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Ethanol Tolerance of Yeasts Isolated from Fruits

T. Annu and V. C. Suvarna
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru-560 065

Abstract

Yeasts were isolated from Amla (*Phyllanthus emblica*), Pineapple (*Ananas comosus*), Burmese Grapes (*Baccaurea ramiflora*), Custard Apple (*Annona reticulata*), Jamun (*Syzygium cumini*) and Carambola (*Averrhoa carambola)*. Isolates (nine) were characterized by colony morphology and microscopic observations. Circular and creamy white colonies were found to be more prevalent and yeasts cell size varied from 4.4 × 2.6 µm to 5.7 × 5.2 µm. Yeast Extract Peptone Dextrose Agar (YEPDA) medium supplemented with different concentrations of ethanol were used to study the ethanol tolerance capacity of each strain. Ethanol tolerance capacity was measured in terms of OD values. The results of this study revealed that A-Y (Amla isolate) strain recorded significantly highest ethanol tolerance level (6 per cent) and J-Y (Jamun isolate) recorded the lowest ethanol tolerance level. Strain A-Y was found to be superior next to standard strain *Saccharomyces ellipsoideus* and it was further utilized for production of alcoholic beverage.

Yeasts are ubiquitous in nature and are frequently isolated from sugar rich sources (Tikka *et al*., 2013). They have been isolated from leaves, flowers, fruits, grains, exudates of trees, insects and dung. The population of microbiota in any niche is greatly influenced by pH of the substrate. Since fruits are acidic in nature, they are predominantly inhabited by yeasts (Deepak, 1994). Yeast strains are associated with fruit surfaces and they convert sugars into alcohol (Ali and Khan, 2014). Hence, yeasts are being exploited in industries. Ethanol tolerance capacity test becomes essential for an efficient alcohol producing strain in industrial ethanol production. Preez *et al.* (1987) pointed out that ethanol tolerance is very important as it decides the alcohol yield during fermentation. Keeping in view the importance of yeasts, the present study was undertaken. Yeasts were isolated from fruits and subsequent characterization of yeast isolates and screening of those yeast strains for ethanol tolerance capacity was attempted. Yeast strains with higher ethanol tolerance capacity were further used for production of alcoholic beverage.

Amla, Pineapple, Jamun and Custard Apple were purchased from Bengaluru markets. Burmese Grapes and Carambola were procured from Pasighat (Arunachal Pradesh) farm. The collected fruit samples were washed and rinsed with sterile water. They were then cut, squeezed and the juice was collected in sterile flasks. Juices were diluted serially up to 10^{-4} and 0.1ml of the diluted sample was subjected to standard plate count method using Yeast Extract Peptone Dextrose Agar medium (YEPDA) and plates were incubated at 30°C for 48 hrs. The isolated yeast cultures were studied for colony morphology and microscopic observations (Table I). Simple staining technique was followed using crystal violet stain for microscopic observations using 24 hrs old culture. Yeast isolates were purified and maintained on YEPDA slants.

Ethanol tolerance of isolates was tested by inoculating one ml of 24 hrs old broth culture having population density of 10^6 cfu / ml of each strain to YEPD broth containing alcohol (v/v) ranging from 4 to 15 per cent. Flasks were incubated at 30°C for 24 hrs. Optical density was recorded at 615 nm using UV-visible spectrophotometer. Yeast strain (*Saccharomyces ellipsoideus*) maintained in the department culture collection was used as reference culture and this yeast culture is generally used in industrial and traditional ethanol fermentation. The experiment was conducted with two way ANOVA.

Nine yeast strains were isolated from Pineapple, Amla, Burmese Grapes, Custard Apple, Jamun and Carambola. Isolates were characterized for colony and cell morphology and the details are presented in Table I. Yeast strains produced different types of colonies such as raised, circular, irregular, slimy, creamy white and white color colonies on YEPDA medium.

TABLE I

Morphological and microscopic observations of yeast isolates

Sources	Isolates	Colony characteristics	Cell morphology	
			Shape	Size* (µm)
Pineapple	Pi_1-Y	Circular, creamy white and small cluster of cell.	Oval, budding	5.1 x 3.5
Pineapple	Pi_2-Y	Circular, creamy white in color and slimy.	Oval, budding	5.4 x 2.8
Burmese Grape	B_1-Y	Circular, raised, creamy white and slimy.	Oval, budding	5.2 x 2.6
Burmese Grape	B_2-Y	Irregular, white, lobate margin and worm like appearance in the middle.	Oval, budding	4.4 x 2.6
Carambola	St-Y	Irregular, lobate margin and white in color.	Oval, budding	5.1 x 3.9
Amla	A-Y	Irregular, lobate margin, white and worm like appearance in the middle.	Oval, budding	5.7 x 5.2
Custard Apple	C_1-Y	Circular, raised, creamy white and slimy.	Oval, budding	5.2 x 4.7
Custard Apple	C_2-Y	Irregular, worm like appearance in the middle and white in color	Oval, budding	4.9 x 2.6
Jamun	J-Y	Irregular and creamy white in color.	Oval, budding	5.2 x 3.4

*Size is a mean of three cells.

