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Isolation, Morphological Characterization and Screening of Yeast Isolates from Different Fruit Samples of Raichur District, Karnataka, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A laboratory experiment was conducted in the Department of Agricultural Microbiology, College of Agriculture Raichur and Bheemarayangudi for isolation, characterization and screening of yeast isolates from different fruit samples of Raichur district. Fruit samples like Fig, Papaya, Pomegranate,

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Dragon fruit and Grapes were collected from different places of Raichur district. In total 26 yeast isolates were isolated from collected fruit samples all yeast isolates showed colour characteristics like dull white, white milky, creamish-white, pink and orange. The population of yeast isolates ranged from 2.00 to 58.50 cfu/ml of fruit samples. All 26 yeast isolates were positive for sugar fermentation test, different carbon utilization test, ethanol, glucose and pH tolerance test. The yeast isolate Raichur Papaya Yeast- 5 shown better results (2.510@10% v/v, 3.055 @30% and 2.862@ pH 6 respectively).

Keywords: Yeast; RPAY- 5; sugar fermentation; ethanol tolerance; glucose tolerance; PH tolerance.

1. INTRODUCTION

Yeast is the one of the most important microorganism in preparation of some food items and in wine industry because of its good characteristics in environment. Yeast is a eukaryotic single celled microorganism classified under the kingdom fungi. Yeast are chemoorganotrophic organisms which utilizes sugars as their carbon source, they convert the sugars into ethanol and carbon dioxide liberating energy by fermentation process (reference). Some of yeasts isolates produces pigments of different colours like pink, orange, yellow and red. They also posses smooth to rough surface having oval to irregular appearance. The cells of yeast are usually identified by their budding nature which have ovoid to ellipsoid shape [1].

Generally, yeast was found in the sugar rich substances such as nectars of flowers and fruits. As fruits are rich sources of sugar in the form of fructose which makes way for the yeast growth. Yeast is widely used in the industrial purpose for preparation of bakery products and in the wine preparation because of its wider adaptability in nature [2].

2. MATERIALS AND METHODS

2.1 Collection of Fruit Samples

Fresh fruits such as Fig, Papaya, Grapes, Pomegranate and Dragon fruits were collected from the orchards of different places in and around of Raichur [3]. Details of the fruit samples collected are presented List 1.

2.2 Isolation of Yeast

Fruits were cut with the sterilized knife and 20 gm of each fruit sample was taken in 250 ml conical flask containing 100 ml of distilled water, crushed and kept for 30 min on a rotary shaker. The YEPD media (20 gm- peptone, 20 gm- dextrose, 10 gm- yeast extract, 15 gm- agar) was used for the isolation of yeast by using the spread plate method. Plates were incubated for 24 h at 30°C [4]. The isolates were maintained in slants containing YEPD media and were kept in the refrigerator at 4°C for further use.

2.3 Morphological Characterization

Morphological features of yeast isolates such as shape and colour of colony were recorded. For the microscopic studies the slides were prepared, selected isolates were mixed in a drop of distilled water, placed on a glass slide then smeared and allowed to dry off. The smear was stained using diluted methylene blue dye then observed under light microscope and recorded shapes of yeast cells [5].

2.4 Screening of Yeast Isolates

Sugar fermentation test: The isolates were inoculated into a test tube containing YEPD broth (15g peptone, 10g yeast extract, 20g glucose and 1000 ml of distilled water) with an inverted Durham tube. The 10 ml of the broth containing test tubes were inoculated with a loopful of fresh yeast cultures and incubated for 48 h at 30°C. The liberation and trapping of gas in Durham's tube indicated the result of each test. The presence of gas was taken as evidence of fermentative activity and the absence of gas was taken as evidence of non- fermentative activity [3].

Ethanol tolerance test: The modified Osho [6] method was used for ethanol tolerance of yeast isolates. One ml of different concentrations of absolute ethanol was taken *i.e.,* 0, 5, 10, 15 and 20 per cent v/v and transferred to different test tubes. A loopful of freshly grown yeast cultures were inoculated into test tubes containing 10 ml of YEDP broth of five different concentrations of ethanol. The initial optical density (OD) of each

List 1. Fruits sample collected for the isolation of yeast isolates

Sl.no	Fruit name	Location	Variety(s)
1)	Grapes	Research plot, Department of Horticulture, MARS, Raichur.	Wine variety I (2A clone),Wine variety II (K. R. white), Manjari Naveen and Medica
2)	Pomegranate	Manvi	Bhagwa
3)	Fig	Katarki (village) in Manvi	Turkey Brown from orchard and Local Bellary fig
4)	Papaya	Turvihal village near Raichur	Red Lady 786
5)	Dragon fruit	MARS, research plot Raichur.	David Brownie

test tube was recorded by spectrophotometer at 600 nm. Blank was made of YEPD medium without yeast inoculation. The OD is directly proportional to the cell mass or growth. All cultures were incubated at 30 °C for two days. The increase in optical density in test tubes was recorded as evidence of growth [7].

