PCR Based Detection and Evaluation of Fermenting Capability of Local Strains of *Saccharomyces cerevisiae*

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Abstract

On Earth there are several organisms that share close relationship with humankind and *Saccharomyces cerevisiae* is one of them. *Saccharomyces cerevisiae* is also known as the brewer's or baker's yeast and has been used for fermentation and production of bread and alcoholic beverages since long time. The purpose of this study was to evaluate the fermentation capability of *Saccharomyces cerevisiae* local isolates. For this purpose 100 fruit samples (mango, sugarcane, orange, honey melon, grapes) were collected from local fruit markets and subjected to identification by standard microbiology tests and advance molecular techniques like Polymerase reaction test (PCR). The results revealed the typical growth, shape and color of *S. cerevisiae* on YPD media. The growth culture subjected to microscopy showed the budding characteristic of *S. cerevisiae*. The initial identified strains by virtue of YPD selective media and microscopy were subjected to PCR. The PCR results revealed the presence of *S. cerevisiae* in 28 samples among all analyzed samples. Importantly, the presence of *S. cerevisiae* was detected 30% in oranges, in mango 27.77%, in sugarcane 35% while in honey melon and grapes it was 13.63%, 31.81% respectively. This study concludes that *S. cerevisiae* was more abundant in sugarcane and grapes analyzed samples. The collected strains of present study may be further used in future studies like ethanol production from raw materials and also used as reference strain when working with samples of unknown nature.

Keywords: *Saccharomyces Cerevisiae*, Local Strains, Ethanol Production, Rotten Fruits, PCR

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INTRODUCTION

In recent decades Pakistan has faced the severe energy crisis which necessitates studying and implementing the alternatives sources of energy (1). In this regard ethanol fermentation serves the best alternative source as raw material required is available in abundance like sugarcane, orange peel other rotten fruits like grapes, mango and melons (2). Production of ethanol is widely investigated as it serves as biofuel and capable to gasoline which is harmful for environment (3). Several countries like Bangladesh and Brazil has adopted the strategies to produce ethanol from fruits and food crops. Sugarcane is one of the important crops which is harvested at large scale in Pakistan and can be used as source of sugar, electricity and importantly ethanol production. Proper utilization of these resources may lead to availability of cheaper energy source and millions of dollars bill of fuel import can be saved (4).

To produce ethanol from raw materials yeast fermentation has been recognized as the most efficient and economical in the biotechnology industry. Almost 80% of the ethanol produced is carried out by help of *Saccharomyces cerevisiae*

which ferments the sugar sources. Recent research advances has made this technology highly sophisticated and efficient.

Some bottle necks like designing the process of fermentation properly, contaminants and scarcity of raw materials are the main factors which significantly affect the alcohol quality and yield. Several studies have reported the fermented ethanol as an alternative of benzene and diesel however, to make it viable option the production should be cost effective and the resources employed should be used most efficiently (5). The ethanol has advantage on fossil fuel as its combustion has very low emission and toxicity of carbon monoxide (6). Molasses which are by product of sugarcane are mostly used as raw material for production of ethanol by yeast fermentation (7). Molasses obtained from sugarcane and sugar beet are very cheap and easily available in agriculture based country like Pakistan. These molasses after processing of sugar contents, it still contain 60% sucrose (8, 9). Beside ethanol production molasses are used to produce several other items like baker's yeast, lactic, citric acids and animal feed additive (10, 11). *S. cerevisiae* is mainly used as fermenting yeast in ethanol production when using the sugar cane or other food/fruits by products as raw material (12, 13). In Pakistan especially in Balochistan no study has been conducted to

evaluate the fermenting potential of local *S. cerevisiae* strains. This pilot study was conducted to isolate the *S. cerevisiae* from rotten fruits. The yeast from collected samples were grown and identified by standard biochemical and advanced molecular techniques. The isolated *S. cerevisiae* strains from different fruit samples can be further used for ethanol production at commercial level.

MATERIALS AND METHODS

In this study *Saccharomyces cerevisiae* was isolated from five different rotten fruits (Sugarcane, Oranges, Grapes, Honey melon and Mango).

Isolation and Screening

Fruit samples (orange, sugarcane, mango, grapes and honey melon) were purchased from local Markets and were grinded. From each sample one gram was soaked in 250ml yeast extract peptone dextrose broth for 3 days at 30°C (15). After incubation, the plates containing YPD media were streaked with 100µl of suspension. YPD media consisted of 15g of bacto agar, 10g of bacto yeast extract, 20g of bacto peptone, 20% glucose and were dissolved in 1 liter water and for 3 days it was aerobically incubated at 30 °C (16). Colonies formed after 3 days were carefully picked and under a microscope the cells were observed.

Maintenance of Culture

Yeast culture was maintained by sub-culturing on YPD solid medium plates, and incubated at 30°C for 48 h. Moreover they were stored at -4°C in refrigerator for future use (16).

Growth and Microscopy of Isolates

In this study, cells appearance and morphology of the selected isolates was examined on YPD (yeast extract peptone dextrose) agar medium (17). The medium was initially autoclaved at 121 °C and 15 psi later on poured on petri dishes and were left to cool down. The plates were streaked with inoculums of *S. cerevisiae* after cooling, and incubated for 48 hours at 30°C. The features of the colony recorded during observation includes surface of colonies color and texture (18). A glass slide was prepared to check the morphology of *S. cerevisiae* and was observed under light microscope. Microscopy showed the budding pattern of *S. cerevisiae* and white creamy colony (18).

