Abundance of lactose assimilating yeasts from Nepalese Murcha (Yeast cake)

Kiran Babu Tiwari1,2*, Manindra Lal Shrestha1, and Vishwanath Prasad Agrawal1,2

1Universal Science College, Pokhara University, Maitidevi, Kathmandu, Nepal
2Research Laboratory for Agricultural Biotechnology and Biochemistry, Maitidevi, Kathmandu, Nepal

Abstract

A study was conducted to elucidate the lactose assimilation by indigenous strains of yeasts in Nepal. A total of 31 strains were isolated from eight murcha (yeast cakes) samples from different localities of Nepal. A higher percentage of isolates (7/31, 22.58%) were found to possess lactose assimilating activity. Among the lactose assimilating isolates 57.14% (4/7) were able to assimilate galactose, a product of β-galactosidase reaction.

Introduction

Murcha is a mixed starter inoculum, used in production of local alcoholic beverages in India, Tibet, Nepal, Bhutan etc. (Tsuyoshi et al. 2005). Murcha is a round cake, which is mildly acidic and has a pH around 5.2 containing 13% w/w moisture and 0.7% w/w ash (dry weight basis). The Murcha cakes contain mixed microbial population viz. molds, yeasts and bacteria (Tamang and Sarkar 1995). Yeasts are the world's premier industrial microorganisms, which have wide exploitation in the production of foods, beverages and pharmaceuticals. The alcoholic beverages are one of the major products in the world's market. Yeasts can contaminate different dairy products because they have relatively low water activity (a_w, 0.88) (Frazier and Westhoff 1995), can easily grow at room temperature and can utilize (assimilate and/or ferment) a variety of carbohydrates (Nahvi and Moeini 2004), eg. pentoses, hexoses, disaccharides and, rarely polysaccharides (Barnett et al. 1990). The capacities of the organisms, thus, can be exploited to manage the biodegradable wastes of the food, dairy and beverage industries. Most of the yeasts, except Saccharomyces spp., can grow on cellulosic materials, however; only few genera are able to degrade starch. Among disaccharides, lactose is one of the most refractory carbon substrate to most of the yeasts. Among hundreds of genera, only few are lactose positive, viz. many Cryptococcus spp. and Trichosporon spp.; some Debaryomyces spp., Kluyveromyces spp. and Myxozyma spp.; and occasionally Bullera spp., Candida spp., Rhodotorula spp. and Tremella spp. (Barnett et al. 1990). Lactose is one of the major components in whey in cheese industry that is non-friendly in the environment (Nahvi and Moeini 2004). As they can grow at 25-30°C, yeasts can be exploited to manage lactose pollution (Sarova and Nikolova 2002).

Materials and Methods

Isolation: Murcha samples were collected from eight different localities in eastern- and mid- zone of Nepal. The pure culture of yeast strains were isolated by serial dilution methods in Potato Dextrose Agar (PDA) (20% potato extract, 2% dextrose, and 1.5% agar, pH 4.5) incubating for 48 hr at 27°C. The purified isolates were stored on PDA slant at 40°C. The pure culture of the isolates was Gram stained for microscopic morphology.

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*Corresponding author: Kiran Babu Tiwari, Research Scientist, Research Laboratory for Biotechnology and Biochemistry, email: kiranbabu.babukiran@gmail.com

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Sugar assimilation test: The sugars used were glucose, fructose, sucrose, mannitol, galactose, lactose, and arabinose using a basal medium (4.5gm yeast extract, 7.5gm peptone and 20gm sugar in 1-lit distilled water) with phenol red (1mg/ml) as indicator. The pH was adjusted to 7.0-8.0. The medium was dispensed into tubes and sterilized by autoclaving. The pure culture of the isolates was incubated at 27°C till 72 hr. in the tubes and the result was indicated by change of color from red to yellow (Shrestha and Sharma 1995). Control tubes were used in each set to monitor contamination.

Citrate utilization test: Citrate test was examined by using Simmon’s citrate agar (0.2gm Magnesium Sulphate, 0.2gm Ammonium dihydrogen phosphate, 0.8gm Sodium Ammonium phosphate, 2gm Sodium citrate, 5gm Sodium chloride, 0.05gm phenol red, 15gm Agar in 1 lit distilled water with pH 7.0). The medium was dispensed into tubes and sterilized. The yeast isolate was inoculated into these tubes and incubated at 27°C till 72 hr. (Shrestha and Sharma 1995). Control tubes were used in each set to monitor contamination.

Results

Among the 31 yeast strains isolated from the eight Murcha samples, seven (7/31, 22.58%) lactose assimilating strains (Fig. 1) comprised two (2/4) from Nagarkot, one (1/5) from Dhulikhel, two (2/8) from Morang and two (2/5) from Bhaktapur. Strains NS4, DS3, MS3 and MS6 possessed umbonate elevation, and NS2 and NS4 possessed creamy white color (Table 1). The biochemical characteristics of the lactose positive yeast isolates are shown in Table 2. All the lactose positive strains were able to assimilate glucose, sucrose and maltose (Fig. 2). Four (4/7, 57.14%) each were able to assimilate fructose and galactose. Three (3/7, 42.85%) were able to assimilate both fructose and galactose.

