Isolation of wine yeast from sugar rich sources

Abstract

Total 21yeasts were isolated from different sugar rich sources (date palm, pomegranate, roselle, orange, grapes and dry date palm juice) collected from different locations of West Bengal, India. All isolated yeasts were identified and differentiated based on their colony morphology and growth pattern on Yeast Extract- Peptone -Dextrose (YEPD) Agar medium. The isolates could continue to survive on the slants up to maximum 6 months at 8-10°C temperature without any contamination. However, these were maintained by transferring to YEPD agar slants after every four months to maintain the purity and maximum viability of cultures. Fermentation was carried out at 26±1°C temperature at total soluble solid 24^{o}Brix and pH 4.70 in molasses medium for all different isolated yeast. The total soluble solid at the end of fermentation in molasses solution were found in the range of 9.00-20.78^{o}Brix in all isolated yeast. The pH of substrate was decreased (0.10-1.12) during fermentation. The cell count after complete fermentation was found in the range of 3.60×10^{8} cfu/ml to 8.79×10^{8} cfu/ml for all the isolated yeast. The concentration of alcohol in the molasses was observed at range from 4.21 to 13.61 %. After screening or checking out the efficiency of yeasts on the basis of highest alcohol production in molasses media, five yeasts ($Y_{4,}$ $Y_{101,}$ $Y_{33,}$ $Y_{102,}$ and Y_{7}) were selected for wine fermentation out of 24 (new isolates 21, previous isolations Y_{4}, Y_{7} and Y_{33}) at the end of 18 days fermentation.

Key Words: Different fruit juice, Fermentation, Sugar, Alcohol, Yeasts

Introduction

Fermentation is one of the oldest forms of food preservation technologies in the world. Indigenous fermented foods such as bread, cheese and wine, have been prepared and

consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities. The development of fermentation technologies is lost in the midst of history (Yokotsuka, 1985). Anthropologists have postulated that it was the production of alcohol that motivated primitive people to settle down and become agriculturists. Some even think the consumption of fermented food is pre-human (Stanton, 1985). The process of fermenting is basically feeding sugars and nutrients in solution to the fermenting yeast, which return the flavour by producing carbon dioxide gas and alcohol (Garrison, 1993). This process goes on until either all the sugar is used or the yeast can no longer tolerate the alcoholic percentage of the beverage. Different yeasts produce different results, and have different tolerance levels (Anon, 2005). Fermentation is a process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound (Klien *et al*., 2005), as opposed to respiration where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain**.** In alcoholic fermentation, the conversion of hexoses into ethanol and Co_2, forms the very basis of a successful wine fermentation.

The health benefits of (moderate) wine drinking are recognized by modern medicine. In Europe, a daily glass of red wine is often recommended to treat mild anemia; a class of chemicals called flavonoids (antioxidants in grape skins) appears to have many beneficial properties; another class of chemicals, called polyphenols, prevents (Angewandte, 2003) heart disease, arteriosclerosis and maybe some cancers (Vejarano and Lujan-Corro, 2022; Yu *et al.*, 2021).

Material and methods

The present investigation was made in respect of the isolation of wine yeast from sugar rich sources, after collected from different place of West Bengal. The experiment was conducted in the laboratory of Post Harvest Technology of Horticultural Crops, Directorate of Research complex, Bidhan Chandra Krishi Viswa Vidayalaya Kalyani, West Bengal. Molasses solution and different fruit juices were used for isolation of different yeast such as date palm, pomegranate, orange, black grape, dry date palm and roselle juice. The date palm juice was collected from different places of West Bengal during the year 2015-16. Completely randomized block design was adopted for statistical analysis of the data.

The isolation of yeasts, preservation and maintenance were estimated by composition of medium (Deak, 1991). Total soluble solids (T.S.S.) were estimated using a pocket refractometer. The titratable acidity was determined by titrating against 0.1N NaOH and expressed as anhydrous citric acid, the ascorbic acid (mg/100g) content of fruit was determined by using 2, 6 dichlorophenol-indophenol dye by visual titration method. Reducing sugars and total sugars content of the fruits were estimated following the standard method described by Ranganna, (2000). Cell count was estimated using a digital microscope. Alcohol content was estimated by pycnometric method using distillation assembly following the procedure expressed by Sadasivum and Munickam, (2008).

