Original Research Article

ISOLATION AND CHARACTERIZATION OF YEASTS FROM JACKFRUIT AND BEET ROOT JUICES

ABSTRACT

Aim: Yeasts are present as natural microflora on surface of fruits and vegetables that have eminent importance from deputing as biocontrol agents to reduce pathogens of fruits and vegetables to the health benefits of humans. Yeast are used as starter cultures in food fermentation

Materials and methods: Yeasts were isolated using standard plate count technique. Colony morphology of yeasts isolates were characterized under microscope for their cell shapes and sizes. Biochemical characteristics of yeasts were characterized for presence of effervescence in catalase test and production of acid and gas in YEPDA broth. Utilization of carbon sources were evaluated by inoculating yeast isolates in YEPDA broth with specific carbon source and phenol indicator. Ethanol tolerance were evaluated inoculating yeast isolates in YEPDA broth having different concentrations of ethanol *viz.,* 0%, 5.0 %, 10.0 %, 12.5 % and 15.0 %.

Results: Population of yeast in jackfruit juices varied from 20.5 x 10^3 to 70.0 x 10^3cfu per millilitre. Population of yeast varied in beet root juices from 34.5 x 10^3 to 43.0 x 10^3 cfu per millilitre. Juice of Chandra variety of jackfruit recorded the highest yeast population (7.0 x 10^4) when compared to others. Juice of Detroit red dark variety of beetroot (4.3 x 10^4) recorded highest yeast population (4.3 x 10^4) when compared to others. Yeasts were also isolated from different fruits of which BRTY-I, JKFY-I, POMY-I, WATY-I, APLY-I, MUSKY-I and PINY-I were able to grow in jackfruit and beet root juices. The colonies of yeast varied from white to cream and few were pink. The cell shape varied from oval to ellipsoidal. All the yeast isolates were catalase positive. Most of the yeast isolates produced acid and gas. Yeast isolates were able to utilize glucose, galactose and sucrose. None of the yeast were able to utilize lactose. The highest ethanol tolerant yeasts were JCVY-I, JSVY-III, BDVY-I, PGY, *Saccharomyces boulardii* and *Saccharomyces cerevisiae* var. *ellipsoideus* NCIM 3207.

Keywords: Yeasts, utilization of sugars, ethanol tolerance, jackfruit juice and beet root juice.

1. INTRODUCTION

Fruits and vegetables provide a suitable niche to several bacteria, yeasts and moulds (Trias *et al.,* 2008). The term yeast was originally derived from the Dutch word “gist” which refers to the foam

formed during the fermentation of beer wort. Yeast are single celled eukaryotic microorganisms belonging to ascomycetes or basidiomycetes genera of fungi that produce vegetatively by budding or fission and form sexual states which are not enclosed in a fruiting body (Boekhout and Kurtzman, 1996).

Jackfruit (*Artocarpus heterophyllus*) is an ancient and most desirable fruit that is known for its therapeutic and nutritive values. Jackfruit contains vitamins A and C, thiamin, riboflavin, calcium, potassium, iron, sodium, zinc, niacin and has antioxidants (Hettiarachchi *et al.*, 2011). The juicy pulp of the ripe fruit is eaten fresh and has wide potential for preparing different food items due to the presence of protein (Elevitch and Manner, 2006). Jackfruit is rich in potassium which helps in lowering blood pressure by reversing the effects of sodium. It is good source of vitamin C essential for the production of collagen that gives firmness and strength to the skin and also maintains oral health. Dietary fibres present in jackfruit makes it a good bulk laxative (Ranasinghe, 2019).

Beet root (*Beta vulgaris*) on other hand is a vegetable loaded with significant levels of calcium, iron, magnesium, fibres and folate as well as vitamins A and C. (Panghal *et al.,* 2017). In contrast to other fruits which are rich in fructose, beet roots have more of sucrose and small amounts of glucose and fructose (Bavec *et al.,* 2010). The juice of beetroot is consumed as a natural remedy for sexual weakness and to reduce kidney and bladder stones. The therapeutic use of beetroot includes its antitumor, carminative, emmenagogue, hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions (Dambalkar *et al.,* 2015).

