

Molecular Characterization of *Saccharomyces cereviceae* using RAPD Molecular Markers

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Abstract: Yeasts are eukaryotic microorganisms including 1,500 species. They are an important model organism in modern cell biology research, and are one of the most thoroughly researched eukaryotic microorganisms. Some yeast can find potential application in the field of bioremediation. In the present investigation the yeast samples were isolated on YPD medium from natural sources like ripened fruits, molasses, milk and milk products. They were identified using standard microbiological techniques. The DNA from the yeast cells was isolated by phenol:chloroform method and the diversity of the yeast samples were determined by Random amplified polymorphism using OPD18, 19 and 20 at an annealing temperature of 35°C. The amplification of the DNA showed multiple banding patterns with different molecular weight and the banding patterns of all the four yeast isolate had different banding pattern and they were identified to be of different strains of the yeast. With primer OPD18, isolate 1 showed only four prominent bands and the other three isolates showed banding pattern with a minimum of 8 prominent bands. The isolates with OPD 19 primer amplified with more than 12 bands except in the isolate 1 which showed only 3 prominent band. The amplified product size was mainly found to be between 200- 1000 kilo Daltons. The amplification was carried out with OPD 20 primer and the isolates showed varied banding patterns. The amplified product length ranged from 300- 2500 kilo Daltons. The isolate 3 and 4 had very few common bands with the same product length.

Keywords: *Saccharomyces cereviceae*, RAPD, PCR, Genetic diversity, Polymorphism.

1. Introduction

The beneficial activities of yeasts are of great economic significance and they have been used for millennia in the production of fermented foods and alcoholic beverages [1]. Yeasts are unicellular fungi and can be classified into two phylogenetic groups, teleomorphic and anamorphic ascomycetous yeasts and teleomorphic and anamorphic basidiomycetous yeasts [2].

Traditional identification methods that are based on laborious morphological and physiological tests often fail to species determination (Arias et al. 2002). These methods lack discriminatory power and, as a consequence, misidentification occurs frequently [3]. In contrast, DNA-based techniques provide complementary methods and, in recent years, the application of these techniques has generated a large number of studies on the identification and typing of, both, industrial and contaminant yeasts [4,5].

Molecular biology study has led to the development for yeast identification based on similarity or dissimilarity of DNA, RNA or proteins. These include allozyme patterns [6], DNA-DNA hybridization [7,8], electrophoretic karyotyping [9,10,11], microsatellite analysis [12], nested-PCR [13], random amplified polymorphic DNA (RAPD) analysis [14,15,16], RFLP of chromosomal DNA [17] or RFLP of mitochondrial DNA [18,19,20].

2. Materials and Methodology

2.1 Isolation of Yeast

Isolation of the yeast was performed on yeast extract peptone dextrose (YPD) agar medium. The yeast isolates were isolated from different natural sources like fruits, milk and milk products Sugarcane molasses etc; which contains

or harbours yeast cells. The natural Samples were inoculated onto YPD agar plates and incubated at 28°C for 24-48 hr. The *Saccharomyces cerevisiae* obtained on the agar plates were identification according to [21, 22, 23] and the were subcultured on the agar slants and maintained in refrigerating condition for further use.

2.2 Isolation of DNA from Yeast Isolates

0.5 ml of broth overnight culture broth and 0.8ml of reagent A was mixed and and Centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and cells were collected. 0.2ml of reagent B and 0.025 ml reagent C was added to the pellet and mixed well. 0.06 ml Tris phenol and 0.06 ml of chloroform and isoamyl alcohol mixture (24:1) was added and centrifuge at 8000 rpm for 10 min. 0.06 ml chloroform was added to the aqueous layer and centrifuged again at 8000 rpm for 10 min. To the upper aqueous phase double volume of cold absolute alcohol was added and incubated for overnight in 4°C. The samples were then Centrifuged at 12,000 rpm for 10 min and the pellet was dissolved in 80-100 µl of TE buffer and Stored at 4°C for further investigation. The qualitative analysis was carried out with 1.0% agarose gel electrophoresis and quantitative estimation was done using Nano Drop 1000.

2.3 Polymerase Chain Reaction

The polymerase chain reaction was carried out for the isolated DNA with primers OPD 18, 19 and 20. The PCR conditions for the primer were standardized. The initial denaturation was carried out at 94° C for 5 min. Final denaturation at 94 °C for 30 seconds, Annealing temperature was 35 °C for 30 seconds and Extension was at 72 °C for 30 seconds. This cycle was repeated for 36 cycles. The final elongation was carried out at 72 °C for 10 mins. The PCR products were determined on 1.5% agaore gel.

