases have been reported from different Streptomyces species harboring in different tropical and temperate regions (Niehaus et al. 1999; Kim et al. 2000b; Lee et al. 2001) but scanty literature sis available from hot arid and semi-arid regions of the western Rajasthan. The climate of arid zones is often characterized as hot and dry summers, sub-humid monsoon and cold dry winters. The high temperature, low relative humidity, high evaporation rate and scanty rainfall are major features of arid regions. The soils of these regions are generally deficient in nitrogen and organic matter (Rajasekar, 2015). These ecosystems are characterized by lack of moisture and nutrition and affect the survival and growth of microorganisms (Fita, 2015; Rajasekar, 2015). However, this region harbours a plethora of Streptomyces diversity ranging from halotolerant to thermo-tolerant species but their exploitation is limited to assess their true potential (Gaur et al. 2012; Sharma et al. 2013a & b; Tiwari et al. 2015). Therefore, the present study was undertaken to investigate the Streptomyces species of the western Rajasthan to assess the production potential of enzymes viz., amylase, protease and lipase.

**Materials and Methods**

**Survey and Collection of Soil Samples**

The soil samples were collected during year 2014 from different locations of Jodhpur district, Rajasthan, India (table 1) at 10-15 cm depth. The soil samples were collected from 10 randomly selected points from each location and mixed thoroughly to form a composite sample, brought to laboratory, sieved to get rid of large debris and kept in refrigerator at 4°C until analyzed.

**Isolation of Streptomyces**

One gram of each composite and sieved soil sample were suspended in 100 ml sterile distilled water and incubated in an orbital shaker at 28°C with shaking at 180 rpm for 1 hr. The mixtures were allowed to settle and then serial dilutions of the soil suspensions were prepared up to 10^-2. From each dilution an aliquot of 0.5 ml was inoculated in a Petri plate containing Actinomyces Isolates Agar (AIA) media in three replications for the isolation of Streptomyces by the dilution plate technique (Seong et al. 2005). The inoculated Petri plates were incubated at 37°C in a BOD incubator for 7 days. The Streptomyces colony forming units (CFU’s) were recorded and purified in Starch Casein Agar (SCA) medium and maintained 4°C in a refrigerator until used.

**Separation of Streptomyces isolates**

The morphological characteristics of spore bearing hyphae and spore chains of the isolates were examined using coverslip culture technique as described by Arifuzzaman et al. (2010). These characteristics were then compared with that of Streptomyces description from Bergay’s manual of Determinative Bacteriology (Holt et al. 1994). The isolates showing non-filamentous bacterial characteristics were separated from the true cultures on SCA medium and typical characteristics of Streptomyces validated.

**Authenticity of Streptomyces isolates**

Twenty one test isolates exhibiting typical characteristics of Streptomyces were previously characterized in our laboratory (Kumar et al. 2016) using PCR amplification and sequencing of 16S rRNA gene and GenBank accession numbers KJ438290-KJ438291 (ITD-5 and ITD-6) and KM215719- KM215737 (ITD-7 to ITD-25). These molecularly well authenticated isolates were used under present study for enzymatic profiling.

**Screening for enzymes production**

1. **Qualitative Screening**

The Streptomyces isolates were screened on the basis of their growth phase for the production of amylase, protease and lipase by inoculating them on Tributyrin agar (TbA) [composition per liter - Beef extract 3g, peptone 5g, tributyrin 15 ml, agar 20g, pH 7.2 ± 0.2],(Skimmed milk agar (SkMA) [composition per liter - Skim milk powder 20g casein enzyme hydrolysate 5g, yeast extract 2.5g, dextrose 1g agar 15g, pH 7.0 ± 0.2] and Starch agar (StA) [composition per liter - Beef extract 3g, peptic digest of animal tissue 5g, soluble starch 2g, agar 15g, pH 7.2 ± 0.1], respectively on to culture plates. Himedia brand readymade culture media were used for the enzymatic assays. The inoculated plates were incubated at 37°C for 24 h and were observed for the production of the enzymes in the form of clear halo zone around the bacterial growth.