RESULT AND DISCUSSION

Over all 21 yeasts were isolated from different sugar rich sources and differentiated based on their colony morphology and growth pattern on Yeast Extract- Peptone - Dextrose (YEPD) Agar medium. The yeasts were isolated from date palm, pomegranate,

roselle, orange, grapes and dry date palm juice under different agro-climatic zones of West Bengal regions in India and enlisted in Table 1.

Table 1: Different yeast isolates from different sources with colony morphology

Sl. No.	**Yeast isolate**	**Source**	**Location (West Bengal)**	**Colony Morphology**
1	Y_{101}	Date palm Juice	Jaguli	Whitish, smooth and shiny
2	Y_{102}	Date palm Juice	Mohanpur	Whitish, rough and dry
3	Y_{103}	Date palm Juice	Hoogly	Whitish, smooth and shiny
4	Y_{104}	Date palm Juice	Bardhaman	Whitish, smooth and shiny
5	Y_{105}	Date palm Juice	D.R.Building	Off white, rough and dry
6	Y_{106}	Date palm Juice	C. Block	Off white, rough and dry
7	Y_{107}	Date palm Juice	Fatehhpur	Whitish, smooth and shiny
8	Y_{108}	Date palm Juice	Sadapare	Off white, smooth and shiny
9	Y_{109}	Date palm Juice	Pukarpare	Whitish, smooth and shiny
10	Y_{110}	Date palm Juice	Raghunathpur	Whitish, smooth and shiny
11	Y_{111}	Date palm Juice	Fatehhpur	Creamy white, smooth and shiny
12	Y_{112}	Date palm Juice	Jaguli	Whitish, smooth and shiny
13	Y_{113}	Date palm Juice	Jaguli Digha	Creamy white, smooth and

				shiny
14	Y_{114}	Date palm Juice	Mubarakpur	Dull white, smooth and shiny
15	Y_{115}	Date palm Juice	Tehatta	Off white, smooth and shiny
16	Y_{116}	Date palm Juice	Gayeshpur	Creamy white, smooth and shiny
17	Y_{117}	Pomegranate Juice	Mohanpur	White, rough and dry
18	Y_{118}	Roselle Juice	C. Block	White, rough and dry
19	Y_{119}	Orange Juice	Anukulmode	Creamy, smooth and shiny
20	Y_{120}	Grape Juice	Anukulmode	Creamy, smooth and shiny
21	Y_{121}	Dry date palm Juice	Anukulmode	White, rough and dry

Table 2: Growth of yeast at 26±1°C temperature on YEPD agar medium

Sl. No.	**Yeast**	**26±1°C**		
		16 hours	**24 hours**	**48 hours**
1	**Y_{101}**	+++	++++	++++++
2	**Y_{102}**	+++	++++	+++++
3	**Y_{103}**	+++	++++	+++++

4	**Y_{104}**	+	++	+++
5	**Y_{105}**	+	++	+++
6	**Y_{106}**	+	++	++
7	**Y_{107}**	+	++	+++
8	**Y_{108}**	+	++	+++
9	**Y_{109}**	++	+++	++++
10	**Y_{110}**	+++	++++	+++++
11	**Y_{111}**	+++	++++	+++++
12	**Y_{112}**	+++	++++	+++++
13	**Y_{113}**	+++	++++	+++++
14	**Y_{114}**	++	+++	+++++
15	**Y_{115}**	+++	++++	+++++
16	**Y_{116}**	++	+++	++++
17	**Y_{117}**	++	+++	++++
18	**Y_{118}**	++	+++	++++
19	**Y_{119}**	Nil	+	++
20	**Y_{120}**	++	+++	++++
21	**Y_{121}**	++	+++	++++

