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Isolation and Taxonomic Characterization of Yeast Strains on the Basis of Maltose Utilization Capacity for Bread Making

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ABSTRACT

A total of 25 samples from different food sources viz., control, citrus juice, dahi and sugarcane juice were collected randomly from different localities of Rawalpindi. Forty yeast strains were isolated using selective medium sabouraud`s dextrose agar (SDA). Differential tests were applied including morphological, cultural and biochemical characteristics, which facilitate the opportunity for identification of the yeasts. The total number of isolated yeast strains from control (saf instant), citrus juice, dahi and sugarcane juice was 1, 14, 13 and 12, respectively. From these 40 strains, 14 were identified as *S. cerevisiae*, 12 as *S. kluyveri*, 4 as *S. exigus* and *S. dairnensis*, 2 as *S. ludwigii*, *S. octosporus* and *S. unisporus*, respectively. Later on, 14 isolates of *S. cerevisiae* were assessed for their maltose utilization capacity for bread making.

Key Words: *S. cerevisiae;* Maltose utilization capacity; Isolation and identification

INTRODUCTION

The importance of strains of yeast genus *Saccharomyces cerevisiae* has been well recognized from the ancient times for fermentation. Today, baker's yeast is used for bread manufacturing through out the world at industrial scale. With the improvement of bread industry, the use of starter culture increased tremendously. At present, the bread industry of Pakistan is solely dependent upon the import of yeast. So, there is a dire need to explore the potential of indigenous strains of *Saccharomyces cerevisiae* in order to meet the national requirements and to save the foreign exchange. There are different sources for the isolation of yeast species. However their presence, were reported mostly from the acidic foods. Among them citrus juice (Arias *et al.*, 2002), dahi (Savova & Nikolova, 2002) and sugarcane juice (Antonnini *et al.*, 2004) are considered to be the best. Citrus juices are acidic beverages (pH 3 - 4) with high sugar content (15^0 Brix) and typical yeast species found in citrus juices are *S. cerevisiae, S. kluyveri, S. exigus* and *S. ludwigii.* Dahi are most important fermented milk product. Yeasts are un-desirable additional microflora in dahi. Dahi are especially favorable environment for growth of yeasts due to the acidic reaction of the medium. Yeast species found in dahi are *S. cerevisiae, S. dairnensis, S. unisporus* and *S. octosporus* for their ability to grow at low temperature and also lactose dissimilation Sugarcane juice is favorable environment for growth of yeasts due to high sugar content. Typical yeast species associated with sugarcane juice are *S. cerevisiae, S. kluyveri, S. exigus* and *S. ludwigii.*

In bread manufacture, there are three sources of fermentable sugars. First, there is the sugar present in dough at the start of the bread-making process (including glucose, fructose, sucrose & maltose naturally present in the flour) and secondly, any fermentable sugars such as sucrose added by the baker. The amount of fermentable sugar added by the baker varies, but can reach to 25% w/w in some sweet doughs (Nagodawithana & Trivedi, 1990). The third source of sugar is maltose produced by the amylolytic breakdown of starch (Evans, 1990). If bread is made without added sugars (plain dough), the pre-existing free sugars are completely fermented with in the first hour, leaving only the starch-derived maltose to sustain fermentation (Oda & Ouchi, 1990; Higgins *et al*., 1999).

Keeping in view the importance of yeast, the present work, following an isolation program of yeasts from different food sources viz., control, citrus juices, dahi and sugarcane juices, several strains of *Saccharomyces cerevisiae* were selected on the basis of maltose fermentation for bread making.

MATERIALS AND METHODS

All the research work was carried out in the Microbiology Laboratory of the Department of Food Technology, University of Arid Agriculture Rawalpindi.

Collection of samples. Samples of sugarcane juice, dahi and citrus juice were collected randomly from local markets of different areas of Rawalpindi in sterilized sample bottles and were transferred to laboratory for further analysis. These samples were allowed to ferment at room temperature for 2 days. One sample was of commercial yeast (Saf instant) used as control.

Isolation. Routine microbiological procedures and selective nutrition medium for isolation of yeasts were used. The most appropriate medium for this purpose was the modified Sabouraud`s dextrose agar with the

following composition: dextrose 4%, peptone 1%, agar 2%, pH was adjusted with 1% HCl at 4.5 for gripping the growth of other microorganisms. After 48th h cultivation at 25 - 28^{0}C, single morphologically well formed colonies were isolated. The appropriate ones were re-cultivated several times for purity.

