Fermentation of starch to ethanol by an amylolytic yeast

*Saccharomyces diasticus* SM-10

S Sharma, M Pandey & B Saharan

Department of Microbiology, CCS Haryana Agricultural University, Hisar 125 004, India

Received 4 December 2000; revised 8 August 2001

A total of fifteen yeast strains were isolated from natural sources including fruits, soil, molasses, honey and a variety of indigenous fermented foods. Screening of these strains for growth, ethanol production and glucoamylase activity led to selection of a yeast strain SM-10 identified as *S. diasticus* having maximum glucoamylase activity (80 units ml⁻¹) and ethanol production from starch (3.5%). Ethanol production from wheat flour was found to be 1.75% which could be increased to 5.2% after treatment of wheat flour with pepsin, diastase and glucoamylase.

There is much interest in the utilization of starch as an alternate source of energy because of its renewable nature and availability throughout the world in large quantities. There are a variety of products that can be obtained from starch biomass via hydrolysis, and ethanol is one of the largest volume of product that can be produced from biomass. Ethanol production from starch involves two steps: (a) hydrolysis of starch to glucose either by amylolytic enzymes or inorganic acids and (b) fermentation of glucose to ethanol by yeast. In this process the conversion of starch to glucose is an expensive and time consuming step. A system of simultaneous saccharification and fermentation has been attempted by use of mixed culture of two yeasts or mold and yeast. Certain amylolytic enzymes along with yeast have also been used for the production of ethanol from starch in a single step fermentation. The biggest drawback of using this system is that yeast, fungi and enzymes do not have similar optimum cultural or reaction conditions.

Common ethanol producing yeast such as *Saccharomyces cerevisiae*, *S. carlsbergensis*, *S. sake* or the bacterium *Zymomonas mobilis*, can not utilize starch because they lack the starch splitting enzymes. In the last few years, several amylolytic yeast species belonging to *Candida*, *Lipomyces*, *Saccharomycopsis*, *Schwanniomycys*, *Filobasidium* and *Trichosporon* have been isolated and studied extensively for direct conversion of starchy substrates to ethanol. This is of considerable importance as direct starch fermentation does not require liquefaction and saccharification. Considering these aspects, yeast strains were isolated from natural sources having fermentative as well as amylolytic activity for production of ethanol from starch (Wheat flour).

**Materials and Methods**

**Yeast cultures**—In the present investigation 15 amylolytic yeast cultures (SM-1 to SM-15) isolated from natural sources were included.

**Media, enzymes and fermentation discs**—Cultures were grown at 30°C on starch, yeast extract and peptone (SYP) medium slants containing 2% peptone, 1% yeast extract, 2% agar. Pepsin (Sisco Laboratories), bacteriological differentiation discs and diastase (Hi-media) and glucoamylase (Kedia castle Industries, Durg) were used as and when required in the experiments.

**Isolation of yeast strains from various sources**—Samples of fruits, molasses, stored honey, soil and fermented foods were inoculated in SYP broth for enrichment at 30°C for 24 hr. The enriched samples were streaked on SYP medium plates and incubated at 30°C for 48 hr. The yeast like colonies appeared and these were purified by further streaking on SYP medium plates and finally transferred on SYP slopes in tubes. A total of 31 samples were plated initially out of which 15 samples showed yeast like colonies.

**Ethanol production by yeast strains**—Ethanol production by isolated strains was determined by standard procedures. Equal biomass (0.5g wet weight per 100 ml) was inoculated in sterilized dextrose fermentation medium (DFM) containing 15% dextrose, 0.5% yeast extract, 0.5% peptone (pH 5.0). Fermentation was carried out at 30°C and ethanol produced was estimated. To study ethanol production from starch,
cultures were grown in starch, yeast extract and peptone medium (SYP) containing 2% starch and incubated on rotary shaker at 30°C. After 24 hr equal number of cells $2 \times 10^6$ were inoculated into 100 ml starch fermentation medium (SFM) containing starch 10%, peptone 0.5% and yeast extract 0.5% (pH 5.0). Fermentation was carried out at 30°C and samples were analysed for ethanol content. Ethanol was estimated spectrophotometrically after distillation of the samples (Fig. 1).