Glucose tolerance test: A loopful of freshly cultured yeast isolates were inoculated into 10 ml of YEDP broth of seven different concentrations of glucose (0%, 5%, 10%, 15%, 20%, 25% and 30% w/v). The inoculated tubes were incubated at 30 °C for two days. The growth of the inoculated yeast isolates was examined and their optical density was recorded at 600 nm using a spectrophotometer [3].

Ph tolerance test: The YEPD liquid medium with different pH was used for the test. A loopful of fresh yeast cultures were inoculated into 10 ml of YEDP broth of seven different pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 pH) levels. The inoculated tubes were incubated at 30°C for two days. Blank was made of YEPD medium without yeast inoculation. The growth of the inoculated isolates was examined and their optical density was recorded at 600 nm by using a spectrophotometer. The increase in optical density in a test tube was recorded as evidence for the pH tolerance [3].

Growth of yeast isolates on different carbon source: Yeast isolates were examined for their ability to grow on different carbon sources *i.e.,* Dextrose, Maltose, Sucrose and L-arabinose. Carbon sources were substituted in the place of glucose in YEPD media then sterilized and poured into the Petri plates followed by streaking with freshly cultured yeast isolates and incubated at 30 °C for 24 h. Results indicated that if growth is seen then it is considered positive and if no growth is seen it is indicated as negative [8].

3. RESULTS AND DISCUSSION

3.1 Isolation of Yeast

In total 26 yeast isolates were isolated from collected fruit samples (Fig-2 Dragon fruit - 4, Pomegranate -3, Papaya - 5 and 12 from Grapes *i.e*., Manjari Naveen - 4, Wine variety 1- 3, Wine variety 2 - 2 and Medica variety -3). Similar results were found by Kasa et al. [9] isolated different yeast strains from papaya and grape fruit pulps. Shikha et al. [4] isolated 13 yeasts (Y1 to Y13) from fruits samples such as banana, citrus, mango, apple and grapes. Most of the yeast isolates were found in fruits because of the high sugar content of fruits.

3.2 Population of Yeast Isolates

Population of the yeast isolates ranged from 2.00 cfu/ml to 58.50 cfu/ml. Yeast colony isolated from grapes and fig showed maximum (Wine variety1- 58.50 cfu/ml, Manjaree Naveen- 43.30 and Fig- 41.50 cfu/ml) count and yeast colony isolated from the Dragon fruit recorded least count (2.00 cfu/ml). Similarly, Chand et al. [10] recorded the densities of yeast isolates on the surface of pear fruits collected from four different places were approximately 7.3 x 10^3, 6.4 X 10^3, 4.1 X 10^3 and 9.9x 10^3cfu/cm, respectively.

3.3 Characterization of Yeast Isolates

All 26 yeast isolates showed different colours like white, dull white, milky white, light brown, pink and orange colours were recorded. The colony appearance of the isolates varied from oval to circular or irregular to round. Results were in accordance with studies of Lakatosova et al. [11] identified different coloured, pure yeast cultures isolated from grapes and yeast cells differ in shape, many of isolated yeasts were entirely convex, however some of them had a reduced margin or pulvinated tip.

3.4 Screening of Yeast Isolates for Its Potentiality

Sugar fermentation test: All 26 yeast isolates showed positive for sugar fermentation test. The results pertaining to the test are represented in Table 1. Similar findings were observed by Melo et al. (2007) out of the 54 yeasts isolated from the fruits of the "umbu" tree, 50 presented high-fermentative ability (gas production ≥ 50% Durham tube after 24 h) and Similarly, Maimer and Busse [12] showed that *Saccharomyces cerevisiae* and *Torulaspora delbrueckii,* these strains also produced gas within a short time period.

Ethanol tolerance test: The Table 2 represents the ethanol tolerance ability of the yeast isolates. In this all 26 yeast isolates tolerated up to 10 per cent v/v ethanol and only two isolates RPAY-5 (Raichur Papaya Yeast- 5 and RDY-5 (Raichur Dragon Yeast- 5) showed highest cell growth 1.395 and 1.283 (OD) respectively at 20 per cent v/v ethanol. There was a gradual decrease observed in the yeast growth due to an increase in ethanol concentration. Ethanol inhibits yeast growth, cell division, decreases cell volume and even the specific growth rate. Whereas the high concentration of ethanol reduces cell vitality and increases cell death [13].