+ = Very poor growth ++ = Poor growth +++ = Slightly moderate growth

++++ = Moderate growth +++++ = Good growth ++++++ = Very good growth

Growth response of different yeasts at 26±1^{o}C temperature in incubation

Data presented in Table 2 on growth of yeasts at 26±1^{o}C temperature indicated that majority of yeast isolates started to grow within 16 hours of incubation at 26±1°C except Y_{119}. Ten isolates viz. Y_{101}, Y_{102}, Y_{103}, Y_{109}, Y_{110}, Y_{111}, Y_{112}, Y_{113}, $Y_{114,}$ and Y_{115} showed good to very good growth at 26±1°C. Yeast Y_{104}, Y_{105}, Y_{106}, Y_{107}, Y_{108}, Y_{116}, Y_{117}, Y_{118}, Y_{120} and Y_{121} showed poor to moderate growth at 26±1°C after 48 hours of incubation. The isolate Y_{119} failed to grow after 16 hours and after 24 hours growth was started at 26±1°C in incubation. The Y_{119} yeast also showed poor growth with respect to colony thickness development on YEPD agar medium at 26±1°C.

While growing the yeast isolates on the YEPD agar medium it was observed that, the isolates could continue to survive on the slants up to 6 months at 8-10°C temperature without any contamination on all slants accept Y_{101}, Y_{102}, $Y_{103,}$ Y_{111}, Y_{112} and Y_{113}. The survival was confirmed through transferring the same to the fresh YEPD agar slant and incubated at 26±1°C. The growth was observed by 24-hour, except the slants of Y_{119}. However, all the yeast isolated were maintained throughout the period of this investigation by transferring it to YEPD agar slants after every four months and preserving those at 8-10°C temperature to maintain the purity and maximum viability of cultures beyond any doubt.

It was also observed that in order to have significant growth on the YEPD agar slants, irrespective of extent of growth it had to be incubated for 24 - 48 hours at 26±1°C except above 48 hours for Y_{119}.

After observing the growth on the medium all the yeasts with prolong viabilities were tried to test their fermentation capability to produce ethyl alcohol. It was apparently confirmed through production of bubbles in the medium and the odour of alcohol, till the end of fermentation. In this study the yeasts Y_{101}, Y_{102}, $Y_{103,}$ Y_{111}, Y_{112} and Y_{113} had shown very distinct bubbling and rest of the yeasts did not give much indication of alcoholic fermentation during the period of this experiment. However, at the end of the period of fermentation the analysis for the production of ethyl alcohol was done for all the yeast, under study, to confirm their alcohol production ability for those even which did not show any physical change of medium. Fermentation was carried out at 26±1°C temperature at pH 4.70 without nutrient supplements in molasses medium. The yeasts had shown sufficient growth at 24°C and might very easily be tried to test their alcohol production ability in this investigation as this range of temperature, was within the range mostly used to produce wine (Joshi *et al.,* 1999b) and this is the range which prevails during most of the time of the year, in the eastern part of the country (Baidya, 2010). Yeasts are active in a very broad temperature range from 0 to 50^{o}C, with an optimum temperature range of 20^{o}C to 30^{o}C (Mountney and Gould, 1988).

Alcohol production efficiency using molasses solution at 26±2^{o}C

The initial data of broth solution have been showed in Table 3.

Table 3: Initial data of broth solution

Sl. No.	**Parameters**	**Source**
1	Total soluble soiled (^{o}Brix)	24.00
2	Reducing sugar (%)	11.76

3	Total sugar (%)	16.66
4	pH	4.70

Changes in total soluble solids (^{o}Brix)

The results shown (Table 4) in this study the residual degree Brix was measured after every 2 days of fermentation to study the sugar utilization by all the yeasts. All yeast isolates could utilize sugar after 24-hour of fermentation. These indicated that these 4 yeasts (Y_{107}, Y_{109}, Y_{111} and Y_{119}) either could not survive on this molasses substrate at 26±1°C or these were not the fermenting agents of molasses.