Several factors could limit viability and survival in juices. As suggested by Tripathi and Giri (2014), the major influencing parameters are intrinsic food parameters, such as pH, titrable acidity, molecular oxygen, water activity, presence of salt, sugar, artificial flavoring, coloring agents, chemical or microbial preservatives like hydrogen peroxide and bacteriocins, processing parameters such as extent of heat treatment, incubation temperature, cooling rate, volume, packaging materials, storage techniques and microbiological factors which mainly includes kind of strains, compatibility of different strains, inoculums proportion and rate. Among all these, pH is one of the chief significant factors affecting the probiotic viability.

Fermentation of fruits and vegetables occur spontaneously on their surfaces. Fermentation of milk, cereals and other substrates are indigenous to many regions of Asia, Africa, Europe, the Middle East and South America to produce beverages with health-promoting properties. Fermentation process increases its shelf life by enhancing nutritional value of substrates, bioavailability of nutrients, enzyme concentration and by neutralizing several toxic substances (Septembre *et al.,* 2018).It is an effective way to preserve perishable food products. Yeast such as *Saccharomyces cerevisiae* are used as starter cultures in food fermentation. Apart from their use as starter cultures, some have probiotic properties, *Saccharomyces cerevisiae* var. *boulardii* and *Kluyveromyces marxianus* which are added to prepare beverages with therapeutic properties (Moradi *et al.,* 2018).

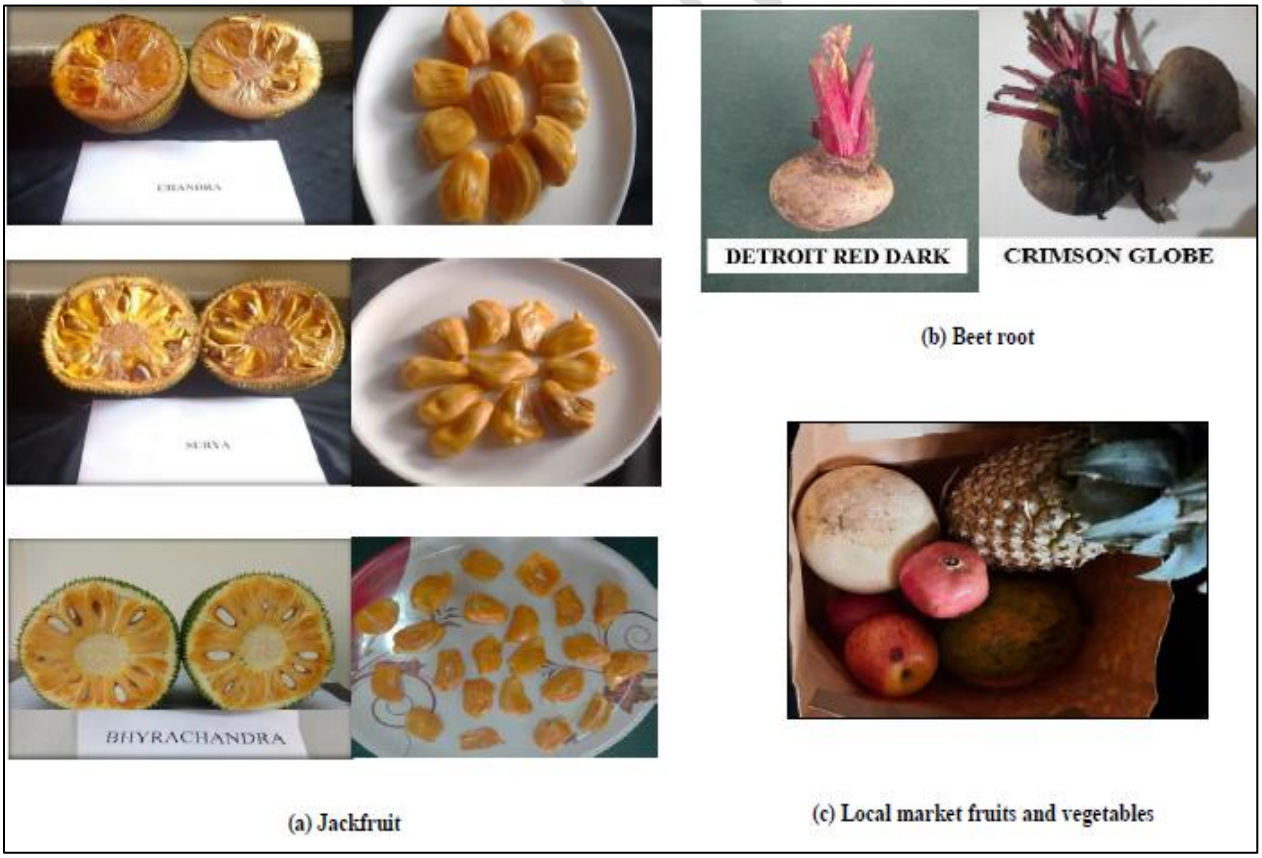
In developing countries, it is assessed that 50 % of production is lost during storage due to infection caused by soft rot bacteria and fungi (Gunny *et al*., 2021). Therefore, the excess fruits and vegetables can be processed and nutrient bioavailability can be enhanced. In recent time several raw materials have been extensively explored to determine if they are appropriate substrates to produce novel non-dairy functional foods (Vasudha and Mishra, 2013).