3. Results

Isolation

Saccharomyces cerevisiae was isolated from different natural sources and 4 different yeast isolates were taken for further studies based on colony and cell morphology (figure-1). These isolates were identified using staining and biochemical techniques. DNA was isolated by Phenol-Chloroform method and Qualitative and Quantitative analysis was done on Agarose Gel electrophoresis (figure-2) and Nanodrop spectrophotometry (figure-3).

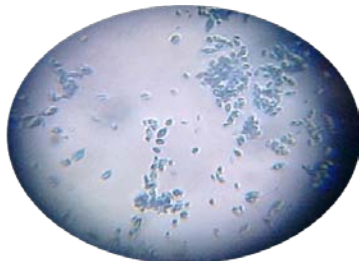


Figure 1: Microscopic view of Yeast



Figure 2: Qualitative Analysis of the Genomic DNA

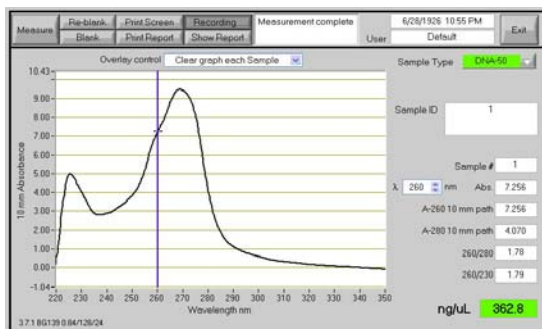


Figure 3: Quantitative Analysis of the DNA

RAPD and PCR: Reproducibility of RAPD and PCR-fingerprinting assays were assessed by performing. High and Low intensity bands were consistently reproducible, and high intensity bands were taken into consideration when the patterns were compared. Data generated, from RAPD assay and dendrogram analysis, showed all four isolates were of different strains which varied from each other.

The isolates showed a varying banding pattern with primer OPD18, where in the isolate 1 showed only four prominent bands and the other three isolates showed banding pattern with a minimum of 8 prominent bands. Isolate 2 and 3 showed a similar kind of banding pattern and in all the isolates the bands were seen below 1000 kilo Daltons (figure-4).

The isolates with OPD 19 primer amplified many products which were prominent and there were more than 12 bands except in the isolate 1 which showed only 3 prominent bands. The isolate 2 and 3 had similar kind of bands with slight difference, which indicated that they were closely related to each other. Isolate 3 and 4 showed bands more than 3000 kilo Daltons. The amplified product size was mainly found to be between 200- 1000 kilo Daltons (figure-5).

The amplification was carried out with OPD 20 primer and the isolates showed varied banding patterns. The amplified product length ranged from 300- 2500 kilo Daltons. The isolate 1 and 2 had a similar kind of banding pattern with little change in few of the amplified product size. The isolate 3 and 4 had very few common bands with the same product length (figure-6).