Similar results were obtained by Tikka et al. [14] isolated seven strains of *S. cerevisiae* obtained from different fruit sources screened for ethanol tolerance showed a range of ethanol tolerance levels between 7 to 12 per cent in all the strains.

Glucose tolerance test: There was an increase in yeast cell growth with an increase in the glucose concentration up to 25 per cent w/v and further there was decrease in the growth as the concentration increased, where only 23 isolates tolerated glucose concentrations up to 25 per cent w/v and showed further decrease in cell growth (Table 3). Other three isolates RPAY-5 (3.055), RDY-5 (2.993) and RGMN-5 (1.910) tolerated glucose concentration of 30 per cent w/v. This implies that the yeast strains can remain metabolically active in the fermentation medium containing glucose and utilize these sugars and convert them to alcohol during fermentation. High sugar centralization prompts the high osmotic weight of yeast which causes low level of yeast development [15].

Similar findings were reported by Ali et al. [16] and Balia et al. [17] reported that isolates with the highest OD at 30 per cent glucose concentration (2.215) gained by *Candida tropicalis*. Similar results were observed by Arekar and Lele, [18] that the isolates FJ 10 and KF 01 showed significant glucose tolerance up to 10 to 25 per cent w/v while a drastic decrease in the biomass was observed thereafter.

pH tolerance test: All 26 yeast isolates showed pH tolerance from 3 to 6 pH and isolates RPAY-5 (2.053 and 2.893 OD) and RDY-5 (2.021 and 2.862 OD) showed better growth at pH 3 and 6 respectively (Table 4). The range of optimum pH is better for the activity of plasma membrane-bound proteins, including enzymes and transport proteins of yeast [19]. Similar results were obtained by Alabere et al. [3] among the 13 isolates tested the five isolates identified were able to tolerate pH range of 3.0 to 4.5. Same result was observed by Jangra et al. (2018) isolated 5 yeast isolates and characterized for pH tolerance showed that tolerance from pH 2 to 5.

Table 1. Sugar fermentation ability of yeast isolates of different fruit samples

Sl.no	Isolates	Sugar fermentation	Sl.no	Isolates	Sugar fermentation
1	RFY-1	+	14	RPOY-3	+
2	RFY-2	+	15	RGMN-1	+
3	RDY-1	+	16	RGMN-2	+
4	RDY-2	+	17	RGMN-3	
5	RDY-3	+	18	RGMN-4	+
6	RDY-4	+	19	RGW1-1	+
7	RPAY-1	+	20	RGW1-2	+
8	RPAY-2	+	21	RGW1-3	+
9	RPAY-3	+	22	RGW2-1	+
10	RPAY-4	+	23	RGW2-2	+
11	RPAY-5	+	24	RGW2-3	+
12	RPOY-1	+	25	RGM-1	+
13	RPOY-2	+	26	RGM-2	+

Note: +: Positive and -: Negative; RFY (Raichur Fig Yeast), RDY (Raichur Dragon Yeast), RPAY(Raichur Papaya Yeast), RPOY(Raichur Pomegranate Yeast)

Table 2. Ethanol tolerance ability of yeast isolates of different fruit samples

Sl.no	Isolates	OD values at 600 nm				
		0%(v/v)	5%(v/v)	10% (v/v)	15% (v/v)	20%(v/v)
1	RFY-1	2.481	2.401	2.029	1.275	0.639
2	RFY-2	2.882	2.572	2.030	1.145	1.083
3	RDY-1	1.280	1.232	1.152	1.012	0.915
4	RDY-2	2.760	2.715	2.301	1.315	0.902
5	RDY-3	2.742	2.632	2.287	2.015	0.505
6	RDY-4	2.950	2.815	2.401	2.083	1.283
7	RPAY-1	2.471	2.342	2.112	1.520	1.087
8	RPAY-2	2.862	2.621	2.415	1.325	1.012
9	RPAY-3	2.431	2.323	2.282	1.246	1.079
10	RPAY-4	2.980	2.853	2.510	2.119	1.395
11	RPAY-5	2.812	2.655	1.754	1.473	1.003
12	RPOY-1	2.561	2.521	2.386	2.052	1.102
13	RPOY-2	1.921	1.752	1.686	1.453	1.015
14	RPOY-3	1.560	1.535	0.982	0.802	0.723
15	RGMN-1	2.250	2.114	1.467	1.075	0.865
16	RGMN-2	2.601	2.493	2.302	2.053	1.013
17	RGMN-3	2.391	2.239	2.192	1.202	0.852
18	RGMN-4	2.240	2.150	2.024	1.388	1.053
19	RGW1-1	2.571	2.438	2.323	1.282	1.094
20	RGW1-2	1.942	1.910	1.789	1.465	1.083
21	RGW1-3	2.761	2.546	2.205	1.659	1.034
22	RGW2-1	2.460	2.242	2.120	1.368	1.062
23	RGW2-2	2.091	1.713	1.683	1.371	0.952
24	RGW2-3	2.532	2.250	2.126	1.260	0.974
25	RGM-1	2.420	2.231	2.023	1.822	0.578
26	RGM-2	2.752	2.713	2.532	1.613	1.047