Table 4: Changes in total soluble solids (°Brix) during fermentation in molasses broth at 26± 1°C

Sl. No.	Yeast	Total soluble solid (°Brix)									
		0 days	2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days	18 days
1	Y_4	24	16.50	15.97	12.83	12.27	11.10	11.00	10.90	10.77	10.67
2	Y_7	24	11.50	10.33	9.87	9.80	9.70	9.63	9.63	9.63	9.50
3	Y_{33}	24	15.43	15.17	10.43	9.53	9.13	9.13	9.10	9.10	9.00
4	Y_{101}	24	15.43	15.37	14.57	13.47	12.33	12.00	11.33	10.87	10.40
5	Y_{102}	24	13.93	10.73	10.43	10.37	10.27	10.27	10.20	9.90	9.87
6	Y_{103}	24	12.40	11.93	9.73	9.63	9.50	9.47	9.47	9.43	9.40
7	Y_{104}	24	19.00	15.37	15.37	15.30	15.20	15.20	15.17	15.17	15.13
8	Y_{105}	24	19.50	16.70	15.40	15.37	15.37	15.30	15.27	15.27	15.23
9	Y_{106}	24	18.57	17.97	16.17	16.16	16.13	15.83	15.70	15.67	15.13
10	Y_{107}	24	23.96	23.57	22.73	22.53	22.13	16.60	16.60	16.60	16.57
11	Y_{108}	24	19.07	17.63	15.47	15.40	15.30	15.20	15.16	14.37	14.37
12	Y_{109}	24	23.67	23.73	23.43	23.13	21.07	18.00	17.00	16.83	16.17
13	Y_{110}	24	20.87	18.00	11.73	11.00	11.00	10.97	10.37	10.23	9.50
14	Y_{111}	24	24.00	23.70	23.03	23.00	22.60	22.40	22.27	21.65	20.78

15	**Y_{112}**	24	19.17	15.80	15.30	13.77	9.23	9.17	9.13	9.08	9.00
16	**Y_{113}**	24	22.87	16.03	15.60	12.83	12.20	10.33	10.23	10.12	9.87
17	**Y_{114}**	24	23.80	23.10	22.43	22.10	21.00	17.10	13.87	13.52	12.88
18	**Y_{115}**	24	22.23	18.90	18.87	18.30	17.27	15.17	15.20	14.93	13.65
19	**Y_{116}**	24	20.87	16.70	15.87	15.73	14.50	13.30	13.23	13.10	12.56
20	**Y_{117}**	24	23.53	20.17	17.17	16.33	16.23	15.80	14.30	14.27	12.60
21	**Y_{118}**	24	23.47	21.03	17.47	15.90	14.87	14.17	12.37	12.37	11.17
22	**Y_{119}**	24	24.00	23.83	23.33	22.00	21.43	20.27	18.97	18.77	17.80
23	**Y_{120}**	24	24.00	22.80	20.67	19.63	17.93	15.07	13.00	12.60	11.33
24	**Y_{121}**	24	23.23	19.90	16.47	15.07	14.87	13.57	11.70	11.57	9.93
SE(m)		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.194
CD at 5%		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.553

The yeast isolates viz. $Y_{7,}$ Y_{33}, Y_{102}, Y_{103}, Y_{110}, Y_{112}, Y_{113} and Y_{121} had utilized significant quantity of sugar after 18 days of study which was reflected from the residual °Brix of 9.00-9.93 respectively. At the end of fermentation period under study (after 18 days) it was revealed that the residual degree brix was in the range of 10.40-15.23 when molasses was inoculated with $Y_{4,}$ Y_{101}, Y_{104}, Y_{105}, Y_{106}, Y_{108}, $Y_{114,}$ $Y_{115,}$ Y_{116}, Y_{117}, Y_{118} and Y_{120} for the purpose of studying alcohol production efficiency by these yeasts. It means that sugar utilized approximately by these

yeasts was in the range of 8.77-13.60 % which was very negligible. This quantity of sugar might have been utilized for their own survival. A gradual decrease in TSS which is a typical nature of fermentation was observed in this experiment by many workers as it was observed by Ezeronye (2004) in their experiment with fruit wine. Molasses solution having 24°brix and pH 4-5 was selected as the most effective and economic substrate for this purpose as it is normally utilized for the industrial alcohol production by many distilleries and by the researchers (Bardiya *et al.,* 1976).

