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Research Article

ISOLATION AND CHARACTERIZATION OF YEAST STRAINS FROM SACCHARINE MATERIALS FOR OSMO-TOLERANCE AND THERMO TOLERANCE SCREENING

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Abstract- An attempt had been made to isolate and characterize yeast strain from different saccharine materials *viz,* apple juice, pineapple juice, mango juice, mosambi juice, orange juice, pomegranate juice and Lichi juice. A total of 7 samples from different kind of fruit juices sources were isolated using selective medium *i.e.*, Yeast Extract Peptone Dextrose Agar (YEPDA). Differential tests were applied including morphological, cultural and biochemical characteristic, which facilitate the opportunity for identification of the yeasts. The strains were tested for growth at different temperature to check their mortality. These strains were further screened and tested on molasses for osmo-tolerant properties. Activated Dry yeast was taken as control. The performance of isolated yeast strains was tested on molasses. Out of different fruit juices strains obtained from apple and orange gave the best results in term of ethanol content 10.28 % & 10.15% and also Fermentation Efficiency 89.39 % and 88.26% respectively which was at par with ADY. Moreover, the yeast strains were also tested for its osmo-tolerance and thermo-tolerance using molasses as raw material for production of ethanol. Result revealed that total reducing sugar (TRS) of 14%, 18%, 21% at all these concentrations the isolated strain growth was observed however apple and orange isolates gave best result in terms of ethanol yield.

Keywords- *Ethanol, Yeast, Fermentation, Isolation of yeast*

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Introduction

India is the Bio- ethanol being energy rich source attracted the interest of the whole world to do the deep studies on its cost and efficient industrial production. Profound research has been carried out for obtaining efficient fermentative strains, low-cost fermentation raw materials and optimum environmental conditions for fermentation process 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 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 different saccharine juice *viz*., fruits juices [5], sugarcane juice [6], molasses [7], sugar mill effluents [8] and fermented foods [9] and fermented pineapple juice[10]. To assess the yeast strains for industrial use, specific physiological properties are required such as osmo-tolerance, thermo tolerance and ethanol tolerance. There is global emphasis in ethanol production by fermentation process.

Traditionally or conventionally alcohol is produced by the fermentation of molasses, the byproducts of cane sugar industry. Saccharomyces cerevisiae the common distiller's yeast is used as fermentation microorganism. Normally this organism produce yield up to 225-250 liter/ ton molasses. However, this microorganism may undergo genetic changes on usage and as there is always a need for the isolation of new strains from various materials in order to search for strains which give better yield and remain tolerant to temperature and also osmo-tolerance which may further be utilized in alcohol production. In the present study isolation of yeast has been carried out from different saccharine sources from various fruit juices *viz*; pineapple, apple, mango, mosambi, litchi, orange. They have been purified by pour plate method and the performance of the isolated strains with regard to their fermentative ability was tested on molasses.

Activated dry yeast was used as a control in the present study for comparing the results. The basic aim of the study was to Screen a yeast strain which is high osmo-tolerant and thermo-tolerant with respect to ethanol yield on molasses

Materials and methods

All the research work was carried out in the Department of Biochemistry, National sugar Institute, Kanpur U.P. 208017. India.

Collection of Sample

Different fruits *viz*; mosambi, pineapple, apple, orange, mango, litchi, pomegranates, were collected from local market for isolation of yeast cells.

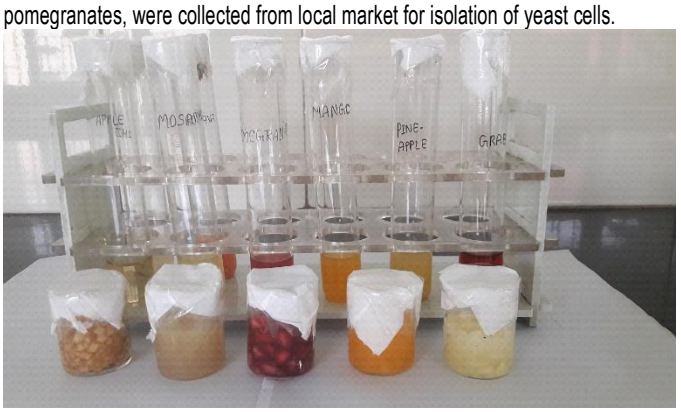

Fig-1 Fruit juices-Sample's

Identification of yeast strains

Pure Yeast culture (isolated and purified from different fruits) was picked up and mounted on a clean slide then observed under the microscope.