**Estimation of glucoamylase activity**—The glucoamylase activity was estimated by the method of Lemmel et al. Cell suspension with an optical density 0.5-0.7 at 610 nm was inoculated into SYP medium containing 2% starch and incubated at 30°C on a rotary shaker. Cell free culture supernatant obtained by centrifugation was used for measuring extra-cellular amylolytic activity by incubating 0.5 ml culture supernatant with 0.5 ml of 1% soluble starch (merck) dissolved in 0.1 M acetate buffer (pH 4.8) at 30°C in a water bath for 3 min to convert starch to sugar which was estimated by DNSA reagent. One unit of amylolytic activity is defined as the amount of enzyme which release 1 μM (0.18 mg) of glucose from starch in 3 min.

**Ethanol production from wheat flour**—The ethanol production from wheat flour was carried out after gelatinization and pretreatment of wheat starch. Gelatinization of wheat starch was done by open cooking method. Wheat flour slurry (25%) was prepared and pH was adjusted to 5.0. It was cooked by boiling for 5 min on magnetic stirrer with continuous agitation and cooled down to 40°C. Pretreatment of wheat starch was carried out with 13.5 mg (40 units) pepsin and 1.3 mg (1690 units) diastase per 12.5 g of wheat flour at 40°C for 2 hr. The pretreated wheat starch was hydrolysed with 2 ml glucoamylase (600 AMG units) at 40°C for 4hr. The pretreated hydrolysed wheat starch was fermented by inoculating with the yeast culture at 15% which was found to be optimum. Fermentation was carried out at 30°C in BOD incubator and ethanol produced was estimated.

**Identification of yeast**—Identification of yeast strains was based on morphological, physiological and biochemical tests by standard procedures.

### Results and Discussion

**Yeast isolates**—Out of 31 samples used for enrichment and isolation, yeast strains were obtained from 15 samples and given code numbers from SM-1 to SM-15 (Table 1). Further selection was based on efficient starch fermenting ability and high glucoamylase activity.

**Growth, glucoamylase activity and ethanol production of different strains in SYP broth**—The growth of different yeast strains was studied by inoculating equal biomass into 100 ml of SYP broth containing 2% starch. Growth was estimated in terms of O.D. after incubation at 30°C under shaking conditions. It

<table>
<thead>
<tr>
<th>Sources</th>
<th>No. of samples</th>
<th>Samples showing growth</th>
<th>Code No. of strain</th>
<th>Colony morphology</th>
<th>Microscopic morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>4</td>
<td>2</td>
<td>SM-1, SM-2</td>
<td>White</td>
<td>Round cells</td>
</tr>
<tr>
<td>Honey</td>
<td>2</td>
<td>1</td>
<td>SM-3</td>
<td>Creamy</td>
<td>Oval cells</td>
</tr>
<tr>
<td>Molasses</td>
<td>2</td>
<td>2</td>
<td>SM-4, SM-15</td>
<td>White, Creamy</td>
<td>Round cells</td>
</tr>
<tr>
<td>Bakery dough</td>
<td>2</td>
<td>2</td>
<td>SM-5, SM-6</td>
<td>Creamy, White</td>
<td>Round cells</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>2</td>
<td>2</td>
<td>SM-8, SM-13</td>
<td>White, Creamy</td>
<td>Round cells</td>
</tr>
<tr>
<td>Rice flour dough</td>
<td>2</td>
<td>2</td>
<td>SM-7, SM-14</td>
<td>White</td>
<td>Round cells</td>
</tr>
<tr>
<td>Fruits</td>
<td>4</td>
<td>2</td>
<td>SM-9, SM-10</td>
<td>White</td>
<td>Round cells</td>
</tr>
<tr>
<td>Soil</td>
<td>4</td>
<td>2</td>
<td>SM-11, SM-12</td>
<td>White, Creamy</td>
<td>Round cells</td>
</tr>
<tr>
<td>Cereal based fermented food</td>
<td>7</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
was found that different yeast strains differed with respect to their growth in SYP medium (Table 2). Better growth in terms of O.D. in the range of 2.300 to 2.900 was observed in case of strains SM-3, SM-10 and SM-14 only. In comparison, strains SM-1, SM-8, SM-15 attained a maximum O.D. only in the range of 0.62-0.90 under similar conditions. Very poor growth was seen in rest of the strains.