Identification. The strains were identified according to the procedures described by Barnett *et al.* (1983) and Kurtzman and Fell (1998). The ability of strains to utilize glucose, sucrose, maltose, galactose and lactose as carbon sources was determined in Durham tubes on YP medium containing the respective sugar plus the pH indicator. For determining the ability of growth at 25^{0}C and 30^{0}C, the yeast strains were cultured in a medium containing 2% (w/v) Glucose-Peptone-Yeast extract Broth (SB). This solution was dispensed into tubes and sterilized at 121^{0}C for 15 min. The tubes were inoculated with actively growing yeast cultures and incubated at 25^{0}C and 30^{0}C for 48 h. A positive reaction was detected by observation of turbidity in the solution. Maltose fermentation test was performed in Durham tubes at room temperature for 7 days and the production of gas and acid was monitored daily. The strains showing strong maltose fermentation were selected.

RESULTS AND DISCUSSION

The results regarding the identification of yeast species have been described under the following section.

Isolation and identification. Forty yeast strains were isolated, purified and identified from different food sources. Differential tests were applied, including morphological and physiological characteristics, which facilitate the opportunity for identification of the yeasts. The sets of these tests allow the information gathering for the studied objects and for determination of their systematic status to species. The morphological, cultural and biochemical data of the investigated strains are described and represented in Table I.

The data from the taxonomic researches (colonial, cell morphology & physiological characteristics) were analyzed according to the findings of Barnett *et al.* (1983) and Kurtzman and Fell (1998). The represented results allowed us to refer the tested cultures to the listed species: (*S. cerevisiae, S. kluyveri, S. exigus, S. ludwigii, S. octosporus, S. unisporus* & *S. dairnensis*). In the studied food samples, the yeast species were reported in different quantities as divulged by Table II. It is clear from the table that *S. kluyveri* comprises 7 (87.5%) from the tested citrus samples, *S. cerevisiae* was 4 (50%), *S. ludwigii, S. exigus* and *S. octosporus* comprises 1 (12.5%), respectively. In the tested dahi samples, the quantity of the strains was as follows: *S. cerevisiae* 5 (62.5%), *S. dairnensis* 4 (50%), *S. exigus* 2 (25%), *S. unisporus* and *S. octosporus* was 1 (12.5%), respectively. *S. kluyveri* comprises 5 (62.5%) from the tested sugarcane samples, *S. cerevisiae* was 4 (50%), *S. ludwigii, S. exigus* and *S. unisporus* comprises 1 (12.5%), respectively. Among all the tested samples, the predominant strain is the *S. cerevisiae.* The possible reason of the higher incidence may be the chemical composition of samples that facilitate its growth. Moreover, the nature of the collected samples also varied from one another depending upon the local microflora coupled with environmental conditions.

Maltose fermentation test. In order to evaluate the potential utilization for bread making of the indigenous strains isolated, a preliminary screening was carried out based on their maltose fermentative capacity. As it is well known, in lean dough the principal fermentable sugar for yeast is maltose liberated from starch of the flour by amylases being generally accepted that a good baker's yeast should be able to rapid ferment maltose. Fourteen isolates of *Saccharomyces cerevisiae* identified from different samples were appraised for their maltose utilization capacity. It was observed that there is a large variation among the tested strains for their utilization of this particular sugar at room temperature.

Table III displayed ability of maltose utilization in selected strains through out the evaluation period of seven days. It is clearly divulged from the table that all strains of dahi and sugarcane showed strong maltose utilization

Table I. Morphological, cultural and biochemical characteristics of yeast strains isolated

	Strains						
Character-istics	**Saccharomyces cerevisiae**	**Saccharomyces kluyeri**	**Saccharomyces ludwigii**	**Saccharomyces exigus**	**Saccharomyces octosporus**	**Saccharomyces unisporus**	**Saccharomyces dairnensis**
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Margin	Circular	Irregular	Irregular	Circular	Irregular	Circular	Irregular
Color	Creamy white	Creamy white	Creamy color	White	White	Creamy white	White/Cream
Cells	Spherical /oval	Spherical	Spherical	Round/oval	Oval	Round	Round
Gram stain reaction	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	W	+	-
Maltose	+	W	-	-	W	W	-
Galactose	+	+	-	+	+	W	+
Lactose	-	-	-	-	-	-	-
Growth at 25^{0}C	+	+	+	+	+	+	+
Growth at 30^{0}C	+	+	+	+	+	+	+