DNA Extraction and PCR Analyses

DNA extraction was done through boiling method as has been described previously (15). An aliquot of cell suspension containing 10⁷ cells/mL of *S. cerevisiae* were transferred to tubes and centrifuged for 3 minutes at 7000rpm.

Supernatant was discarded and pellet was dissolved in 400 microliter TE buffer was added to it. The tubes were then kept in water bath whose temperature was already raised to 95°C, then again the tubes were centrifuged and the supernatant containing DNA was transferred to new tubes. After DNA extraction PCR analyses were done.

RESULTS AND DISCUSSION

A total of 100 samples of five different fruits were collected from fruit markets of Quetta city. All of them were processed and analysed for the presence of *Saccharomyces cerevisiae*. The plating of samples was done on YPD media and growth was observed after 48 hours as shown in figure 1. Based on the characteristics of their colony (creamy and white texture), budding pattern of colonies, and ovoid microscopic shape confirmed that the isolated organism belongs to *Saccharomyces*. The isolate was further tested through PCR in which 28 samples were found positive for the presence of *S. cerevisiae*.

Fig. 1. Growth of *Saccharomyces cerevisiae* on YPD solid media

After plating of samples the initial identification of *S. cerevisiae* was done through microscopy as shown in Fig. 2.

Fig. 2. microscopic identification of *S. cerevisiae*. The typical budding colonies were confirmed by microscopy.

After microscopy the DNA was isolated from morphologically confirmed samples and were subjected to PCR identification. A total of 18 samples of oranges were collected

and subjected to PCR. The results confirmed 6 positive samples of *S. cerevisiae* as shown in Fig. 3 (A and B)

Fig. 3. PCR based detection of *S. cerevisiae* in oranges. The 1200bp amplification of targeted gene of *S. cerevisiae* is shown in sample numbers 7, 9, 11, 14, 16 and 18. M denotes the marker while +C and -C represents the positive and negative control respectively.

Similarly 22 collected samples of rotten grapes were analysed by PCR. Out of 22 samples 7 were found positive for *S. cerevisiae* as shown in figure Fig. 4 (A and B).

Fig. 4. PCR based detection of *S. cerevisiae* in grapes. The 1200bp amplification of targeted gene of *S. cerevisiae* is shown in sample numbers 19, 23, 29, 35-37 and 40 were successfully amplified for the targeted gene (1200bp) of *S. cerevisiae*. Marker is denoted as "M" denotes the while positive and negative control are represented by the +C and -C respectively. All other samples shown in figure were found negative.

The samples (n=18) collected from rotten mango were also subjected to PCR and results demonstrated that *S. cerevisiae* was present in 5 samples as shown in Fig. 5 (A and B).

Fig. 5. Mango samples analysis by PCR to detect *S. cerevisiae*. The sample numbers 43, 46, 53, 54 and 58 were found positive for *S. cerevisiae* while the remaining 13 samples were found negative. The successful amplification of the targeted gene (1200bp) of *S. cerevisiae* is shown in figure 5A and 5B.

Total number of samples collected from sugarcane (n=20) were subjected to PCR. Out of 20 samples 7 were positive and the rest were negative for the presence of *S. cerevisiae*. As shown in Fig. 6 (A and B).

The other fruit selected for the isolation of *S. cerevisiae* was honey melon (*Cucumis melo* species). There are two members of honey melon species (cantaloupe and honeydew melon). A total of 20 samples from both members of the species were collected from rotten honey melon and all of them were processed and analyzed by PCR. The PCR result showed only

3 samples were positive for *S. cerevisiae* results as shown in Fig. 7 (A and B).

Fig. 6. Detection of *S. cerevisiae* from sugarcane samples. *S. cerevisiae* was found in 59, 62, 64, 67, 68, 74 and 75 numbered samples of sugarcane as shown above. Marker is represented by "M" while +C and -C represents the positive and negative control respectively.

Fig. 7. PCR based detection of *S. cerevisiae* in honey melon. The 1200bp amplification of targeted gene of *S. cerevisiae* was performed in all collected samples. Only sample number 81, 94 and 99 were amplified successfully while all other were found negative. Marker is denoted as "M" denotes the while positive and negative control are represented by the +C and -C respectively. All other samples shown in figure were found negative.

These results demonstrated that *S. cerevisiae* can be isolated from different fruits but every rotten fruit does not contain *S. cerevisiae*. Only 28 samples out of 100 analyzed samples were found positive for *S. cerevisiae*. Furthermore, results indicated that sugarcane samples were the most contaminated with *S. cerevisiae* (n=07/100) followed by grapes in which 07 samples out of 22 analyzed samples were positive for *S. cerevisiae*. Similarly, 06 and 05 out of 18 samples of each orange and mango samples respectively were found positive for *S. cerevisiae*. The least detected *S. cerevisiae* was in honey melon samples in which only 03 samples were found positive out of 22 analyzed samples.

This study concludes that local strains of *S. cerevisiae* can be successfully isolated from rotten fruits sold in markets. The identified and isolated strains in this study can be further used for industrial fermentation purpose and ethanol production.

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