2. MATERIAL AND METHODS

Isolation of yeasts

Yeasts were isolated from jackfruit and beet root samples collected from nearby villages. Yeasts were also isolated from pomegranate, apple, watermelon, papaya, pineapple and muskmelon obtained from local markets. Standard plate count method was used for isolation of yeasts.

One milliliter (ml) of samples were suspended in 100 ml sterile distilled water blanks. Diluted samples of 0.1 ml were spread on solidified Yeast Extract Peptone Dextrose Agar (YEPDA) media plates for isolation of yeasts. The dilutions from 10^{-1} to 10^{-3} were used in the isolation of yeasts. The petridishes containing yeasts were incubated for 24-48 hours at room temperature. Reference strains of *Saccharomyces cerevisiae* var. *ellipsoideus* NCIM 3207 and Commercial strain of *Saccharomyces boulardii* (Econorm) were streaked on YEPDA.



(a) Jackfruit

(b) Beet root

(c) Local market fruits and vegetables

Fig 1: Isolation of yeast from different sources

The yeast cultures were streaked on YEPDA agar plates to obtain pure cultures. The cultures were also maintained on agar slants for further analysis. The observations regarding colony characteristics were recorded and also compared with the reference and commercial strains. The total soluble solids of the samples (TSS) were also assessed with the help of 'ERMA' hand refractrometer having a range of 0-32˚ Brix at room temperature.

Morphological characterization of yeasts

The colony morphology of isolated yeasts were studied by streaking on YEPDA media and incubating it for 48 hours at 28° C. After incubation, the colony morphological characters such as colour, texture and margins were observed as tools for preliminary identification. Cell shape of 1 day old cultures were observed under microscope (40X and 100X) using wet mount method. The observations were recorded as ellipsoidal or oval in shape (Aneja, 2018).

Biochemical characterization of yeasts

Catalase test

A drop of yeast culture grown in YEPDA broth was added to a clean grease free slide, on it 0.3% hydrogen peroxide solution was added. Observation of immediate effervescence was recorded. Formation or absence of bubbles were indicated as catalase positive or catalase negative respectively (Balazevic and Ederes, 1975).