All the fifteen strains (SM-1 to SM-15) were screened for glucoamylase activity. Only three strains SM-3, SM-10 and SM-14 exhibited higher glucoamylase activity of 49, 80 and 22 units ml⁻¹, respectively. In other strains very low glucoamylase activity (10 units ml⁻¹ or less) was observed (Table 2).

Ethanol production by different yeast strains when tested in SFM medium containing 10% starch with equal inocula, strain SM-10 produced maximum amount of 3.5% ethanol after 72 hr while strains SM-3 and SM-14 produced only 1.5% and 2.5% under similar conditions. Other strains were found to produce less than 1% ethanol.

The amount of ethanol produced by different yeast strains in fermentation medium containing 15% dextrose was studied by inoculating equal biomass @ 0.5g per 100 ml (wet weight). Three strains SM-3, SM-10 and SM-14 were able to produce a maximum of 5.0, 6.5 and 6.0% ethanol, respectively after 72 hr which was much higher than the amount of ethanol produced by these strains in starch medium. However, yeast strain SM-10 was found to be better with respect to growth in starch, glucoamylase activity and ethanol production from starch as well as dextrose (Table 2). Hence this strain was used further for ethanol production from wheat flour.

Ethanol production by yeast strain SM-10 from wheat flour—Wheat flour slurry obtained after gelatinization by boiling for 5 min on a magnetic stirrer was inoculated at different inoculum levels @ 15, 30 and 45%. Fermentation was carried out at 30°C and amount of ethanol produced was estimated. It was found that yeast strain SM-10 produced only 1.75% ethanol using 15% inoculum which was increased to 2.25% by using inoculum at the level of 45% (Table 3).

Ethanol production by yeast strain SM-10 from wheat flour using commercial enzymes—Wheat flour hydrolysate was treated with different concentrations of commercial enzymes pepsin, diastase and glucoamylase as shown in Fig. 1. Wheat flour hydrolysate was inoculated at the level of 15% with an actively growing yeast inoculum of SM-10. Fermentation was carried out at 30°C and ethanol produced was estimated. It was found that treatment of wheat hydrolysate with commercial enzymes increased the ethanol production.

| Table 3—Ethanol production by yeast strain SM-10 from wheat flour |
|---------------------|---------------------|---------------------|
| Inoculum (%)     | 24 hr   | 48 hr   | 72 hr   |
| 15            | 0.25 | 0.75 | 1.75 |
| 30           | 0.48 | 1.10 | 2.10 |
| 45           | 0.53 | 1.23 | 2.25 |

Growth, glucoamylase activity and ethanol production were estimated after 72 hr.
Identification of yeast strain SM-10—Various morphological, physiological and biochemical characteristics of yeast strain SM-10 confirm well with the characteristics of Saccharomyces diastaticus and hence this strain was identified as S. diastaticus SM-10 (Tables 5 and 6).

Thus, after a thorough screening of various amylolytic yeasts isolated from different sources an amylolytic yeast S. diastaticus SM-10 was obtained. This strain had the ability to assimilate and ferment starch as well as alcohol production from starch. Further work on standardization of conditions for high glucoamylase activity as well as characterization and purification of glucoamylase is continued.

References