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ISOLATION AND CHARACTERIZATION OF LOCAL YEAST STRAINS FROM WASTE FRUIT JUICES, JAGGERY AND DAHI SAMPLES

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ABSTRACT

A total of 14 samples from different food sources viz., dahi, jaggery and different kinds of fruit juices were collected randomly from different localities of Kolkata, West Bengal, India. Thirty-three yeast strains were isolated using selective medium, Martin's Rose Bengal Agar. 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 was 12 from dahi, 2 each from apple juice, pineapple juice, mango juice, musambi juice, grape juice, orange juice, jaggery and 7 from sugarcane juice. These strains were found to produce various extra cellular enzymes and could ferment various carbon sources for the production of alcohol.

Key words: Ethanol, Yeast, Fermentation, Isolation of yeast.

INTRODUCTION

The useful physiological properties of yeast have led to their use in the field of biotechnology, fermentation of sugars by yeast being the oldest and largest application of this technology. Many types of yeasts are used for making many foods: baker's yeast in bread production; brewer's yeast in beer fermentation; yeast in wine fermentation and for xylitol production. A worldwide interest in the utilization of bio-ethanol as an energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production. Intense research has been carried out for obtaining efficient fermentative organisms, low-cost fermentation substrates and optimum environmental conditions for fermentation to occur. Traditionally, ethanol production is usually accomplished by microbial conversion of carbohydrates present in agricultural products[1]. As, few yeast strains have been found to possess appreciable characteristics for ethanol production[2,3], there is a

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dire need to explore the potential of indigenous strains of yeasts to meet the national requirements and to save the foreign exchange. There are different sources for the isolation of yeast species[4]. However their presence, were reported mostly from the citrus juice[5], dahi[6] and sugarcane juice[7], molasses[8], sugar mill effluents[9] and fermented foods[10] and fermented pineapple juice[11]. As there is global emphasis in ethanol production by fermentation process.

In the present study, several local samples of dahi, various fruit juices and jaggery were analyzed for isolation and subsequent characterization of yeast strains, which may further be utilized in alcohol production.

EXPERIMENTAL

Materials and methods

All the research work was carried out in the Microbiology Laboratory of the Department of Zoology, Molecular Biology & Genetics, Presidency University, Kolkata, India.

Collection of samples

Samples of dahi, waste juices of apple, pineapple, mango, musambi, grape, sugarcane, orange and jaggery were collected randomly from local markets of different areas of Kolkata in sterile bottles and kept at 4°C. These samples were screened for isolation of the yeast strains.

Isolation of yeast strains

The samples were serially diluted, plated on a selective medium, Martins Rose Bengal agar Medium (33.6 gm /liter) and were incubated at 28°C for 48 hours. The colonies appeared were further purified. Pure colonies were isolated and tested for further characterization.

Morphological characterization

The strains were stained by lactophenol-cotton blue, carbol fuchsin and seen under phase contrast microscope. The colonies were checked for their colour, outline and other features.

Determination of enzyme synthesizing ability

To detect their ability to assimilate various polysaccharides, the strains were grown

in basal medium composed of (gL^{-1}): peptone 0.9; $(NH_4)_2HPO_4$ 0.4; KCl 0.1; $MgSO_4.7H_2O$ (pH 7) at 28°C for 18 hours, supplemented with starch, carboxy methyl cellulose, xylan and pectin (0.5) respectively. For in situ detection of amylase and cellulase & xylanase activities, plates were flooded with iodine solution (I_2 + KI) and congo red solution respectively. The catalase activity was checked by dropping few drops of H_2O_2 on the culture. The quantitative assay of cellulase, amylase, xylanase and pectinase was done by dinitrosalicylic acid method[12]. For checking the efficacy of the strains to grow and produce alcohol, each strain was cultivated in basal medium supplemented with various sugars and organic and inorganic nitrogen sources.

Determination of alcohol producing ability

To detect their alcohol producing ability, quantitative estimation of ethanol by potassium dichromate method[13] was followed. The supernatant (1 mL) of centrifuged culture broth of glucose-grown yeast strain was made up the volume to 5 mL with distilled water. Then 1 mL of Potassium dichromate reagent was added. All the test tubes were kept in ice water and 4 mL of conc. sulfuric acid added to each tube gently through the walls. Then the optical density was measured at 660 nm. Alcohol standard was prepared by dissolving absolute ethanol in water to get 10 mg/mL concentration.$K_2Cr_2O_7$ solution was prepared by dissolving 10 gm of $K_2Cr_2O_7$ in distilled water in a 100 ml standard flask and make up the volume to mark.