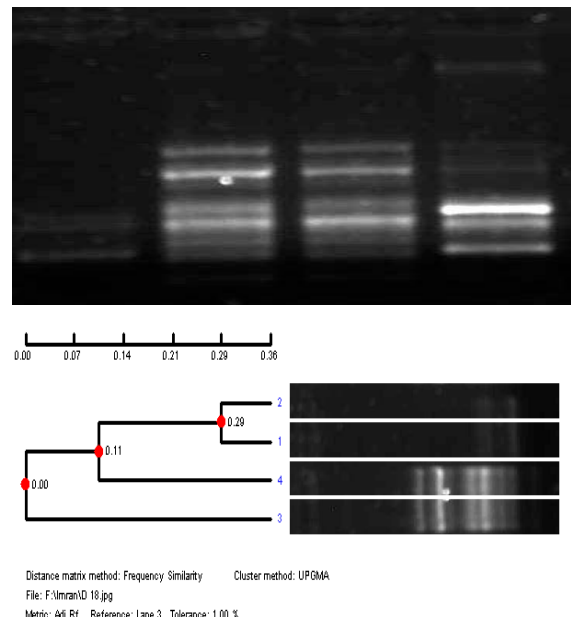


Figure 4: DNA fingerprint and Dendrogram for D18

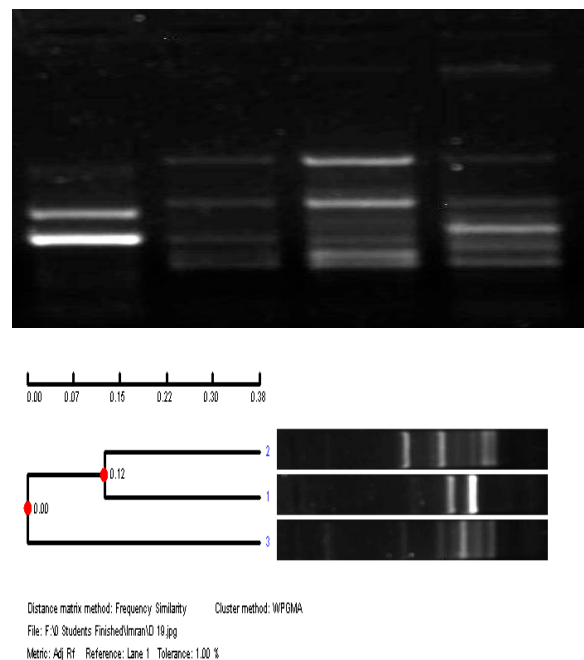


Figure 5: DNA fingerprint and Dendrogram for D19

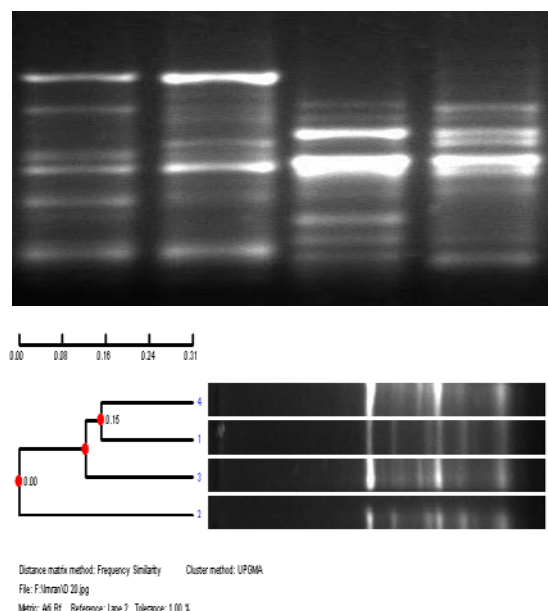


Figure 6: DNA fingerprint and Dendrogram for D20

4. Discussion

Yeasts most frequently isolated from contaminated soft drinks are from the genera *Saccharomyces* and *Candida*, but species of the genera *Pichia*, *Hansenula*, *Torulopsis*, *Rhodotorula*, *Zygosaccharomyces*, *Brettanomyces* and *Dekkera* are also found [24].

[25], discriminated the isolated species level of strains of the genus *Saccharomyces* with simple repeat primers such as (GAC) 5 (GTG) 5 and M13 core sequence.

[26], identified the isolates *Malassezia* yeasts using three molecular tools, amplified fragment length polymorphism (AFLP), denaturing gradient gel electrophoresis (DGGE) and random amplified polymorphic DNA (RAPD) analysis which is similar to the present study where in the RAPD was carried out with 3 primers OPD18, 19 and 20. The AFLP showed that four genotypes could be distinguished within *M. furfur*. AFLP genotype 4 contained only isolates from deep human sources, and ca. 80% of these isolates were from patients with systemic disease. Most of the systemic isolates belonged to a single RAPD genotype. This suggests that systemic conditions strongly select for a particular genotype.

[27], investigated and carried out studies on *Y. lipolytica* strains at the genetic level by means of RAPD-PCR using the primers M13 and RF2. The dendrogram derived from the combined analysis of the M13 and RF2 RAPD-PCR profiles showed the presence of three distinctive clusters and one independent strain.

[28], investigated the potential value of microsatellite length polymorphism with a panel of 91 isolates and tested for seven polymorphic regions in a subgroup of 58 unrelated strains identified a total of 69 alleles (6 to 13 per locus) giving 52 different patterns with a discriminatory power of 99.03%. Similarly in the present investigation there were different multiple banding patterns with all the RAPD primers and each of the isolates showed 8-15 bands of different molecular weights.