Changes in reducing sugar, total sugar and pH

Reducing sugar and total sugar were decreased during the fermentation of molasses. After 18 days of fermentation reducing sugar and total sugar were found in the range of 3.06-7.67 per cent and 3.73-13.34 per cent in all isolated yeasts respectively. The maximum reducing and total sugar were found in Y_{109} (7.67%) and (13.23%) and minimum in Y_{110} (3.06%) and (3.73%) after 18 days of fermentation respectively.

During the period of study, in most of the cases pH was changing and later it became stable. The pH of substrate generally 0.10-1.12 was decreased by over a period of 18 days during fermentation by various yeasts but pH was 4.81-5.02 i.e., 0.11-0.32 increase from initial pH of 4.70 and it was found in case of Y_7, Y_{33} and Y_{103} pH was slightly increased as that of initial pH of 4.70 during fermentation periods (Table 5).

It is due to the production of organic acids by the yeasts and the stability of the pH was probably due to efficient buffering of protons in the fermenting medium by the weak acids produced (Abiose and Adedji, 1994; Elijah *et al.,* 2007).

Table 5: Changes in reducing sugar, total sugar and pH during fermentation using molasses at 26± 1°C after 18 days of fermentation

Sl. No.	Yeast	Reducing sugar (%)	Total sugar (%)	pH
1	**Y_4**	5.11	8.58	4.33
2	**Y_7**	3.56	7.89	4.81
3	**Y_{33}**	3.28	7.65	5.02
4	**Y_{101}**	3.23	9.40	4.48
5	**Y_{102}**	3.15	8.23	4.03
6	**Y_{103}**	3.18	7.46	4.94
7	**Y_{104}**	6.27	12.03	3.92
8	**Y_{105}**	5.75	12.12	3.87
9	**Y_{106}**	5.38	12.45	4.04
10	**Y_{107}**	6.52	13.24	4.17
11	**Y_{108}**	6.33	12.00	4.09
12	**Y_{109}**	7.67	13.34	3.95
13	**Y_{110}**	3.06	3.73	4.60
14	**Y_{111}**	7.03	8.60	3.84

15	**Y_{112}**	6.53	6.80	4.08
16	**Y_{113}**	5.90	7.27	3.81
17	**Y_{114}**	6.37	8.80	3.58
18	**Y_{115}**	6.23	7.22	3.94
19	**Y_{116}**	6.67	7.13	4.10
20	**Y_{117}**	5.84	6.80	4.21
21	**Y_{118}**	6.08	6.54	4.24
22	**Y_{119}**	6.10	13.31	4.11
23	**Y_{120}**	5.59	6.37	4.30
24	**Y_{121}**	4.48	7.01	4.24
SEm(±)		0.082	0.136	0.062
CD at 5%		0.235	0.387	0.176

Production of alcohol and cell count from substrate

As shown in Table 6, a steady increase in alcohol content was observed in the molasses throughout the period of fermentation with the test yeast strains. At the end of the 18 days fermentation, the concentration of alcohol in the molasses was observed at range from 4.21 to 13.61 %. In Y_4 the highest and lowest alcohol levels were observed in the presence of Y_{109} isolated from date palm juice.