Utilization of different carbon sources

Yeast isolates were inoculated to test tubes containing yeast extract peptone broth with a specific carbohydrate (Glucose, dextrose, lactose, mannitol, D-sorbitol and sucrose) and phenol red indicator (pH indicator). Red colour of phenol indicator at neutral pH changes to yellow at pH of 6.8 and below. Durham's tube were also added for detection of gas production. The test tubes were incubated at room temperature and observations were recorded after 48 hours (Nwachukwu *et al.,* 2015).

Ethanol tolerance

The experiment was conducted to evaluate the ethanol tolerance of yeast isolates at different concentrations of 0 (control), 5, 10 and 15 per cent (v/v). The yeast isolates were grown in YEPDA medium with varying quantities of ethanol with initial cell density of 2×10^6 cfu/ml and were incubated at 30 °C. Ethanol tolerance was measured in terms of turbidity (OD values) after different intervals for a period of 24 and 48 hours using spectrophotometer at 600 nm (Khaing et al., 2008).

3. RESULTS

Isolation of yeasts

Jackfruit varieties, beet root varieties and other fruits and vegetables were collected from different locations of Karnataka and local super markets respectively. Yeasts were isolated from the juices of jackfruit, beet root and other fruits and vegetables *viz.,* pomegranate, apple, watermelon, papaya, pineapple and muskmelon (Plate 1) by employing Standard Plate Count Method.

Population of yeast varied from 4.1 to 7.0 x 10^4 in jackfruit juice. Population of yeast varied from 4.1 to 4.3 x 10^4 in beet root juice. Juice of Chandra variety of jackfruit recorded the highest yeast population of 7.0 x 10^4. Juice of Detroit red dark variety of beetroot recorded the highest yeast population of 4.3 x 10^4 when compared to others. The results are presented in Table 1.

Table No. 1: Yeast and lactic acid bacterial (LAB) population on different varieties of jackfruit and beet root

S. No.	Sources	Name of Varieties	TSS (°Brix)	Yeast population (x10^4 CFU/ml)	Location
1.	Jackfruit	Chandra	26.5°	7.0	Tubagere, Doddaballapur
		Surya	25.7°	6.2	Hosahalli, Kunigal
		Bhyrachandra	26.2°	6.8	GKVK, Bengaluru
2.	Beet root	Detroit red dark	10.0°	4.3	Sulibele, Hoskote
		Crimson globe	9.2°	4.1	Nandi, Chikkaballapura

Morphological characterization of yeast

Colony colour of yeast isolates varied from whitish to cream and few were pink**.** Whereas, cell shape of yeast varied from oval to ellipsoidal when observed under microscope (100 X). The results are presented in table 2.

Biochemical characterization of yeast

The catalase test was conducted to identify catalase positive and catalase negative yeast isolates. The presence of effervescence is noted as catalase positive (+) and absence of effervescence as catalase negative (-). All the yeast isolates were catalase positive (+). Most of the yeast isolates produced acid and gas except JCVY-IV, JSVY-III, JBVY-I, JBVY-II, BDVY-IV, AY and MMY. The results are presented in table 3.

All the yeast isolates were able to utilize glucose. Most of the yeast isolates were able to utilize galactose and sucrose. None of the yeast were able to utilize lactose. The results are presented in table 4. The ethanol tolerance of yeast isolates were tested at different concentrations *viz.,* 0%, 5.0 %, 10.0 %, 12.5 % and15.0 %. The highest ethanol tolerant yeasts were JCVY-I, JSVY-III, BDVY-I, PGY,

Saccharomyces boulardii and *Saccharomyces cerevisiae* var. *ellipsoideus* NCIM 3207. The results are presented in table 5.

