Starch degrading amylolytic enzymes are of greater significance in biotechnological applications ranging from food, textile and paper industries (Pandey et al., 2000). Amylase is the name given to glycoside hydrolase enzymes that breakdown starch into maltose molecules and has been derived from several sources such as plants, animals and microbes. Amylases from fungal and bacterial sources have dominated in their application in industrial sector. A large number of them are available commercially and they have completely replaced chemical hydrolysis of starch processing industries. The major advantage of using microorganisms for production of amylase is due to their ability for easy manipulation to obtain enzymes of desired characteristics.

**MATERIAL AND METHODS**

**Collection of sample**

Spent mushroom substrate (SMS) samples for isolation of bacteria were collected in different sites of mushroom farms situated around Gandhigram Rural University, Gandhigram, Dindigul (DT), Tamil Nadu.

**Isolation of Bacterial SMS**

The SMS samples were collected in clean, sterilized Petri plates mixed thoroughly and subjected for bacterial isolation by standard dilution technique and spread plate method. Further the bacterial isolates were identified using morphological characteristics gram staining, motility test and biochemical tests (Cappucino et al., 2005)

**Starch hydrolysis assay**

Bacteria isolated from SMS were subjected for starch hydrolysis assay by plating the diluted samples on Lama et al., (1991) isolating medium and incubating the petri dishes at 35°C for 3 days. Composition of Lama et al., (1991) medium is KH₂PO₄, 3.10 g;
(NH₄)₂SO₄, 2.5 g; MgSO₄·7H₂O, 0.2 g; yeast extract 2.0 g; starch 5.0 g and distilled water to make final volume 1000 ml. pH 7.2. Pure line cultures of the bacterial flora isolated from SMS were established on nutrient agar. Starch hydrolysis assay (Case & Johnson, 1985) using gram’s iodine was performed with all the isolates to confirm their amylase production capability. The bacterial isolates that exhibited widest zone of clearance indicated their high amylase production capability was tested for extra cellular amylase production.

**Extra cellular production of amylase by the potential bacterial isolates of SMS**

The amylase producing potential of the isolates was carried out by inoculation 1.0% (0.1 OD) of isolate to Lama et al broth (1991) and incubated at 50°C for 120 hrs. After the production of enzyme, the cultures were centrifuged at 5300 rpm for 10min and the supernatant was tested for amylase assay.

Amylase assay was taken up using phosphate buffer (0.1m, pH 7.0); starch solution (1%) and dinitrosalicylic acid (DNSA) reagent and standard maltose. The reaction mixture contained 0.5ml of starch solution, 0.3ml of phosphate buffer and 0.2ml of enzyme. The control was run without adding any enzyme. The tubes were incubated at 50°C for 5minutes and later 3ml of DNSA was added to each tube. The tubes were immersed in boiling water bath for 15min and after cooling the absorbance were measured at 540 nm. By drawing the standard graph; the enzyme activity which represents the amount of maltose produced in the reaction mixture per ml per unit time was calculated.

**RESULTS**

Bacterial colonies of spent mushroom substrate samples were recorded as 86,110 at the 10⁻⁵, 10⁻⁶ dilutions with 178.18x10⁻⁵ CFU/ml. Seven different colonies were isolated and identified at genus level on the basis of biochemical tests (Table 1). The bacterial colonies isolated from SMS varied from even nature to opaque nature; their colour also varied from yellow, white to green. The bacterial isolates of SMS such as *Micrococcus* spp, *Bacillus* spp(B1), *Bacillus* spp (B2) and *Staphylococcus* spp were found to be gram positive and *Pseudomonas* spp.

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Organisms</th>
<th>Gram Stain</th>
<th>Motility test</th>
<th>MR Reaction</th>
<th>VP Reaction</th>
<th>Indole production</th>
<th>MR Reaction</th>
<th>Citrate test</th>
<th>Gelatin Liquefaction</th>
<th>Acid Gas production</th>
<th>Acid only, the broth has turned Yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>Micrococcus</em> spp</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td><em>Bacillus</em> spp (B1)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C</td>
<td><em>Bacillus</em> spp (B2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
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<td>A</td>
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<tr>
<td>D</td>
<td><em>Staphylococcus</em> spp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A</td>
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<td>E</td>
<td><em>Staphylococcus</em> spp</td>
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</tr>
<tr>
<td>F</td>
<td><em>E. coli</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td><em>Proteus</em> spp</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>-</td>
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</tr>
</tbody>
</table>
E. coli, Proteus spp as gram negative. Among the seven isolates Bacillus spp (B1), Pseudomonas spp, E. coli and Proteus spp were found to be motile and the rest of the species were nonmotile.

In starch hydrolysis assay, bacterial isolates such as Bacillus spp (B1), Pseudomonas spp, Bacillus spp (B2) exhibited widest zone of clearance indicating their high amylase producing efficiency.

High enzyme activity which is recorded from the amount of maltose production, was 245 µg/ml in Bacillus spp (B2), 214 µg/ml in Bacillus spp (B1) and 180 µg/ml in Pseudomonas spp.

**DISCUSSION**

Introduction of amylase for the commercial purpose represents a milestone in the food, pharmaceutical and textile industries (Rajnikanth & Ravi, 1998). Commercial mushroom compost that is inhabited by wide consortium of microbes would be a new novel source to isolate microbes (Sharma et al., 2006). In the present study also, spent mushroom substrate (SMS) was recorded as a potential source to isolate bacteria for exploring amylase production. Viji et al., (2002) reported a number of bacterial flora like B. subtilis, B. licheniformis, E. coli, Pseudomonas aeruginosa from SMS. In the present study among the seven bacterial isolates of SMS Bacillus spp (1 & 2) and Pseudomonas spp were recorded their efficiency to hydrolyse starch by producing amylase. Similar results stating the production of extra cellular amylase from Bacillus subtilis, B. licheniformis were recorded by Yoneda (1974). Sharma & Khosla (2002) have reported that there is a good correlation between amylase forming activity and tendency to lyse. The activation of autolytic enzymes near the cellwall supposedly takes place when growth and oxidative metabolism slows down during the stationary phase; apparently, this permits the liberation of extracellular amylase. This study shows that SMS can be explored as a new habitat for isolating potent enzyme producing bacterial strains.

**REFERENCES**