Table-1 *Morphological characteristics of the Yeast strains isolated from 10 selected fruit juices and ADY*

Characteristics	ADY	Apple	Orange	Mosambi	Mango	Pomegranate	Litchi	Pine-Apple
Colour	Wh	OW	OW	Wh	Red	OW	OW	OW
Surface	Rou	Sm	Sm	Rou	Rou	Sm	Sm	Sm
Margin	Cir	Cir	Cir	Undu	Cir	Cir	Cir	Cir
Cell-shape	Ellip	R.	R/O.	Ellip	R.	R/O	O	O
Ascospore	A	A	P	A	A	P	P	P
Ballistispore	A	A	A	A	A	A	A	A
Pseudo-mycelium	A	P	A	A	P	A	A	A
Elevation	Sl.Co	Co	Co	Co	Co	Co	Sl.Co	Co
True mycelium	A	A	A	A	A	A	P	A

Responses: Wh=White; OW=Off white; Rou=Rough; Sm=Smooth; Cir=Circular; Undu=Undulating; Sl.Co= Slightly Convex; Ellip= Ellipsoid; Sph= Spherical; R= Round; O=Oval; A=Absent; P=Present

Table-2 *Biochemical test of the Yeast strains isolated from 10 selected fruit juices and ADY*

Test Name	ADY	Apple	Orange	Mosambi	Mango	Pomegranate	Litchi	Pine-Apple
Starch hydrolysis	-	-	-	-	-	-	-	-
Gelatin test	-	-	-	-	-	-	-	-
MR test	+	+	+	+	+	+	+	+
VP test	+	+	+	+	+	+	+	+
Simmon's citrate	-	-	-	-	-	-	-	-
Urease test	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+

Table-3 *Biochemical test of the Yeast strains isolated from 10 selected fruit juices and ADY*

Fermentation in carbon source	ADY	Apple	Orange	Mosambi	Mango	Pomegranate	Litchi	Pine-Apple
D-glucose	++	++	++	+	+	+	+	+
D-galactose	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Lactose	N	N	N	N	N	N	N	N

Responses: +=positive; -= Negative; +(-)=Mostly positive with some negative; -(+)= Mostly negative with some positive; vw= very weak; w= weak; N= Not determine

Table-4 *The growth of different yeast isolates at different temperatures*

Growth at temperature	ADY	Apple	Orange	Mosambi	Mango	Pomegranate	Litchi	Pine-Apple
30°C	++	++	++	++	++	++	++	++
35°C	++	++	++	++	++	++	++	++
40°C	-	-	-	-	-	-	-	-
45°C	-	-	-	-	-	-	-	-

Responses: +=Positive; -= Negative

The yeast was identified based on colony characteristics, staining methods and by various biochemical tests.

Physiological and Biochemical tests

The physiological and biochemical test were conducted following the method of Somasegaran and Hoben and Josey et al., respectively, as described by cappuccino and sherman to identify the yeast [11].

Molasses

In the manufacturing process of sugar, the syrup is being boiled and during its third boiling of sugar yields dark, viscous fluid known as molasses. When maximum sugar from the original juice has crystallized and removed. Molasses was obtained from Experimental sugar factory attached to National Sugar Institute. The molasses was analyzed for Brix, Pol Purity, Ash, TRS, RS, UFS, Ethanol percent and FE using isolated strains. For seeing osmo-tolrance TRS of molasses was kept at different concentration *viz.,* 14%, 18% and 21%. The respective cultures were added at the rate of 5% (v/v). And for thermo tolerance the plates were kept at different incubation temperature *viz*., 25°C, 35°C, 40°C and 45°C.

Determination of total soluble solids in was carried using brix spindle method, Determination of pol % was carried out through Polari meter and estimation of total reducing sugars in molasses was done by lane & eynon method.

Ethanol Production

After analyzing the total reducing sugars in molasses it was diluted to 14%, 18% and 21% of TRS. 200 ml of each diluted molasses also called wort was fortified with nutrients like potassium hydrogen phosphate and ammonium sulphate. The pH was adjusted to 4.5 and sterilized at 15 p.s.i.g for 15 minutes. It were now inoculated with yeast strains (*Saccharomyces cerevisiae*) with 20ml broth developed from already isolated strains from different fruit juices. Each set of strains isolates were kept for fermentation for 48 hrs, in incubator at different temperature *viz*., 36°C, 40°C and 45°C each. After fermentation wash was analyzed for residual sugars and alcohol % in wash based on which fermentation efficiency and ethanol yields were calculated for each set.

Results and Discussion

Morphological characteristics of most of the isolated colonies exhibited smooth surfaces with circular margins. The colour of the colonies showed variation of white, off white and red. Most of the isolated colonies exhibited smooth surfaces with circular/entire margins. The cells were found to be of various shapes such as round; oval, and ellipsoidal [Table-1].

To prove that the isolates were of yeast strains, biochemical test were performed which are presented in [Table-2]. These strains were incubated at 30°C for 18 hours for their biochemical analysis. Starch hydrolysis, Gelatin test, Simmon's citrate, Urease test and Indole test showed negative test and only Catalase test, MR, VP test were positive by all the isolated strains of the Yeast and ADY.

The observations regarding biochemical analysis of the strains isolated from various fruits showed that, all the seven strains and ADY could grow in presence of sugars and fermented them except D- galactose and lactose. The results indicate that in this yeast galactose is a non-fermentable carbon source, and also yeast do not have the lactase enzyme and therefore cannot break down lactose. Thus, the yeast isolates were not able of metabolizing the sugar lactose [12].