Table No. 2: Colony morphology and cell shape of yeast isolates

S.No.	Name of Yeast Isolates	Isolates code	Colony colour	Margin	Cell shape
1.	Jackfruit Chandra variety yeast- I	JCVY-I	Whitish cream	Rough	Oval
2.	Jackfruit Chandra variety yeast- II	JCVY-II	Light creamish	Smooth	Ellipsoidal
3.	Jackfruit Chandra variety yeast- III	JCVY-III	Creamish	Smooth	Ellipsoidal
4.	Jackfruit Chandra variety yeast- IV	JCVY-IV	Light Creamish	Smooth	Ellipsoidal
5.	Jackfruit Surya variety yeast- I	JSVY-I	Whitish	Smooth	Oval
6.	Jackfruit Surya variety yeast- II	JSVY-II	Light creamish	Smooth	Oval
7.	Jackfruit Surya variety yeast- III	JSVY-III	Creamish	Smooth	Ellipsoidal
8.	Jackfruit Bhyrachandra variety yeast- I	JBVY-I	Light creamish	Smooth	Oval
9.	Jackfruit Bhyrachandra variety yeast- II	JBVY-II	Whitish	Smooth	Oval
10.	Jackfruit Bhyrachandra variety yeast- III	JBVY-III	Light creamish	Smooth	Ellipsoidal
11.	Beet root Detroit red dark variety yeast- I	BDVY-I	Creamish	Smooth	Oval
12.	Beet root Detroit red dark variety yeast- II	BDVY-II	Creamish	Smooth	Oval
13.	Beet root Detroit red dark variety yeast- III	BDVY-III	Light creamish	Smooth	Oval
14.	Beet root Detroit red dark variety yeast- IV	BDVY-IV	Whitish	Smooth	Oval
15.	Beet root Crimson globe variety yeast- I	BCVY-I	Creamish	Smooth	Oval
16.	Beet root Crimson globe variety yeast- II	BCVY-II	Light creamish	Smooth	Oval
17.	Beet root Crimson globe variety yeast- III	BCVY-III	Whitish	Rough	Oval
18.	Pomegranate yeast	PMY	Whitish	Smooth	Oval
19.	Water melon yeast	WMY	Creamish	Smooth	Oval
20.	Apple yeast	AY	Creamish	Smooth	Oval
21.	Musk melon yeast	MMY	Whitish	Smooth	Oval
22.	Pineapple yeast	PY	Creamish	Smooth	Oval
23.	Beet root yeast –I	BRY- I	Whitish	Smooth	Oval
24.	Beet root yeast –V	BRY- V	Pinkish	Smooth	Oval
25.	Jackfruit yeast	JFY	Whitish	Smooth	Oval
26.	*Saccharomyces boulardii* (Probiotic strain)	*S. boulardii*	Whitish	Smooth	Oval
27.	*Saccharomyces cerevisiae var. ellipsoideus* NCIM 3207 (Reference strain)	NCIM 3207	Whitish	Rough	Ellipsoidal

Table No. 3: Biochemical characteristics of yeast isolates

S.No.	Yeast isolates	Catalase Test	Acid production	Gas production
1.	JCVY-I	+	+	+
2.	JCVY-II	+	+	+
3.	JCVY-III	+	-	+
4.	JCVY-IV	+	-	-
5.	JSVY-I	+	+	-
6.	JSVY-II	+	-	+
7.	JSVY-III	+	-	-
8.	JBVY-I	+	-	-
9.	JBVY-II	+	-	-
10.	JBVY-III	+	+	-
11.	BDVY-I	+	+	-
12.	BDVY-II	+	+	+
13.	BDVY-III	+	+	+
14.	BDVY-IV	+	-	-
15.	BCVY-I	+	+	-
16.	BCVY-II	+	+	+
17.	BCVY-III	+	+	+
18.	PMY	+	+	+
19.	WMY	+	+	-
20.	AY	+	-	-
21.	MMY	+	-	-
22.	PY	+	+	+
23.	BRY- I	+	+	+
24.	BRY- V	+	+	-
25.	JFY	+	+	+
26.	*S. boulardii* (Probiotic strain)	+	+	+
27.	*Saccharomyces cerevisiae* var. *ellipsoideus* 3207 (Reference strain)	+	+	+