TABLE II

Optical density of yeast isolates and cultures at 24 hrs in broth supplemented with varying alcohol concentrations

	OD values (615 nm)						
	Alcohol concentration (%)						
Strains	0	4	6	8	10	12	15
Pi_1-Y	1.147 ^{dA}	1.053 ^{eB}	0.916 ^{eC}	0.290 ^{cD}	0.157 ^{cE}	0.118 ^{cF}	0.049 ^{dG}
Pi_2-Y	1.150 ^{dA}	1.027 ^{fB}	1.025 ^{bB}	0.183 ^{fC}	0.069 ^{gD}	0.066 ^{fE}	0.015 ^{hF}
B_1-Y	1.144 ^{dA}	0.936 ^{gB}	0.907 ^{fC}	0.286 ^{dD}	0.140 ^{dE}	0.067 ^{fF}	0.023 ^{fG}
B_2-Y	1.108 ^{fA}	0.932 ^{hB}	0.891 ^{gC}	0.113 ^{iD}	0.067 ^{gE}	0.061 ^{gF}	0.014 ^{hG}
St-Y	1.184 ^{cA}	1.095 ^{cB}	1.002 ^{dC}	0.176 ^{gD}	0.112 ^{E}	0.108 ^{dF}	0.094 ^{cG}
A-Y	1.258 ^{aA}	1.172 ^{aB}	1.133 ^{aC}	0.504 ^{bD}	0.178 ^{bE}	0.175 ^{bE}	0.109 ^{bF}
C_1-Y	1.073 ^{gA}	0.920 ^{iB}	0.576 ^{hC}	0.152 ^{hD}	0.052 ^{hE}	0.36 ^{hF}	0.019 ^{gG}
C_2-Y	1.116 ^{eA}	1.101 ^{bB}	1.000 ^{dC}	0.242 ^{eD}	0.103 ^{fE}	0.082 ^{eF}	0.040 ^{eG}
J-Y	1.004 ^{hA}	0.665 ^{jB}	0.365 ^{iC}	0.092 ^{jD}	0.042 ^{iE}	0.028 ^{iF}	0.010 ^{igG}
Reference strain (SY_2)	1.198 ^{bA}	1.072 ^{dB}	1.008 ^{eC}	0.970 ^{aD}	0.823 ^{aE}	0.240 ^{aF}	0.193 ^{aG}
Source		S. Em+			CD		
Alcohol concentration		0.001			0.003		
Strains		0.001			0.009		
Interaction(A x B)		0.003			0.009		

Note - Small letters in superscripts refer to the difference between strains and capital letters refer to alcohol concentration.

However, circular and creamy white colonies were found dominating. Similar results were observed by Chatterjee *et al.* (2011). All the nine isolates exhibited budding character and were of oval shape (Table I). The size of the yeast cells varied from 4.4 × 2.6 µm to 5.7 × 5.2 µm.

It was observed that all the strains showed good growth in broth supplemented with alcohol up to 6 per cent. Further, with the increase in ethanol concentration, growth decreased. Previous studies conducted by Ndip *et al*. (2001) have revealed that, yeast isolates grew profusely at 6 per cent ethanol concentration and no growth was observed at 15 to 20 per cent concentration. The results pertaining to the effect of ethanol on growth are presented in Table II. Variation in ethanol tolerance was observed among yeast strains. The ethanol tolerance levels of all the isolates with reference strain were studied in the range of 4 to 15 per cent. Even though all the strains could tolerate 15 per cent alcohol, growth was very less. Ethanol at higher concentration has a denaturing effect on proteins and it affects the enzyme activity (Sevda and Rodrigues, 2011). Tikka *et al*. (2013) screened seven yeast isolates, identified as *Saccharomyces cerevisiae* for alcohol tolerance and the results showed a range of tolerance between 7-12 per cent in all the strains. They also reported that ethanol decreased the rate of growth and cell viability. At 24 hrs, significantly highest tolerance level was observed in A-Y strain up to 6 per cent but, tolerance was reducing from 8 per cent onwards compared to reference stain. Reference strain (SY_2) gave significantly highest OD at 8, 10, 12 and 15 per cent. J-Y showed significantly lowest ethanol tolerance at 15 per cent with OD value of 0.01. This study showed that the isolated yeast strain A-Y could tolerate alcohol better than all other isolates. However, it was found to be superior next to standard strain *Saccharomyces ellipsoideus*.

The isolation of high ethanol tolerant yeast strains from fruits has revealed that the need to look further into other locally available fruits harbouring better alcohol producing strains. Among all the isolates, A-Y exhibited highest tolerance next to the standard strain. So, it can be further identified at molecular level and can be used for alcoholic fermentation to develop an alcoholic beverage.

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