Table 3. Glucose tolerance ability of yeast isolates of different fruit samples

Sl.no	Isolates	OD values at 600nm						
		0 %(w/v)	5% (w/v)	10% (w/v)	15% (w/v)	20% (w/v)	25% (w/v)	30% (w/v)
1	RFY-1	0.153	1.164	1.762	1.797	1.960	1.976	1.890
2	RFY-2	0.335	2.405	2.541	2.624	2.702	2.822	1.903
3	RDY-1	0.180	1.604	1.641	2.392	2.591	2.633	1.832
4	RDY-2	0.074	1.548	2.136	2.230	2.823	2.521	1.782
5	RDY-3	0.154	2.341	2.412	2.513	2.902	2.015	1.694
6	RDY-4	0.360	2.544	2.910	2.925	2.961	2.980	2.993
7	RPAY-1	0.321	1.448	1.914	1.953	1.972	2.015	1.293
8	RPAY-2	0.094	1.947	2.295	2.386	2.732	2.913	1.321
9	RPAY-3	0.320	1.043	1.075	1.082	1.234	1.291	0.990
10	RPAY-4	0.475	2.702	2.963	2.982	2.990	3.023	3.055
11	RPAY-5	0.207	2.418	2.750	2.822	2.843	2.892	1.905
12	RPOY-1	0.232	1.503	1.832	1.882	1.975	2.892	1.903
13	RPOY-2	0.134	1.173	1.815	1.833	2.210	2.154	1.713
14	RPOY-3	0.301	1.764	1.854	1.891	1.903	1.972	1.673
15	RGMN-1	0.200	0.490	0.863	0.868	0.902	0.951	0.742
16	RGMN-2	0.342	1.667	2.591	2.795	2.851	2.903	1.620
17	RGMN-3	0.092	0.447	0.918	1.220	1.815	2.072	1.374
18	RGMN-4	0.135	1.011	1.211	1.514	1.586	1.643	1.910
19	RGW1-1	0.019	1.037	1.404	2.400	2.532	2.854	1.843
20	RGW1-2	0.067	0.290	0.649	0.732	0.854	0.995	0.732
21	RGW1-3	0.234	0.281	1.075	1.240	1.865	1.945	1.284

Sl.no	Isolates	OD values at 600nm						
		0 %(w/v)	5% (w/v)	10% (w/v)	15% (w/v)	20% (w/v)	25% (w/v)	30% (w/v)
22	RGW2-1	0.102	0.847	1.410	1.522	1.750	1.831	1.415
23	RGW2-2	0.084	1.583	1.642	1.693	1.764	1.782	1.325
24	RGW2-3	0.158	1.765	1.982	1.990	2.401	2.643	1.110
25	RGM-1	0.073	0.942	1.084	1.225	1.819	2.486	1.289
26	RGM-2	0.142	0.470	0.510	0.632	1.605	2.093	1.144