2. **Quantitative Screening**

The microbial isolates found positive for test enzyme production were subjected to qualitative screening of the
three enzymes amylase, protease and lipase. The microbial isolates positive for enzyme production were inoculated onto nutrient broth (NB) [Composition composition per liter-Peptone 10g meat extract, 10g, NaCl 5g, pH 7.3±0.1] tubes and were incubated at 37°C for 12 h under shaking conditions.

Two per cent of 12 h old seed culture of *Streptomyces* isolate was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of amylase (composition per liter : Beef extract 3g, peptic digest of animal tissue 5g, soluble starch 2g, pH 7.2 ± 0.1) or protease (composition per liter- Skim milk powder 20g, casein enzyme hydrolysate 5g, yeast extract 2.5g, dextrose 1g, pH 7.0 ± 0.2)or lipase (composition per liter:Peptone 10g, yeast extract 5g, sodium sulphate 2g, K₂HPO₄ 3g, KH₂PO₄ 1g, MgSO₄·7H₂O 0.1g, glucose 2g, olive oil 10 ml, pH 7.0 ± 0.2) production medium. The inoculated flasks were incubated at 37°C and shaking of 150 rpm for 48 h.

The cells of each culture were removed from respective fermentation broth by centrifugation at 10,000 rpm for 10 minutes after 48 h of incubation and supernatants were collected and examined for enzyme activities. The enzyme activities were determined using two approaches viz., Gel diffusion assay and Quantitative enzyme assay.

**Gel diffusion assay**

The supernatants of each culture were inoculated in the wells bored in Tba (Tributyrin agar), SKMA (Skimmed milk agar) or Sta (Starch agar), depending on the expected enzyme produced by a particular culture based on the result of the qualitative screening.

**Quantitative assay**

The enzymatic activities of the test isolates for amylase, protease and lipase were determined by the methods suggested by Sudharhsan et al. (2007), Meyers and Ahearn (1977) and Winkler and Stuckmann (1979), respectively.

**Results and Discussion**

In the present study, 21 *Streptomyces* isolates isolated from the soil samples collected from Jodhpur district were assigned culture codes (ITD-5 to ITD-25). The results of qualitative screening shown in the form of gel diffusion assay clearly indicated the production of the three enzyme *viz.* amylase, protease and lipase by different *Streptomyces* strains. The sizes of hydrolytic zones are approximately in accordance with the quantitative production of these enzymes. Out of these 21 *Streptomyces* isolates, 10 isolates (ITD-5, 6, 7, 11, 16, 17, 20, 22, 24 and 25) produced the all three enzyme *i.e.* amylase, protease and lipase, whereas nine isolates (ITD-8, 9, 10, 12, 13, 15, 19, 21 and 23) produced amylase and protease, one isolate (ITD-14) showed only amylase activity while isolate ITD-18 had lipase activity (table 2).

Different strains belonging to the same *Streptomyces* species (ITD-10 & 18, ITD-12 & 25, 19 & 20) produced different enzymes while same enzymes produced by different strains of taxonomically identical species (ITD-5 & 6, ITD-8, 9 & 15, ITD-16 & 22). This inconsistency of biochemical enzymes assays indicates the strain specific enzyme production ability within *Streptomyces* species. Strain specific biochemical potential of metabolites production has been reported by other researchers (Krieg, 2005; Larsen et al. 2005; Mangamuri et al. 2016).

The 16S rRNA sequences polymorphism might occur at isolates level making it useful for phylogeny, evolution and biogeographically diversity studies (Rajwar and