RESULTS AND DISCUSSION

Fourteen samples of dahi, jaggery and fruit juices were screened from where thirty three yeast strains were isolated and purified (Table 1). Maximum yeast strains were isolated from dahi samples, followed by sugarcane juice. Most of the isolated colonies exhibited smooth surfaces with circular margins. The colour of the colonies showed a wide variation of creamy white and pinkish. The cells were found to be of various shapes such as round; oval, spherical and ellipsoidal (Table 2).

Table 1: Total number of isolates from dahi, various fruit juices and jaggery

Sources tested	No. of samples isolated	No. of strains isolated	Alcohol production
Dahi	6	12	++
Apple juice	1	2	+

Cont…

Sources tested	No. of samples isolated	No. of strains isolated	Alcohol production
Pineapple juice	1	2	+
Mango juice	1	2	+
Musmambi juice	1	2	+
Grape juice	1	2	+
Sugar cane juice	1	7	+
Orange juice	1	2	+
Jaggery	1	2	+
Total	**14**	**33**	

Table 2: Morphological characteristics of the Yeast strains isolated from dahi samples

Strains	Characteristics			
	Surface	**Margin**	**Colour**	**Cells**
D1	Rough	Circular	Creamy white	Round/Oval
D2	Smooth	Irregular	Pinkish	Round/Oval
D3	Smooth	Circular	Creamy white	Spherical/Oval
D4	Smooth	Circular	Creamy white	Round/Oval
D5	Smooth	Circular	Creamy white	Ellipsoidal
D6	Smooth	Circular	Creamy yellow	Round
D7	Smooth	Circular	Creamy white	Round
D8	Smooth	Circular	Creamy white	Spherical
D9	Rough	Irregular	Light pink	Spherical
D10	Smooth	Circular	Creamy white	Ellipsoidal
D11	Smooth	Irregular	Creamy white	Oval
D12	Rough	Circular	White	Oval

The biochemical analysis of the strains isolated from dahi samples showed that all the twelve strains could grow in presence of sugars and urea and ferment them (Table 3). Four of them, namely D1, D2, D3 and D4 were found to produce alcohol of significant quantity. The strains showed highest alcohol production in presence of glucose and fructose. Table 4 represents the morphological and cultural analysis of the strains isolated from different fruit juices and jaggery. The surface, margin and colour of the colonies isolated from the various samples differed from each other. However, smooth, circular and creamy white colonies were found to be more prevalent. These strains were incubated at 28^{o}C for 18 hours for their biochemical analysis (Table 5). The best result in alcohol production was given by pineapple and grape juices in the medium containing glucose, fructose, maltose, sucrose and urea.

Table 3: Biochemical analysis of yeast strains isolated from dahi samples

Yeast strains	Carbon sources											
	Glucose		Fructose		Sucrose		Maltose		Urea		Peptone	
	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc
D1	++	++	++	++	++	++	++	++	+	+	+	+
D2	+	+	++	++	++	++	++	++	++	++	++	+
D3	++	++	+++	+++	++	+	+	+	+	+	++	++
D4	+++	+++	++	++	+++	+++	+	+++	+++	+	++	++
D5	+	+	+	+	+	+	+	+	+	+	+	+
D6	+	+	+	+	+	+	+	+	+	+	+	+
D7	+	+	+	+	+	+	+	+	+	+	+	+
D8	+	+	+	+	+	+	+	+	+	+	+	+
D9	+	+	+	+	+	+	+	+	+	+	+	+
D10	+	+	+	+	+	+	+	+	+	+	+	+
D11	+	+	+	+	+	+	+	+	+	+	+	+
D12	+	+	+	+	+	+	+	+	+	+	+	+

Alc.: Alcohol production; Gr.: Growth; +: moderate; ++: very good; +++: extremely good

Table 4: Morphological characteristics of yeast strain isolated from different fruit juices

Source	Yeast strains	Surface	Margin	Colour	Cells
Apple Juice	FJ1	smooth	circular	Creamy white	Oval
Apple Juice	FJ2	smooth	Circular	Creamy white	Spherical
Pineapple juice	FJ3	smooth	Circular	White	Round
Pineapple juice	FJ4	rough	Circular	Creamy white	Oval
Mango juice	FJ5	smooth	Circular	Creamy white	Round
Mango juice	FJ6	rough	Circular	Creamy white	Round
Sugarcane juice	FJ7	smooth	irregular	Pinkish white	Spherical
Sugarcane juice	FJ8	smooth	circular	Creamy white	Round
Sugarcane juice	FJ9	smooth	circular	Creamy white	Ellipsoidal
Sugarcane juice	FJ10	rough	irregular	Creamy white	Round
Sugarcane juice	FJ11	smooth	circular	Creamy white	Ellipsoidal
Sugarcane juice	FJ12	Smooth	circular	Pinkish	Round
Sugarcane juice	FJ13	Rough	irregular	Yellow white	Round
Musambi juice	FJ14	Smooth	circular	White	Oval
Musambi juice	FJ15	Smooth	circular	Creamy white	Spherical
Grape juice	FJ16	Rough	irregular	Pinkish	Oval
Grape juice	FJ17	smooth	circular	Creamy white	Round
Orange juice	FJ18	rough	circular	Light pink	Oval
Orange juice	FJ19	rough	circular	Pinkish	Round
Jaggery	JG1	smooth	circular	Creamy white	Ellipsoidal
Jaggery	JG2	rough	irregular	White	Ellipsoidal