$Y_{109,}$ Y_{107} and Y_{116} produced less alcohol at the end of fermentation and more amount of alcohol being produced by $Y_{4,}$ $Y_{101,}$ $Y_{33,}$ $Y_{102,}$ Y_{7} and Y_{112} were found 13.61, 12.01, 11.88, 11.06, 10.35 and 10.01 per cent respectively. In this study the highest capability of alcohol production was observed in the isolates $Y_{4,}$ $Y_{101,}$ $Y_{33,}$ $Y_{102,}$ Y_{7} and $Y_{112.}$

The cell count after 18 days fermentation was found in the range of 3.60×10^{8} cfu/ml to 8.79×10^{8} cfu/ml for all the isolated yeast. Highest cell count was reported in Y_{33} (8.79×10^{8} cfu/ml) and Y_{114} (8.78×10^{8} cfu/ml) followed by Y_{113} (8.17×10^{8} cfu/ml) and lowest in Y_{104} (3.60×10^{8} cfu/ml) after till of fermentation. In general, the percentage alcohol produced from the respective fruits at the end of fermentation by the test yeast strains was above 11%, which is comparable with moderate grape wines (Ayogu, 1999; Querol *et al.*, 2003; Okunowo *et al*., 2005). Production of ethyl alcohol depends upon the type of yeast used in fermentation as it was observed by Bajaj *et al.* (2001) in their experiment with 24 different yeast strains collected from the natural sources. Inoculum dose was 2×10^{7} cfu/ml in an experiment by Kundu *et al.* (1981) in preparation of grape wine.

Table 6: Production of alcohol (in per cent) and cell count of yeasts in fermenting molasses broth at 26± 1°C after 18 days

Sl. No.	Yeast isolate	Alcohol (%)	Cell count cfu/ml ($\times10^{8}$)
1	**Y_4**	13.61	6.01
2	**Y_7**	10.35	6.93
3	**Y_{33}**	11.88	8.79

4	**Y_{101}**	12.01	5.14
5	**Y_{102}**	11.06	6.21
6	**Y_{103}**	8.20	5.84
7	**Y_{104}**	5.44	3.60
8	**Y_{105}**	5.78	5.78
9	**Y_{106}**	7.02	5.68
10	**Y_{107}**	4.28	6.21
11	**Y_{108}**	9.41	6.10
12	**Y_{109}**	4.21	5.45
13	**Y_{110}**	8.55	7.60
14	**Y_{111}**	5.44	6.57
15	**Y_{112}**	10.01	7.13
16	**Y_{113}**	6.09	8.17
17	**Y_{114}**	8.96	8.78
18	**Y_{115}**	7.23	8.14
19	**Y_{116}**	4.65	7.69

20	**Y_{117}**	8.55	7.26
21	**Y_{118}**	7.55	6.84
22	**Y_{119}**	5.91	4.74
23	**Y_{120}**	7.90	6.30
24	**Y_{121}**	8.06	6.52
SEm(±)		0.121	0.094
CD at 5%		0.344	0.268

Conclusion

Molasses solution and different fruit juices were used for isolation of different yeast such as date palm, pomegranate, black grape and roselle, juice under different agro-climatic zones of West Bengal regions in India. Yeast isolate numbers Y_{104}, Y_{105}, Y_{106}, Y_{107}, Y_{108}, Y_{116}, Y_{117}, Y_{118}, Y_{120} and Y_{121} showed poor to moderate growth at 26±1°C after 48 hours of incubation. Isolate numbers Y_{101}, Y_{102}, Y_{103}, Y_{109}, Y_{110}, Y_{111}, Y_{112}, Y_{113}, Y_{114}, and Y_{115} showed good to very good growth at both 26±1°C. The isolate Y_{119} failed to grow after 16 hours and after 24 hours growth was started at 26±1°C in incubation. The total soluble solid after end of fermentation in molasses solution were found in the range of 9.00-20.78^{O}Brix in all isolated yeast. After 18 days of fermentation reducing sugar, total sugar and pH were found in the range of 3.06-7.67 per cent, 3.73-13.34 per cent and 0.10-1.12 in all isolated yeasts respectively. The cell count after complete fermentation was found in the range of 3.60×10^{8} cfu/ml to 8.79×10^{8} cfu/ml for all the isolated yeast. In this study the highest alcohol production was observed in the isolates Y_{4}, Y_{101},

$Y_{33,}$ $Y_{102,}$ and Y_7 at the end of the 18 days fermentation. After screening or checking the efficiency of yeast in molasses media, five yeasts were selected for wine fermentation.

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