+ = Positive result (Acid production) - = Negative result (No acid production) W = Weak acid production

Table II. Total number of isolates from citrus juice, dahi and sugarcane juice

Samples	No. of Samples	No. of isolates							
		S. cerevisiae	S. kluyveri	S. ludwigii	S. exigus	S. octosporus	S. unisporus	S. dairnensis	Total
Control (Saf instant)	1	1	-	-	-	-	-	-	1
Citrus juice	8	4 (50%)	7 (87.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	-	-	14
Dahi	8	5 (62.5%)	-	-	2 (25%)	1 (12.5%)	1 (12.5%)	4 (50%)	13
Sugarcane juice	8	4 (50%)	5 (62.5%)	1 (12.5%)	1 (12.5%)	-	1 (12.5%)	-	12
Total	25	14	12	2	4	2	2	4	40

Table III. Ability of selected strains to ferment maltose throughout the evaluation period of seven days

		Maltose fermentation						
Samples	Strains identified	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Saf Instant powder	Control	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	CY_1	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
Citrus	CY_2	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	CY_3	W	W	W	+(S)	+(S)	+(S)	+(S)
	CY_4	W	W	W	W	+(S)	+(S)	+(S)
	RY_1	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
Dahi	RY_2	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	RY_3	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	RY_4	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	RY_5	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	SY_1	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
Sugarcane	SY_2	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	SY_3	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)
	SY_4	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)	+(S)

+(S) = Strong Fermentation W = Weak fermentation

Table IV. Ability of selected strains to produce gas throughout the evaluation period of seven days

		Gas production						
Samples	Strains identified	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Saf Instant powder	Control	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve
	CY_1	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve
Citrus	CY_2	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve
	CY_3	+ve	+ve	+ve	3+ve	3+ve	3+ve	3+ve
	CY_4	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve
	RY_1	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve
Dahi	RY_2	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve
	RY_3	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	RY_4	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve
	RY_5	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	SY_1	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve	3+ve
Sugarcane	SY_2	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve	2+ve
	SY_3	+ve	+ve	3+ve	3+ve	3+ve	3+ve	3+ve
	SY_4	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve	1+ve

+ = Very low gas production 2+ve = Medium gas production
1+ve = Least gas production 3 +ve = Highest gas production

through out the evaluation period of seven days. In case of citrus strains, two strains, CY_1 and CY_2 remained strong maltose utilizing strains through out the evaluation period. However, two strains of citrus, CY_3 and CY_4 exhibited a quite different pattern in utilization of maltose. Till the 3rd day of evaluation, they remained weak utilization strains; however, they displayed strong maltose fermentation behaviour, on day 4 and day 5, respectively. Both strains remained strong fermentation strains till the last day (day 7) of the evaluation period.

It is clearly revealed from the Table IV that CY_2, RY_2 and SY_1 produced highest gas through out the evaluation period of seven days, however, CY_1, RY_1 and SY_2 exhibited medium gas production through out the evaluation period. The least gas production observed in CY_4, RY_4 and SY_4 through out the evaluation period. CY_3 remained very low gas producing strain till day 3. From day 4, CY_3 displayed highest gas production up to 7 days. SY_3 remained very low gas producing strain till day 2. From day 3, SY_3 displayed highest gas production up to 7 days. RY_3 and RY_5 remained very low gas producing strains through out the evaluation period of 7 days. These strains might be very low gas production capabilities and their own genetic make up. On the basis of above results, after 7 days the best maltose fermenting strains were screened.

These results are in line with the findings of Almeida

and Pais (1996). They characterized the yeast population from traditional corn and rye bread dough. They selected the *Saccharomyces cerevisiae* strains on the basis of maltose utilization capacity. The variation in our results may be due to their genetic combination and climatic conditions. These results provide us an opportunity to access their potential in bread making.

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