JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2011. Vol. **5**(1), p. 313-316

Isolation, Characterization of Ethanol Tolerant Yeast

V.P. Abbinayha Priya, S. Amutha and H. Mangalam

Department of Microbiology, Meenakshi Mission Hospital and Research Centre, Madurai – 625 107, India.

(Received: 02 May 2010; accepted: 20 June 2010)

The present study was conducted to isolate the yeast strain (*Saccharomyces cerevisiae*) from the sources such as Grapes, Molasses, Musambi, Cashew Apple, Distillary Effluent, Sorghum, Sugarcane and it is used for the study of ethanol tolerance at various concentrations from 6-14.5%. Among these the yeast strains isolated from Molasses and Cashew apple showed highest tolerance upto 12.5% and strains from Sorghum had least with 10%. Molecular characterization of yeast strain also studied using RAPD-PCR, among 63 bands produced, 6 bands were monomorphic and 57 bands were polymorphic and genetic distance of yeast strains were performed based on cluster analysis reveals that the highest genetic difference between Sugarcane and Molasses at average of 0.5 and the least genetic difference showed between Sorghum, Cashew apple and Distillary effluent at average of 0.10.

Key words: Yeast strains, Ethanol tolerance, Cluster analysis, RAPD-PCR.

Yeasts are also one of the most widely used model organisms for genetics and cell biology. The yeast *Saccharomyces cerevisiae* is now recognized as a model system representing a simple eukaryote whose genome can be easily manipulated. *Saccharomyces* sp are the safest and most effective micro organisms for fermenting sugars to ethanol and traditionally have been used in industry to ferment glucose based agricultural products to ethanol (Nancy W.Y.*et al.*, 1998).

* To whom all correspondence should be addressed.
Tel.: +91-9976647159,
E-mail: abbinayha_priya@yahoo.co.in

During fermentation sugars lead to the production of ethanol and carbon dioxide. A highly ethanol tolerant *Saccharomyces* wine strain is able, after growth is in the presence of ethanol, to efficiently improve the ethanol tolerance of its membrane. A high tolerance occurs only after the addition of energy and does not take place in the presence of cycloheximide (Jimenez *et al.*, 1986).

Molecular genetic techniques can be used to discriminate between yeast strains that have similar physiological characteristics. RAPD analysis is faster, technically less demanding and more economical than the other genomic typing methods like RFLP, AFLP. Further, this technique elucidates the biodiversity in a group of isolates (Hansel *et al.*, 1998). The purpose of this study was to screen the isolated yeast strains for ethanol tolerance and to characterize these yeast strains using molecular markers.

MATERIAL AND METHODS

The present investigation on screening of yeast strains for ethanol tolerance and molecular characterization were carried out in the Department of Biotechnology, UAS, GKVK, Bangalore-560065, India.

Isolation and identification of yeast

Yeast Extract Peptone Dextrose Agar (YEPDA) media was used for isolation of yeast strains. Samples such as Grapes, Molasses, Musambi, Cashew Apple, Distillary Effluent, Sorghum, and Sugarcane were collected from different market places in and around Bangalore.

The collected fruits were washed and rinsed many times in distilled water. They were then cut, squeezed and the juice collected in separate sterile flasks and allowed it to fermentation for seven days. From that samples of the juice were diluted serially and 0.1 ml of the diluted samples was plated on YEPDA medium. The plates were incubated at 30^0C for 24 to 48 hours. The isolated cultures were identified at genus level through colony characters and cell morphological characters.

Screening of the isolated yeast strains for ethanol tolerance

Ethanol has three major effects on yeast. It decreases the rates of growth and of fermentation, and it reduces overall levels of cell viability. Ethanol tolerance of each strain was studied by allowing the yeasts to grow in liquid YEPD having different concentrations of ethanol (Osho, 2005). Yeasts were inoculated into 10ml tubes containing 5ml portion of liquid YEPD and Incubated for 24 hours at 30^0 C.10 µl portions were then inoculated into 10ml tubes containing 4ml portion of YEPD supplemented with 6,7,8,9,10,11,12,12.5, 13,13.5,14 and14.5% ethanol and incubated for 24 hours at 30^0C.Growth expressed as generation time, was determined by measuring the optical density of cultures at 595 nm. The initial optical density of each tube was read spectrophotometrically at 595 nm against the medium as blank. The inoculated tubes were transferred to a gyrorotatory shaker set at 30^0 C for 24 to 48 hours. The increase in optical density in a tube was recorded as evidence for growth of the yeast. The concentration of alcohol at which the yeast just inhibited was assessed as the ethanol tolerance of yeast.

Molecular Characterization of Yeast strains using RAPD marker

Isolated *Saccharomyces* sp strains from different samples were used for RAPD–PCR characterization.

Isolation of DNA

Yeast DNA was isolated by using protocol provided in Sambrook and Russell, (2001) with slight modification. Instead of acid washed glass beads, glass powder (supplied by HiMedia) was used.

Randomly Amplified Polymorphic DNA analysis (RAPD– PCR)

Amplification conditions

For fingerprinting and diversity analysis, PCR amplification conditions were optimized based on the protocol outlined by Echeverrigaray *et.al.*,(2000).

Selection of primers

Primer screening was carried out using DNA obtained from the *Saccharomyces* sp isolates. 8 primers were screened; from these 8 primers finally 4 primers (5'GGG GTG ACG A 3', 5' GAC GGA TCA G 3', 5'GGA CCC AAC C 3', 5'GTG TGC CCC A 3') producing sharp, intense bands were selected for the RAPD analysis.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplification product using 1.5 % agarose in 1X TBE buffer, 0.5µg/ml of ethidium bromide, and loading buffer (0.25% Bromophenol Blue in 40% sucrose). 5 µl of the loading dye was added to 25µl of PCR products and loaded to the agarose gel. Electrophoresis was carried at 65V for 4 hours. The gel was visualized under UV light and documented using Gel documentation unit.