Note: '+'= Growth;'-'= No growth

Table No. 4: Utilization of different carbon sources by yeast isolates

S.No.	Yeast Isolates	Glucose	Galactose	Lactose	Mannitol	D-Sorbitol	Sucrose
1.	JCVY-I	+	-	-	-	-	+
2.	JCVY-II	+	+	-	-	-	+
3.	JCVY-III	+	+	-	-	-	+
4.	JCVY-IV	+	+	-	-	-	+
5.	JSVY-I	+	+	-	-	-	-
6.	JSVY-II	+	+	-	-	+	-
7.	JSVY-III	+	+	-	-	-	-
8.	JBVY-I	+	+	-	+	-	+
9.	JBVY-II	+	+	-	-	-	+
10.	JBVY-III	+	+	-	-	-	-
11.	BDVY-I	+	+	-	-	+	+
12.	BDVY-II	+	+	-	-	-	+
13.	BDVY-III	+	+	-	-	-	-
14.	BDVY-IV	+	+	-	-	-	-
15.	BCVY-I	+	+	-	-	-	-
16.	BCVY-II	+	+	-	-	-	+
17.	BCVY-III	+	+	-	-	-	-
18.	PGY	+	+	-	-	-	+
19.	WMY	+	+	-	-	-	+
20.	MMY	+	+	-	-	-	+
21.	AY	-	+	-	-	-	-
22.	PY	+	+	-	-	-	+
23.	BRY-I	+	+	-	+	+	+
24.	BRY-V	+	+	-	-	-	+
25.	JFY	+	+	-	-	+	+
26.	*Saccharomyces boulardii* (Probiotic strain)	+	+	-	-	-	+
27.	*Saccharomyces cerevisiae* var. *ellipsoideus* 3207 (Reference strain)	+	+	-	-	-	+

Note: '+'= Growth;'-'= No growth

Table No. 5: Ethanol tolerance of yeast isolates

S.No.	Yeast isolates	OD value @ 600 nm				
		Ethanol concentration %				
		0%	5.0 %	10.0 %	12.5 %	15.0%
1.	JCVY-I	0.65	0.62	0.59	0.52	0.16
2.	JCVY-II	0.46	0.44	0.37	0.16	0.02
3.	JCVY-III	0.45	0.42	0.38	0.02	0.10
4.	JCVY-IV	0.45	0.43	0.34	0.18	0.06
5.	JSVY-I	0.44	0.42	0.37	0.19	0.14
6.	JSVY-II	0.45	0.43	0.36	0.11	0.09
7.	JSVY-III	0.54	0.46	0.39	0.38	0.13
8.	JBVY-I	0.46	0.43	0.41	0.10	0.06
9.	JBVY-II	0.44	0.42	0.38	0.09	0.05
10.	JBVY-III	0.45	0.43	0.33	0.20	0.07
11.	BDVY-I	0.57	0.55	0.51	0.46	0.14
12.	BDVY-II	0.45	0.44	0.32	0.16	0.03
13.	BDVY-III	0.44	0.43	0.37	0.14	0.09
14.	BDVY-IV	0.45	0.44	0.37	0.13	0.07
15.	BCVY-I	0.44	0.43	0.42	0.12	0.06
16.	BCVY-II	0.45	0.44	0.34	0.23	0.05
17.	BCVY-III	0.47	0.42	0.40	0.12	0.05
18.	PGY	0.55	0.54	0.48	0.43	0.11

19.	WMY	0.54	0.53	0.42	0.12	0.03
20.	MMY	0.55	0.53	0.56	0.44	0.04
21.	AY	0.54	0.52	0.37	0.05	0.11
22.	PY	0.45	0.5	0.42	0.36	0.13
23.	BRY-I	0.44	0.43	0.36	0.35	0.15
24.	BRY-V	0.35	0.34	0.38	0.31	0.05
25.	JFY	0.54	0.52	0.55	0.46	0.13
26.	*Saccharomyces boulardii* (Probiotic strain)	0.72	0.73	0.63	0.50	0.17
27.	*Saccharomyces cerevisiae* var. *ellipsoideus* 3207 (Reference strain)	0.77	0.73	0.75	0.58	0.21