**Table 1: Streptomyces isolates collected from different locations of Jodhpur district, Rajasthan.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Isolate</th>
<th>Name of isolate</th>
<th>Collection site</th>
<th>Soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ITD-5</td>
<td><em>Streptomyces rochei</em></td>
<td>University campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>2</td>
<td>ITD-6</td>
<td><em>S. rochei</em></td>
<td>AFRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>3</td>
<td>ITD-7</td>
<td><em>S. espinosus</em></td>
<td>AFRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>4</td>
<td>ITD-8</td>
<td><em>S. gancidicus</em></td>
<td>CAZRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>5</td>
<td>ITD-9</td>
<td><em>S. gancidicus</em></td>
<td>CAZRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>6</td>
<td>ITD-10</td>
<td><em>S. werraensis</em></td>
<td>CAZRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>7</td>
<td>ITD-11</td>
<td><em>Streptomyces sp.</em></td>
<td>CAZRI campus</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>8</td>
<td>ITD-12</td>
<td><em>S. albogriseolus</em></td>
<td>Soorsagar</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>9</td>
<td>ITD-13</td>
<td><em>S. variabilis</em></td>
<td>Soorsagar</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>10</td>
<td>ITD-14</td>
<td><em>S. enissocaesilis</em></td>
<td>Mandore</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>11</td>
<td>ITD-15</td>
<td><em>S. gancidicus</em></td>
<td>Mandore</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>12</td>
<td>ITD-16</td>
<td><em>S. griseorubens</em></td>
<td>Mandore</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>13</td>
<td>ITD-17</td>
<td><em>S. coelicolor</em></td>
<td>Kailana</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>14</td>
<td>ITD-18</td>
<td><em>S. werraensis</em></td>
<td>Kailana</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>15</td>
<td>ITD-19</td>
<td><em>S. cyaneus</em></td>
<td>Machiya Safari Park</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>16</td>
<td>ITD-20</td>
<td><em>S. cyaneus</em></td>
<td>Mathania</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>17</td>
<td>ITD-21</td>
<td><em>S. flavomaculatus</em></td>
<td>Tiwri</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>18</td>
<td>ITD-22</td>
<td><em>S. griseorubens</em></td>
<td>Tiwri</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>19</td>
<td>ITD-23</td>
<td><em>Streptomyces sp.</em></td>
<td>Tiwri</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>20</td>
<td>ITD-24</td>
<td><em>S. rubrogriseolus</em></td>
<td>Osian</td>
<td>Sandy</td>
</tr>
<tr>
<td>21</td>
<td>ITD-25</td>
<td><em>S. albogriseolus</em></td>
<td>Osian</td>
<td>Sandy</td>
</tr>
</tbody>
</table>
The amylase production activity was detected in all the Streptomyces isolates tested except S. werraensis (ITD-18). Similarly, protease production was observed with all the isolates except S. enissocaesilis (ITD-14) and S. werraensis (ITD-18) showing the prevalence of amylase and protease enzyme within the Streptomyces isolates of desert ecosystem of Rajasthan. The lipase production was observed in lesser isolates as compared to amylase and protease. It was present in ten out of 21 Streptomyces isolates (table 2). Among these S. espinosus, S. werraensis, S. albogriseolus, S. variabilis, S. enissocaesilis, S. griseorubens, S. flavomacrosporus and S. rubrogriseus have not been reported for amylase production earlier. The highest amount (208 U/ml) of amylase was produced by S. albogriseolus ITD-12 followed by S. flavomacrosporus ITD-21 (196 U/ml) and S. griseorubens ITD-22 (148 U/ml). These enzyme titer values are significant and show that they are potential candidates for industrial production of amylase. Out of 19 protease producers, three isolates S. espinosus, S. flavomacrosporus and S. rubrogriseus are new reports. Among these new producers the highest (135.3 U/ml) titer value for protease is from S. flavomacrosporus ITD-21, followed by S. espinosus ITD-5 (20.7 U/ml), S. rubrogriseus ITD-24 (12.4 U/ml). The highest protease production was observed from S. rochei ITD-5 (155.4 U/ml). There are six new producers of lipase viz. S. espinosus ITD-7, S. griseorubens ITD-22, S. cyaneus ITD-20, S. rubrogriseus ITD-24 and S. albogriseolus ITD-25. Among these the highest (8.6 U/ml) titre value for lipase is from S. espinosus ITD-7. However, among all the lipase producers, the highest (10.1 U/ml) titre value for lipase is recorded from S. rochei ITD5.

### Conclusion

In all, 21 molecularly characterized Streptomyces isolates were tested for their biocatalytic potential of industrial important enzymes viz., amylase, protease and lipase. Several Streptomyces isolates are hitherto unknown for production of these important enzymes and are being reported for the first time for their industrial potential. The findings are of significance to microbial biocatalytic potential.

### References