Thermo-tolerance test was performed for isolation the thermo-tolerant yeast isolates. All the isolated strains were grown at different temperature as presented in [Table-4]. It was observed that none of the isolate grown beyond 35°C.

Characteristics of Molasses procured from NSI sugar factory was done and is presented as [Table-5]. Before keeping for fermentation molasses were tested for TRS, RS, reducing sugar, UFS, fermentable sugar and theoretical yield for all the selected osmotolerant content based on 15TRS%, 18TRS% & 21TRS% [Table-6, 8 & 10]. The results of ethanol yield and fermentation efficiency are presented in [Table-7, 9 & 11] with TRS content 15%, 18% & 21% respectively.

Table-5 *Characteristics of NSI cane molasses*

SNo	Particulars	Test values
1	Brix	87.0-89.0
2	Moisture Content (%)	15.0-21.5
3	Total Suspended Solid	3.5-7.0
4	Total Dissolved Solid	74.0-84.0
5	pH of molasses	6.2

With TRS *i.e.*, 15% it was observed that alcohol content and fermentation efficiency of apple and orange isolates were comparatively good as compared with other fruits isolates and was at par with ADY Control isolate [Table-7]. Other fruits isolates did not give good result both in terms of alcohol and fermentation efficiency

Table-6 *Molasses analysis 15% TRS content*

SNo	Particulars	Test values
1	TRS	14.85
2	RS (Residual Sugar)	0.88
3	FS	13.97
4	Theoretical yield %	8.99

Table-7 *Ethanol yield and fermentation efficiency are presented with TRS content 15%*

Strains name	Alcohol%	Fermentation Efficiency%
ADY	7.85%	87.32%
Apple	7.15%	79.53%
Orange	6.85%	76.19%
Mosambi	4.95%	55.06%
Mango	1.95%	21.69%
Pomegranate	1.85%	20.57%
Litchi	0.94%	10.56%
Pine-Apple	0.85%	9.45%

With TRS *i.e.*, 18% it was observed that alcohol content was 8.70 % & 8.52 % and fermentation efficiency 87% & 85.2% of apple and orange isolates respectively which was were at par with ADY Control isolate (8.82 % & 88.2%) [Table-9]. Other fruits isolates did not give good result both in terms of alcohol and fermentation efficiency

Table-8 *Molasses analysis 18%TRS content*

SNo	Particulars	Test values
1	TRS	17.33
2	RS (Residual Sugar)	0.44
4	FS	16.89
5	Theoretical	10.87

Table-9 *Ethanol yield and fermentation efficiency are presented with TRS content 18%*

Strains name	Alcohol%	Fermentation Efficiency%
ADY	9.82%	90.15%
Apple	9.70%	89.20%
Orange	8.88%	81.60%
Mosambi	6.54%	60.10%
Mango	3.54%	32.50%
Pomegranate	2.97%	27.30%
Litchi	1.10%	10.10%
Pine-Apple	1.40%	12.80%

Table-10 *Molasses analysis 21%TRS Content*

SNo	Particulars	Test values
1	TRS	19.25
2	RS (Residual Sugar)	1.79
4	FS	17.86
5	Theoretical%	11.50

The same trend was also observed with high TRS *i.e.*, 20% it was observed that alcohol content and fermentation efficiency of 89.39% apple and 88.26% orange isolates were at par with ADY Control isolate (90.17%) [Table-11]. Other fruits isolates did not give good result both in terms of alcohol and fermentation efficiency

Table-11 *Ethanol yield and fermentation efficiency are presented with TRS content 20%*

Strains name	Alcohol%	Fermentation Efficiency%
ADY	10.37%	90.17%
Apple	10.28%	89.39%
Orange	10.15%	88.26%
Mosambi	8.30%	72.17%
Mango	4.62%	40.17%
Pomegranate	4.45%	38.69%
Litchi	2.42%	21.04%
Pine-Apple	2.45%	21.30%

It was observed that yeast strain developed from apple and orange was capable of producing 10.28 % &10.15% ETOH corresponding to 89.39 % and 88.26% Fermentation Efficiency with 20%TRS. Other isolates could not tolerate even 15% TRS and their both Ethanol and FE decreased showing sugar losses. The above result of apple and orange isolates revealed that the results of yeast cells when compared to that of ADY showed at par ETOH yield and FE.

Conclusion

The result of this study indicated that most of the indigenous yeasts, isolated from fruit juice samples especially from apple and oranges juices showed good fermentation attributes, which might enhance ethanol yield that would contribute for the cost-effective role in the production of bio-alcohol.

Application of research: Isolation of yeast strain using different saccharine materials. The performance of isolated yeast strains was tested on molasses to check their efficiency with respect to osmo-tolerance and thermo-tolerance.

Research Category: Fermentation, Yeast

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