Production of alcohol by yeast isolated from apple, orange and banana

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The purpose of this study was to isolate wild yeast strains present in different fruits (apple, orange and banana) and to determine the yeast growth and the amount of alcohol production at various glucose concentrations. Three fruits namely apple, banana and orange were selected as natural sources for yeast isolation. Medium used for isolation of yeast from fruits was consisting of 50 glucose, 3 malt extract, 3 yeast extract, 5 peptone and 15g/L agar. For fermentation MGYP medium used with different glucose concentrations of 5, 10, 30, 50 and 70g/L. Inocula were prepared by loop transfers from stock slants to 50 mL of the 20 percent medium in 500-mL Erlenmeyer flasks. The results showed that with higher concentration of glucose (30g/L) higher amount of alcohol (0.22g/100mL) was produced. Similarly, the yeast isolated from apple showed maximum yeast biomass (0.38g/100mL) at 70g/L glucose concentration and minimum on 50g/L (0.03g/100mL) glucose concentration. In conclusion, the wild yeast produce higher ethanol amount in case of banana fruits.

Keywords: Alcohol, fruit fermentation, yeast growth, glucose concentrations, malt extract, wild yeast

INTRODUCTION

Yeast is a unicellular eukaryotic fungus, very common in the environment and is mostly saprophytic. It has been classified as Ascomycetes or basidomycetes under fungi taxonomy Kreger-van, et al., 1984) and there are about 1500 species of yeast (Kurtzman, et al., 2006, and Barnett, et al., 1990). The commercial importance of strains of yeast species Saccharomyces cerevisiae has made it a model organism of study in both research and industrial applications (Legras, et al., 2007). Fermenting wild yeast species are being isolated from the natural sources for over decades and is being used in various fermentation processes. Yeast has been isolated from variety of natural sources like leaves, flowers, fruits etc (Spencer, et al., 1997; Davenport, et al., 1980; Tourn, 2005; Li et al., 2008). Being a sugar-loving microorganism, it is usually isolated from sugar rich materials. Fruits contain high sugar concentration and hence yeast species are naturally present on these and can be easily isolated from fruits. Distinct wild yeast species are supposed to be present and associated with different fruits in natural environments (Spencer, et al., 1997). Because of yeast fermentative characteristic, there is always a need for yeast strains with better features of fermentation especially high ethanol tolerance for production of ethanol as bio fuel on commercial scale (Colin, et al., 2006).
Moreover, besides *S. cerevisiae*, there is always a search on for new wild/non toxic-fermentative yeast species for their further industrial exploitation in fermentation industry, baking industry, therapeutic production etc (Alvarenga, 2011; Legras, et al., 2007). The present study was intended to determine the potential of the waste of ripe banana, orange and apple fruits for alcohol production. The outcome of this study may expand the utility of banana, orange and apple wastes. That would not only ensure a cleaner environment but also create more job opportunities, reduce seasonal losses of the fruits and serve as a substitute for produced alcohol by increasing their production. This research study reports on ethanol production from the juices of apple, orange and banana.

MATERIALS AND METHODS

All chemicals used in the experiment were of highest purity and were obtained from Sigma Chemical Company (St. Louis, MO), Merck limited.

Yeast source, Isolation and medium

Fruit samples (banana, apple and orange) were obtained from local sources of Lahore Pakistan and mostly naturally decaying/fermented samples were preferred. Fruit sample 100g was taken in a sterile mortar and crushed to a fine paste by mixing with sterile water. Then mixture was kept for overnight at normal room temperature so that natural wild yeast present on fruit samples to grow. A loop full of liquid portion from each sample was streaked (Quaternary streaking) in plate (with replica) containing MGYP medium (yeast extract 3, malt extract 3, peptone 5, glucose 10 and agar 30g/L), pH 6.4 (phosphate buffer system) and incubated at 26°C for 2 days.

Optimization of Fermentation Medium

The fermentation media was optimized by varying the concentration of glucose (5, 10, 30, and 50g/L) as fermentation media were made up in approximately 100, 200, and 300 g/L glucose concentration and 5, 10, and 15 g/L yeast extract, respectively. Inocula were prepared by loop transfers from stock slants to 50 ml of the 20 per cent medium in 500-ml Erlenmeyer flasks. These were incubated at 30°C for 72h on a rotary shaker (250 rpm). Inoculum (3.5mL) was used with 50 ml of fermentation medium in 500-ml Erlenmeyer flasks. These were incubated at 35°C. Instead of closing the Erlenmeyer flasks with cotton plugs, a double disc was used to provide a more uniform material for gas passage. Analyses were made at 24h intervals required for the fermentation. Fermentation and incubation was continued until most of the glucose had been used.

Analytical methods:

Evaporation during the fermentation was variable depending to a large extent on aeration rate and length of fermentation. Before analyses were made, the fermentation broth was diluted to the original volume. The media of yeast cells was centrifuged. The supernatant was used for further analyses. Glucose was determined by the copper reduction method of Shaffer and Somogyi, (1933). Ethanol was determined by the Conway micro diffusion method as described by Neish (1952).

RESULTS

Yeast isolated from the various fruits (apple, orange and banana) were established in a pure culture on MGYP medium. After 48h, white to cream colored colonies appeared on agar plates. The colonies were smooth, shiny and flat with entire margin. Observation of the colonies under microscope showed that the obtained budding/ oblong shaped colonies were of yeast and belonged to the same morphological group. The cells were globes to ovoid, mostly sub globes, occurring singly
Table 1. Yeast fermentation (Apple, Orange and Banana biomass) with respect to glucose concentrations after 4 days.