Table 1: Colonial morphologies of lactose positive yeast isolates (n=7)

<table>
<thead>
<tr>
<th>Strain</th>
<th>NS2</th>
<th>NS4</th>
<th>DS3</th>
<th>MS3</th>
<th>MS6</th>
<th>BS3</th>
<th>BS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Creamy white</td>
<td>Creamy white</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Margin</td>
<td>round</td>
<td>round</td>
<td>round</td>
<td>round</td>
<td>round</td>
<td>round</td>
<td>round</td>
</tr>
<tr>
<td>Elevation</td>
<td>raised</td>
<td>raised</td>
<td>umbonate</td>
<td>umbonate</td>
<td>umbonate</td>
<td>raised</td>
<td>raised</td>
</tr>
</tbody>
</table>

Table 2: Carbon assimilation tests of lactose positive yeast isolates (n=7)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Glu</th>
<th>Fru</th>
<th>Man</th>
<th>Gal</th>
<th>Ara</th>
<th>Suc</th>
<th>Mal</th>
<th>Starch</th>
<th>Cit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NS4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DS3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BS3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BS5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</table>
Yeasts are one of the important organisms having a wide industrial application. Most of the genera are unable to assimilate lactose as carbon source (Barnett et al. 1990). A search of lactose positive yeasts was conducted to elucidate a potential use of indigenous species in Nepal. A higher abundance of the yeasts capable to produce β-galactosidase and thus assimilate lactose explored the importance of Nepalese yeast cakes (Murcha). Nahvi and Moeini (2004) reported three (3/30, 10.0%) β-galactosidase positive strains in Iran. In a report, Sarova and Nikolova (2002) reported 66.67% (8/12) of strains capable to utilize lactose in Bulgaria. Among disaccharides, lactose is one of the most refractory carbon substrate to most of the yeasts. Barnett et al. (1990) mentioned 71 out of 590 compiles yeast species. The complete biochemical data showed that only few are lactose positive, viz. many Cryptococcus spp. and Trichosporon spp.; some Debaryomyces spp., Kluyveromyces spp. and Myxozyma spp.; and occasionally Bullera spp., Candida spp., Rhodotorula spp. and Tremella spp. (Barnett et al. 1990). Among lactose positive genera, almost of Bullera spp., Cryptococcus spp., Debaryomyces spp., Fellomyces spp. and Trychosporon spp. are starch positive. Cryptococcus spp. are inhabitants of birds drooping and are mostly associated with opportunistic infections in immunocompromised individuals. Most of the starch negative Candida spp., Hansenula spp., Mastigomyces spp., Mrakia spp. and Rhodotorula spp. are citrate positive. Out of 95 Pichia spp., only two viz. P. abadiaceae and P. mexicana are starch negative and sucrose positive, however, P.
*abadiaceae* is citrate positive. Among the *Myxozyma* spp., only *M. geophila* is citrate negative but sucrose negative. Most of the *Kluyveromyces* spp., *P mexicana* and *Leucosporidium lari-marini* correspond to the biochemical tests as shown in Table 2. One of the most industrially exploited yeast, *Saccharomyces* spp, is lactose negative, however, *Kluyveromyces* marxianus, a lactose variable species, was known previously as *Saccharomyces fragilis* (Lodder 1984).

In this study, many lactose positive strains were also found to be galactose (4/7, 57.14%) positive. Galactose negative lactose assimilating yeasts (3/7, 42.85%) in this study was found to be higher than documented by Barnett *et al.* (1990) (11/590, 1.86%). These strains can be used for removal of whey pollutants, Single cell protein (SCP) and ethanol production and treatment of lactose in dairy industry (Nahvi and Moeini 2004). Whey is the aqueous fraction of milk generated a by-product of cheese manufacturing which is produced in large amounts. The main solute in cheese whey is lactose present at a concentration of about 4.5-5% (Rohm *et al.* 1992, Zadow 1992). Because of its high organic content, dumping directly to the environment causes serious contamination problems. As a solution, bioconversion of whey into single cell protein SCP or ethanol has been performed in several countries (Gonzales 1996, Irvine and Hill 1985, Mawson 1994). SCP could be produced from whey with employing of yeasts from different species including *Kluyveromyces* spp., *Candida* spp. and *Trichosporon* spp. as they are naturally able to metabolize lactose (Castillo 1990). Although species of yeasts may differ considerably in their physiology, those of industrial importance have enough physiological characteristics in common to permit generalizations, provided that it is kept in mind that there will be exceptions to every statement made (Frazier and Westhoff 1995). The findings explored that these yeasts may be new strains and lead to a search of yeast strains having high ß-galactosidase activity (Nahvi and Moeini 2004) as more efficient bioactive agents.

**References**


