Original Research Article

Isolation, characterization and screening of yeast isolates from different fruit samples of Raichur district.

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, Dragon fruit and Grapes were collected from different places of Raichur district. Totally 26 yeast isolates were isolated from collected fruit samples. In this all yeast isolates showed colour characters like dull white, white milky 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 showed positive for sugar fermentation test, in this 95 per cent grown on different carbon source and in ethanol, glucose and pH tolerance test the yeast isolate RPAY 5 shown better results (2.510@10% v/v, 3.055 @30% and 2.862@ pH 6 respectively).

Key words – Yeast, RPAY- 5

Introduction

Yeast is the one of the most important microorganism in preparation of some food items and 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. Some of yeasts isolates produces pigments of different colours like pink orange yellow and red, also they have 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 (Ribereau *et al*. 2006).

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 it's wider adaptability in nature (Rainieri and Pretorius, 2000).

II. Materials and methods

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 (Alabere *et al*. 2020). Details of the fruit samples collected are presented in list 1.

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

Sl.no	Fruit name	Location	Variety(s)

Comment [J1]: Isolation, morphological characterization and screening of yeast isolates from different fruit samples obtained from Raichur district.

Comment [J2]: In total, 26 yeast species were isolated from

Comment [J3]: Colour characteristics

Comment [J4]: Milky white, pinkish-white, orange

Comment [J5]: were

Comment [J6]: what is RPAY-5?

Comment [J7]: the sentence is not clear. Recast

Comment [J8]: The procedure used has to be captured briefly in your abstract

Comment [J9]: Use five keywords in all

Comment [J10]: Revisit your abstract and if possible rewrite it in details

Comment [J11]: And in wine industry

Comment [J12]: Reference

Comment [J13]: Pink, orange, yellow and red.

Comment [J14]: They also possess smooth to rough

Comment [J15]: (Ribereau et al., 2006).

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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

Isolation of yeast

Fruits were cut with the sterilized knife and 20 gm of each fruit sample was taken in 250 ml flask containing 100 ml of distilled water, smashed 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 following the spread plate method. Plates were incubated for 24 h at 30°C (Shikha *et al*., 2020). The isolates were maintained in slants containing YEPD media and were kept in the refrigerator at 4°C for further use.

Comment [J18]: State the type of flask

Comment [J19]: Use the right word and not smashed

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Comment [J21]: 30 °C

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Morphological characterization

Morphological characters of yeast isolates such as shape and colour of colony was recorded. 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 (Hospet *et al*. 2019).

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Screening of yeast isolates

Sugar fermentation test

The isolates were inoculated into a test tube containing YEPD broth (15 g peptone, 10 g yeast extract, 20 g 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 (Alabere *et al*. 2020).

Comment [J26]: (Alabere et al., 2020).

Ethanol tolerance test

The modified Osho (2005) method was used for ethanol tolerance of yeast isolates. One ml of various 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 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 (Iticha and Taye, 2016).

Comment [J27]: One ml each of different concentrations of absolute ethanol was taken *i.e.*, 0, 5, 10, 15 and 20

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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 (Alabere et al., 2020).

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 pHs (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 (Alabere et al., 2020).

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 (Easterling et al., 2009).

Results and discussion

Isolation of yeast

Totally 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*., (2015) isolated different yeast strains from papaya and grape fruit pulps. Shikha *et al*., (2020) 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 it's because of the sugar content of fruits.

Comment [J30]: Kasa et al. (2015), who isolated

Comment [J31]: Shikha et al. (2020) isolated

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Comment [J33]: combine your paragraphs to make it one paragraph

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*. (1996) 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.

Comment [J34]: reference is too old. Update and write it properly, see other corrections above

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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*., (2014) identified different coloured, pure yeast cultures isolated from grapes and yeast cells differ in shape, many of the isolated yeasts were entirely convex, however some of them had a reduced margin or pulvinated tip.

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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 (1992) showed that *S. cerevisiae* and *T. delbrueckii*, these strains also produced gas within a short time period.

Comment [J37]: too old

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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 and RDY-5** showed highest cell growth 1.395 and 1.283 (OD) respectively at 20 per cent v/v ethanol.

Comment [J41]: what is PRAY-5 and RDY-5

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 (Kechkar *et al*. 2019).

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Similar results were obtained by Tikka *et al*. (2013) 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.

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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 (Arroyo *et al*. 2009).

Similar findings were reported by Ali *et al*., (2014) and Balia *et al*., (2018) reported that isolates with the highest OD at 30 per cent glucose concentration (2.215) gained by *C. tropicalis*. Similar results were observed by Arekar and Lele, (2015) 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.

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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 (Nasir *etal*. 2017).

Comment [J45]: (Nasir et al., 2017).

Similar results were obtained by Alabere *et al*., (2020) 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.

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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 (Sandven, 1990).

Comment [J47]: update reference and write it correctly

The results obtained are in accordance with findings of Hospet *et al*., (2019) 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*., (2010) reported that yeast isolated from the pomegranate fruits have the ability to assimilate different carbon sources like Glucose, Galactose, Sucrose and Maltose.

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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

Comment [J49]: interpret all your coding properly below the table i.e. RFY-1 meaning etc

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

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		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
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.

		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

Comment [J51]: Note: + = Positive - = Negative, D = Dextrose, L = L-arabinose, M = Maltose, S = Sucrose

Conclusion

A total of 62 morphologically different yeast isolates were isolated from collected fruit samples *i.e.*, Fig, Grapes, Pomegranate, Papaya and Dragon fruits. Yeast colonies showed different colour dull white, white, white milky white pink and orange. Cell shape of the yeast varied from ovoid to elongate 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 as yeast growth 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.

Comment [J52]: species

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Comment [J54]: morphological appearance

Comment [J55]: ranging from dull white, white, white milky white pink to orange

Comment [J56]: elongated

Comment [J57]: indicating that yeast grows

References

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Comment [J58]: General comments
1. The usage of English has to be improved on in the writing
2. The procedure should be detailed enough and reproducible by someone else
3. Most of the references have to be updated
4.The choice of paragraph has to be corrected
5. Sentence formation must be improved on for better understanding by the reader