Table 4. pH tolerance ability of yeast isolates of different fruit samples

S.no	Isolates	OD values at 600 nm						
		pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
1	RFY-1	0.182	0.293	0.317	0.726	0.613	0.261	0.142
2	RFY-2	0.634	1.703	2.652	2.821	2.551	1.102	0.653
3	RDY-1	0.554	1.062	2.023	2.231	2.102	1.335	0.592
4	RDY-2	0.762	1.610	2.431	2.802	2.725	1.125	0.280
5	RDY-3	0.732	1.521	2.615	2.785	1.983	1.365	0.532
6	RDY-4	0.851	2.021	2.678	2.853	2.862	1.463	0.754
7	RPAY-1	0.532	1.034	1.504	1.915	1.580	1.062	0.315
8	RPAY-2	0.529	1.725	2.605	2.642	1.776	0.843	0.256
9	RPAY-3	0.493	1.182	1.843	1.932	1.421	0.654	0.351
10	RPAY-4	0.905	2.053	2.782	2.881	2.893	1.596	0.802
11	RPAY-5	0.530	1.043	2.540	2.161	1.523	0.995	0.341
12	RPOY-1	0.523	1.965	2.163	2.384	1.755	1.253	0.223
13	RPOY-2	0.533	1.285	1.456	1.801	0.840	0.563	0.321
14	RPOY-3	0.543	2.003	2.384	2.535	1.462	1.021	0.412
15	RGMN-1	0.793	1.762	1.932	1.956	0.773	0.362	0.261
16	RGMN-2	0.563	1.984	2.463	2.142	0.945	0.812	0.703
17	RGMN-3	0.752	1.945	2.563	2.785	1.703	1.092	0.371
18	RGMN-4	0.523	1.391	1.441	1.563	0.952	0.823	0.335
19	RGW1-1	0.531	1.262	2.151	2.193	1.892	1.076	0.402
20	RGW1-2	0.541	1.124	2.349	2.544	1.575	1.052	0.392
21	RGW1-3	0.753	1.690	2.344	2.452	1.791	1.225	0432
22	RGW2-1	0.521	1.962	2.310	2.492	2.034	1.375	0.442
23	RGW2-2	0.523	1.200	1.754	2.351	2.015	1.112	0.221
24	RGW2-3	0.514	1.972	2.359	2.421	2.034	0.985	0.402
25	RGM-1	0.721	2.002	2.535	2.632	1.642	1.021	0.363
26	RGM-2	0.517	0.972	1.943	1.982	1.954	0.841	0.323

Table 5. Utilization of different carbon sources by yeast isolates of different fruit samples

Sl.No	Isolates	D	L	M	S	Sl.No	Isolates	D	L	M	S
1	RFY-1	+	+	+	+	14	RPOY-3	+	+	+	+
2	RFY-2	+	-	+	+	15	RGMN-1	+	+	+	+
3	RDY-1	+	+	+	+	16	RGMN-2	+	+	-	+
4	RDY-2	+	-	+	+	17	RGMN-3	+	+	+	+
5	RDY-3	+	+	-	+	18	RGMN-4	+	+	+	+
6	RDY-4	+	+	+	+	19	RGW1-1	+	-	+	+
7	RPAY-1	+	+	+	+	20	RGW1-2	+	+	+	+
8	RPAY-2	+	-	+	+	21	RGW1-3	+	+	-	+
9	RPAY-3	+	+	+	+	22	RGW2-1	+	+	+	+
10	RPAY-4	+	+	+	+	23	RGW2-2	+	-	+	+
11	RPAY-5	+	+	+	+	24	RGW2-3	+	+	+	+
12	RPOY-1	+	-	+	+	25	RGM-1	+	+	+	+
13	RPOY-2	+	+	+	+	26	RGM-2	+	+	+	+

Note: + = Positive - = Negative, D = Dextrose, L = L-arabinose, M = Maltose, S= Sucrose

Growth of yeast isolates on different carbon source: All the isolates tested showed growth on all the carbon sources except the isolates RDY-3, RPAY-2, RPOY-4, RGW1-2 and RGW2-2 which did not show any growth on L- arabinose media plates and isolates RDY-4, RGMN-2 and RGW1-4 did not show any growth on the maltose containing media. The results are represented in Table 5. Where lack of growth indicates a lack of enzymes for utilizing the test sugar or carbon. The results obtained are in accordance with findings of Hospet et al. [5] reported that five yeast isolates exhibited good Glucose fermentation activity and good growth dynamics in the utilization of several sugars such as Sucrose, Fructose, Maltose and Xylose these isolates were preliminarily characterized as *Saccharomyces spp*. Similarly, Matapathi et al. [20] reported that yeast isolated from the pomegranate fruits have the ability to assimilate different carbon sources like Glucose, Galactose, Sucrose and Maltose.

4. CONCLUSION

A total of 62 morphologically different yeast species were isolated from collected fruit samples which includes Fig, Grapes, Pomegranate, Papaya and Dragon fruits. Yeast colonies showed different morphological appearance, colour ranging from dull white, white, white milky white pink and orange. Cell shape of the yeast varied from ovoid to elongated and all isolates showed budding nature. All 26 isolates were positive for gas production which indicates that 26 isolates have better fermentation ability. All the yeast isolates tolerated up to 10 per cent v/v ethanol and only two isolates RPAY-5 and RDY-5 showed highest cell growth at 20 per cent v/v ethanol. All the isolates showed tolerance from 3 to 6 pH indicating that yeast grows well at acidic pH. Whereas, 95 per cent of isolates showed positive growth on different carbon sources, so the isolates which showed positive result can also use Maltose, L- arabinose, Sucrose and Dextrose as carbon sources for their growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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