Table 5: Biochemical analysis of the yeast strain isolated from different fruit juices and jaggery

Strains	Carbon sources											
	Glucose		Fructose		Maltose		Sucrose		Urea		Peptone	
	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc	Gr	Alc
FJ1	+	+	+	+	+	+	++	++	++	++	+	+
FJ2	+	+	+	+	+	+	+	+	+	+	+	+
FJ3	++	++	++	++	++	++	+	+	+	+	+	+
FJ4	++	++	++	++	++	++	++	++	+	+	+	+
FJ5	++	++	+	+	+	+	+	+	+	+	+	+
FJ6	++	++	++	++	+	+	+	+	+	+	+	+
FJ7	++	++	+	+	+	+	+	+	+	+	+	+
FJ8	++	++	+	+	+	+	+	+	+	+	+	+
FJ9	++	++	++	++	+	+	+	+	+	+	+	+
FJ10	++	++	++	++	+	+	+	+	+	+	+	+
FJ11	++	++	++	++	+	+	+	+	+	+	+	+
FJ12	++	++	++	++	+	+	+	+	+	+	+	+
FJ13	++	++	++	++	+	+	+	+	+	+	+	+
FJ14	++	++	+++	+++	+++	+++	++	++	+	+	+	+
FJ15	++	++	++	++	+	+	+	+	+	+	+	+
FJ16	+++	+++	+++	+++	+++	+++	++	+++	++	++	++	++
FJ17	+++	+++	+++	+++	++	++	++	++	++	+	+	+
FJ18	++	++	++	++	+	+	++	++	+	+	+	+
FJ19	++	++	++	++	+	+	++	++	+	+	+	+
JG1	++	+	+++	+++	++	+	+	++	+	+	+	+
JG2	++	+	+++	++	++	++	+	++	++	+	+	+

Alc.: Alcohol production; Gr.: Growth; +: moderate; ++: very good; +++: extremely good.

The physiological researches of each yeasts strain were carried out by using over 5 tests for assimilation of carbon and their catalase activity (Table 6 & 7). The utilization of four polysaccharides namely carboxy methyl cellulose (CMC), starch, xylan and pectin was tested. All the strains isolated were found to assimilate the named polysaccharides and produce detectable amount of extra cellular cellulase, amylase, xylanase and pectinase respectively. Moreover, the strains also exhibited catalase ability of various degrees.

The present study dealing with the isolation and characterization of about thirty three yeast strains with interesting features such as extra cellular enzyme and alcohol production would facilitate the opportunity for identification of the yeasts. The result of this study indicated that most of the indigenous yeasts, isolated from dahi and juice samples showed good fermentation attributes, which might enhance ethanol yield that would contribute for the cost effective role in the production of bioalcohol and enzymes of industrial importance.

Table 6: Physiological characteristics of the yeast strains isolated from dahi

Strains	Assimilation				Catalase activity
	CMC	Xylan	Pectin	Starch	
D1	++	+	+	+	+
D2	+	++	+	++	++
D3	+	+	+	+	+
D4	+	+	+	+	+
D5	++	++	+	++	++
D6	+	+	+	++	+
D7	+	+	+	+	+
D8	++	+	+	+	++
D9	+	+	++	+	+
D10	++	+	+	+	+
D11	+	++	+	++	+
D12	+	+	++	+	++

+ = Growth on the respective plate

Table 7: Physiological characteristics of the yeast strains isolated from different fruit juices & jaggery

Strains	Assimilation				Catalase Activity
	CMC	Xylan	Pectin	Starch	
FJ1	+	+	++	+	+
FJ2	+	+	+	+	+
FJ3	++	+	+	++	++
FJ4	+	++	+	+	+
FJ5	+	+	+	+	+
FJ6	++	+	+	++	++
FJ7	+	+	+	+	+
FJ8	++	+	+	+	+
FJ9	+	+	+	+	+
FJ10	+	++	+	++	++
FJ11	+	+	+	+	+
FJ12	++	+	++	+	+
FJ13	+	+	+	+	+
FJ14	++	+	+	+	+
FJ15	+	++	+	+	+
FJ16	++	++	+	++	++
FJ17	+	+	+	+	+
FJ18	++	+	++	+	++
FJ19	+	+	+	+	+
JG1	++	+	+	++	+
JG2	+	+	+	+	+

+ = Growth on the respective plate

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