Note: '+'= Growth;'-'= No growth

4. DISCUSSION

In the present study, 25 yeast isolates were isolated from different varieties of jackfruit, beet root and other fruits. Yeasts were isolated from the juices of jackfruit, beet root and other fruits. Population of yeast and varied from 4.1 to 7.0 x 10^4 in jackfruit juice. Population of yeast varied from 4.1 to 4.3 x 10^4 in beet root juice. The microbial population on raw fruits and vegetables are estimated to be 10^5 to 10^7 CFU/g. Yeasts are most dominant group having population between 10^2 to 10^6 CFU/g. The most commonly found yeasts genera are *Saccharomyces, Candida, Pichia, Rhodotorula, Torulaspora, Cryptococcus, Hansenula* and *Debaromyces* (Pimentel *et al*., 2021). Dushyantha *et al.,* (2011) isolated yeasts from jackfruit phyllosphere, perianth lobes and juice. Yeast JFY1 was found to be effective strain in jackfruit wine making.

Colony colour of yeast isolates were mainly white or cream. Whereas, the cell shape of yeast varied from oval to ellipsoidal when observed under microscope (100 X). Ali and khan (2014) reported on seven yeast species from soil samples, fruits and fermented products and studied their morphological characteristics. Colonies formed by yeast isolates were round, smooth and cream, white to whitish cream colored. Individual cells were oval, elongate and ovoid to spherical when young and hexagonal when mature.

All the yeast isolates were catalase positive (+). Most of the yeast isolates produced acid and gas except JCVY-IV, JSVY-III, JBVY-I, JBVY-II, BDVY-IV, AY and MMY. Andreolli *et al.,* (2021) investigated on catalase activity of 13 basidiomyceteous yeast strains isolated from grapes, apples and relative juices and few identified in previous investigations. The results revealed that catalase activity was maximum in *Papiliotrema flavescens* Yz3 and *Papiliotrema terrestris* Yvp3.

Most of the yeast isolates utilized glucose, galactose and sucrose. None of the yeast were able to utilize lactose. The ethanol tolerant yeasts were JCVY-I, JSVY-III, BDVY-I, PGY, *Saccharomyces boulardii* and *Saccharomyces cerevisiae* var. *ellipsoideus* NCIM 3207 in varying levels of ethanol concentrations *viz.,* 0%, 5.0 %, 10.0 %, 12.5 % and15.0 %. Senapati *et al.,* (2021) reported on 12 yeasts species isolated from fruits of local markets of Assam that were catalase positive. The isolates had carbohydrate utilization ability to ferment different sugars namely glucose, lactose and mannitol. All strains were able to ferment glucose and mannitol. Isolates J7 and L1 were observed to be grown in 5%, 10%, 12%, 15% and 18% ethanol concentrations which is a very important character of the isolates to utilize them in fermentation process.

5. CONCLUSION

Juices of Chandra variety of jackfruit with 26.5 ºBrix and Detroit red dark variety of beetroot with 10.0 ºBrix recorded highest yeast population of 7.0 x 10^4 and 4.3 x 10^4 respectively when compared

to others varieties. The colonies of yeast isolates varied from white to cream and few were pink. Whereas, the cell shape varied from oval to ellipsoidal. All the yeast isolates were catalase positive. All the yeast isolates were able to utilize glucose. Most of the yeast isolates were able to utilize glucose, galactose and sucrose. None utilized lactose. The ethanol tolerance of yeast isolates were tested and highest ethanol tolerant yeasts were JCVY-I, JSVY-III, BDVY-I, PGY, *Saccharomyces boulardii* and *Saccharomyces cerevisiae* var. *ellipsoideus* NCIM 3207.

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