<table>
<thead>
<tr>
<th>Glucose conc. (g/100mL)</th>
<th>Apple</th>
<th>Orange</th>
<th>Banana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight</td>
<td>Final weight</td>
<td>Initial weight</td>
</tr>
<tr>
<td></td>
<td>Yeast biomass</td>
<td>(g/100mL)</td>
<td>Yeast biomass</td>
</tr>
<tr>
<td>Control</td>
<td>0.11</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>1.16</td>
<td>0.06</td>
<td>3.29</td>
</tr>
<tr>
<td>1</td>
<td>1.17</td>
<td>0.19</td>
<td>0.95</td>
</tr>
<tr>
<td>3</td>
<td>1.20</td>
<td>0.10</td>
<td>2.68</td>
</tr>
<tr>
<td>5</td>
<td>0.72</td>
<td>0.03</td>
<td>1.66</td>
</tr>
<tr>
<td>7</td>
<td>2.05</td>
<td>0.38</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Table 2. Alcohol productions in Apple, Orange and Banana Samples

<table>
<thead>
<tr>
<th>Glucose conc. (g/100mL)</th>
<th>Apple</th>
<th>Orange</th>
<th>Banana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcohol produce</td>
<td>Alcohol produce</td>
<td>Alcohol produce</td>
</tr>
<tr>
<td></td>
<td>(g/100mL)</td>
<td>(g/100mL)</td>
<td>(g/100mL)</td>
</tr>
<tr>
<td>Control</td>
<td>0.001</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>0.5</td>
<td>0.069</td>
<td>0.072</td>
<td>0.042</td>
</tr>
<tr>
<td>1</td>
<td>0.074</td>
<td>0.088</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>0.082</td>
<td>0.091</td>
<td>0.031</td>
</tr>
<tr>
<td>5</td>
<td>0.086</td>
<td>0.035</td>
<td>0.133</td>
</tr>
<tr>
<td>7</td>
<td>0.412</td>
<td>0.362</td>
<td>0.252</td>
</tr>
</tbody>
</table>

or in pairs. Short, branched chains of cells were also identified. But pseudo hyphae and true hyphae were missing. Yeast cell biomass and alcohol were prepared from fruits wastes. Yeast isolated from fruits were than fermented by optimizing the medium (MGYP) by varying the glucose concentration (5, 10, 30, 50, 70g/l) in an incubating shaker at 30°C in 250mL flasks, after 72 h. The yeast isolated from apple showed maximum yeast biomass (0.38g/100mL) at 70g/L glucose concentration and minimum on 50g/L (0.03g/100mL) glucose concentration. The variation of yeast biomass at different glucose concentration was in Table 1. Similar results have led to the tentative application of orange fruit biomass production ranged from 70g/L glucose (0.77g/100mL) to 50g/L (0.03g/100mL) glucose concentration. The variation in ethanol yield obtained from fermentation of orange isolate was shown in table 2. Lastly, the alcohol estimation from banana isolate fermentation gave maximum ethanol yield (0.362g/100mL) at 70g/L of glucose concentration and 0.072g/100mL) at 50g/L of glucose concentration. The variation in ethanol yield obtained from fermentation of banana isolate was given in table 2.

DISCUSSION

Apple, orange, banana and other fruits locally available and thus serve as readily available raw materials for the separation of ethanol yeasts. Eghafona (1999) isolated various strains of indigenous yeasts capable of producing ethanol from local fermented pineapple juice. Bansal and Singh (2003) and Hossain et al (2014) did comparative study on ethanol production from molasses using Saccharomyces cerevisiae and Zymomonas mobilis.
Glucose with different amounts (50, 10, 30, 50, and 70g/l) was used as a sole sugar in the MGYP medium; the consequences showed that the maximum yeast biomass and maximum ethanol yield was obtained at high glucose concentration. Similar result was shown by Xin, et al. 2003. Viability of Saccharomyces spp also studied by Moaris (1996) and Aguilar (2010). In 50% glucose, reported a viability of 10-98.8% in different strains of yeast.

The proximate analysis of various fruits (Apple, orange and banana) was not determined in this study, but Essein et al (2005) recorded crude protein and crude fat contents of 78 and 116g/L, respectively in banana peels. Protein is an essential nutrient for yeast growth while fat is vital for the structure and biological functions of the cell and can be utilized as alternative source of energy by the cells.

The isolates exhibited high flocculation ability, and fermented higher amount of glucose to yield appreciable amount of ethanol. In present experiment, the alcohol estimation from banana isolate fermentation gave maximum ethanol yield (0.362g/100mL) at 70gL of glucose concentration and minimum yield (0.072g/100mL) at 50g/l of glucose concentration. Brooks (2008), isolated yeast strains from ripe banana peels for ethanol production and found, that isolates fermented 40% glucose at 30°C to yield 3.6 and 5.8% ethanol respectively.

Temperature is one of the major constrains that determines the ethanol production. In this study the experiment was carried out at 30°C with 70gL glucose concentration. The maximum amount of ethanol produced by yeast cells using 70gL glucose was 1.36g/100mL. Sree (2000) were also carried out fermentation with initial glucose concentration of 150, 200 and 250g /litre at 30°C the maximum amount of ethanol produced by yeast cells using 150,200 and 250g/L glucose was 72.5,83 and 93g ethanol per litre at 30°C after 48h. Similarly, Yadav et al (1997) also obtained high ethanol yield (40g/l/h) at 30°C. So present study was also carried out at 30°C in 250 mL conical flasks for 72h and obtained the highest ethanol yield (0.412g/100mL) at 7 percent glucose concentration which was the maximum concentration used in this experiment.

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REFERENCES


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