Analysis of RAPD data

The bands were manually scored '1' for the presence and '0' for the absence and the binary data were used for statistical analysis. The scored band data (Presence or absence) was subjected to cluster analysis-using STATISTICA. The dendrogram was constructed by Ward's method of clustering using minimum variance algorithm. The dissimilarity matrix was developed using Squared Euclidean Distance (SED), which estimated all the pair wise differences in the amplification product. Only clear and unambiguous

bands were taken into account and the bands were not scored if they were faint or diffused, as such fragments posses poor reproducibility. The band sizes were determined by comparing with the 100 bp. DNA ladder, which was run along with the amplified products. The Genetic distance was computed as:

$$\Sigma^{n}=1 \quad dj^{2}$$

where $dj = (X_{ik} - X_{jk})$

Where X_{ik} refers to binary code of i^{th} tree for allele "k" and X_{jk} refers to the binary code of the j^{th} tree for allele "k". Dendrogram was computed based on Ward's method of clustering, using minimum variance algorithm.

RESULTS AND DISCUSSION

Isolation and identification of yeast

Raised and creamy with white colour colonies were isolated and further purified on YEPDA plates. Isolated strains were studied for further identification. *Saccharomyces* have typical oval cell shape when compared with other genus of yeast strains.

Ethanol tolerance of yeast isolates

Seven isolates were screened for ethanol tolerance levels from 6-12.5%. Benitez.T *et al.,* (1983) reported that the *Saccharomyces cerevisiae* strains with ethanol tolerance varying from 4-12% (v/v) isolated from grapes. In the present study strains isolated from Grapes, Musambi, Mollasses, Cashew apple and Distillary effluent was tolerated above 12.5%. Strains isolated from Molasses, Cashew apple are tolerated from 10 -12.5%. Strains isolated from Sorghum were tolerated below 10%.

RAPD characterization

A total of 63 RAPD bands produced from the selected 4 primers were used for fingerprinting and for estimation of genetic diversity among eight isolates of *Saccharomyces* strains. The number of bands scored for each primer varied from 1 to 8 with an average of 9.3 bands per primer. Out of 63 amplification bands, 6 bands were monomorphic and 57 bands were polymorphism, which were informative in revealing the relationship among the genotypes.

Cluster analysis and genetic distance of *Saccharomyces* strains

The Cluster analysis based on 63 RAPD bands revealed the seven yeast isolates examined. The dendrogram has clearly depicted that all the 7 yeast isolates formed two major clusters. Among the two major groups, there were five sub clusters. Isolates YCA, YDE, formed the first group, isolates YSM, YMI, YMS, YSC and YGP formed the second group. Strains from Grapes and Molasses getting genetic difference at average of 0.40 and strains form Sorghum, cashew apple and Distillary effluent getting genetic difference at average of 0.10 and strains form Sugarcane and Molasses getting genetic difference at average of 0.5. Strains from Musambi and Sorghum getting genetic difference at average of 0.2 (Fig. 1).

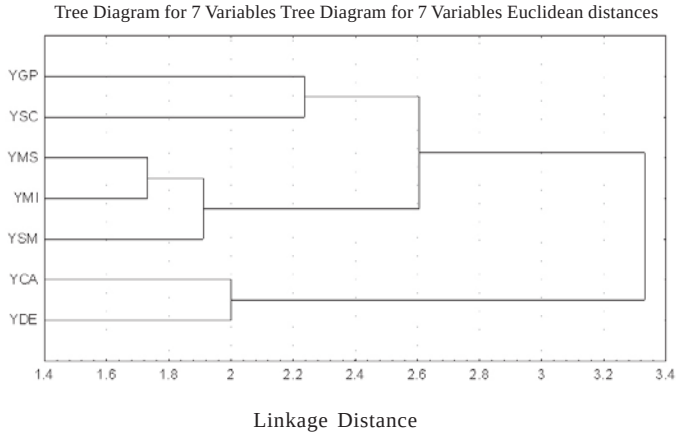

Fig. 1. Dendrogram based on RAPD profile of *Saccharomyces* strains obtained from different samples

REFERENCES

1. Echeverrigaray, S., Paese-toresan, S. and Carrau, J.l., RAPD marker polymorphism among commercial winery yeast strains, *World J. Microbiol Biotechnol.,* 2000; **16**: 143-146.
2. Hansel, B.M., Damgoard, P.H., Elinberg, J. and Pederson, J.C., Molecular and phenotypic characterization of *Bacillus thuringeinsis* isolated from leaves and insects. *J. Invertebrate. Pathol.,* 1998; **71**: 106-114.
3. Jimenez, J. and Benitez, T., Characterization of yeasts for ethanol production. *Appl. Microbiol. Biotechnol.,* 1986; **25**: 150-154
4. Nancy, W. Y. Ho, Zhengdao Chen and Adam P. Brainard., Genetically Engineered *Saccharomyces* Yeast Capable of Effective Cofermentation of Glucose and Xylose. *Appl Environ Microbiol.,* 1998; **64**(5): 1852-1859.
5. Osho., Ethanol and sugar tolerance of wine yeast isolated from fermenting cashew apple juice. *Af.Jr.Biotech.* 2005; **4**(7):160-163.
6. Sambrook J and Russell DW., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NewYork. 2001.
7. Thahia Benitez, Lucas Del Castillo, Andres Aguilera, Jaime Conde, and Cerdaolmedo. E., Selection of Wine Yeasts for Growth and Fermentation in the presence of Ethanol and Sucrose. *Appl. Environ. Microbial,* 1983; **45**(5): 1429-1436.