References

- [1] Dedk, T. & Beuchat, L. R. (1996). *Handbook of Food Spoilage Yeasts*. Boca Raton, FL: CRC Press.
- [2] Kurtzman, C. P. & Fell, I. W. (1998). *The Yeasts - a Taxonomic Study*, 4th edn. Amsterdam: Elsevier.
- [3] Versavaud, A. & Hallet, I. N. (1995). Pulsed-field gel electrophoresis combined with rare-cutting endonucleases for strain identification of *Candida famata*, *Kloeckera apiculata* and *Schizosaccharomyces pombe* with chromosome number and size estimation of the two former. *Syst Appl Microbiol* 18, 303-309.
- [4] Belloch, C., Barrio, E., Uruburu, F., Garcia, M. D. & Querol, A. (1997). Characterization of four species of the genus *Kluyveromyces* by mitochondrial DNA restriction analysis. *Syst Appl Microbiol* 20, 397-408.
- [5] Romano, A., Casaregola, S., Torre, P. & Gaillardin, C. (1996). Use of RAPD and mitochondrial DNA RFLP for typing of *Candida zeylanoides* and *Debaryomyces hansenii* yeast strains isolated from cheese. *Syst Appl Microbiol* 19, 255-264.
- [6] Lopandic, K., Prillinger, H., Molndr, O. & Gimenez-Jurado, G. (1996). Molecular characterization and genotypic identification of *Metschnikowia* species. *Syst Appl Microbiol* 19, 393-402.
- [7] Naumov, G. I., Naumova, E. S. & Sniegowski, P. D. (1997). Differentiation of European and far-east Asian populations of *Saccharomyces paradoxus* by allozyme analysis. *Int J Syst Bacteriol* 47, 341-344.
- [8] Tjrek, T., Rockhold, D. & King, A. D. (1993). Use of electrophoretic karyotyping and DNA-DNA hybridization in yeast identification. *Int J Food Microbiol* 19, 63-80.
- [9] Vaughan Martini, A. & Martini, A. (1985). Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces sensu stricto*. *Int J Syst Bacteriol* 35, 508-511.
- [10] Vaughan Martini, A. & Martini, A. (1987). Taxonomic revision of the yeast genus *Kluyveromyces* by nuclear deoxyribonucleic acid reassociation. *Int J Syst Bacteriol* 37, 380-385.
- [11] Guillamon, M., Barrio, E., Huerta, T. & Querol, A. (1994). Rapid characterization of four species of the *Saccharomyces sensu stricto* complex according to mitochondrial DNA patterns. *Int J Syst Bacteriol* 44, 708-714.
- [12] Nadal, D., Colomer, B. & PiAa, B. (1996). Molecular polymorphism distribution in phenotypically distinct populations of wine yeast strains. *Appl Environ Microbiol* 62, 1944-1950.
- [13] Quesada, M. P. & Cenis, J. L. (1995). Use of random amplified polymorphic DNA (RAPD)-PCR in the characterization of wine yeasts. *Am J Enol Vitic* 46, 204-208.
- [14] Baleiras Couto, M. M., van der Vossen, I. M., Hofstra, H. & 't Veld, I. H. (1994). RAPD analysis: a rapid technique for differentiation of spoilage yeasts. *Int J Food Microbiol* 24, 249-260.
- [15] Perez, L., Martinez, P., Codon, A. C. & Benitez, T. (1995). Physiological and molecular characterization of flor yeasts: polymorphisms of flor yeast populations. *Yeast* 11, 1399-141.

- [16] Querol, A., Barrio, E., Huerta, T. & Ramon, D. (1992a). Molecular monitoring of wine fermentations conducted by active dry yeast strains. *Appl Environ Microbiol* 58, 2948-2953.
- [17] Schuetz, M. & Gafner, I. (1993). Analysis of yeast diversity during spontaneous and induced alcoholic fermentations. *J Appl Bacteriol* 75, 551-558.
- [18] Baleiras Couto, M. M., Eijssma, B., Hofstra, H., in 't Veld, I. H. & van der Vossen, J. M. (1996). Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. *Appl Environ Microbiol* 62, 41-46.
- [19] Ibeas, I. I., Lozano, I., Perdignes, L. & Jimenez, I. (1996). Detection of *Dekkera/Brettanomyces* strains in sherry by a nested PCR method. *Appl Environ Microbiol* 62, 998-1003.
- [20] Ibeas, J. I., Lozano, I., Perdignes, F. & Jimenez, I. (1997). Dynamics of flor yeast populations during the biological aging of sherry wines. *Am J Enol Vitic* 48, 75-79.
- [21] Arias, C.R., Burns, J.K., Friedrich, L.M., Goodrich, R.M. and Parish, M.E. (2002) Yeast species associated with orange juice: Evaluation of different identification methods. *Applied and Environmental Microbiology* 68, 1955–1961.
- [22] Van der Vossen, J.M.B.M. and Hofstra, H. (1996) DNA based typing, identification and detection systems for food spoilage microorganisms: development and implementation. *International Journal of Food Microbiology* 33, 35–49.
- [23] Guillamon, J.M., Sabate, J., Barrio, E., Cano, J. and Querol, A. (1998) Rapid identification of wine yeast species based on the RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Archives of Microbiology* 169, 387–392.
- [24] Thomas, D. (1993) Yeasts as spoilage organisms in beverages. In *The Yeasts*, 2nd edn, Yeast Technology, Vol. 5. ed. Rose, A. And Harrison, J. pp. 517–561. London, UK: Academic Press.
- [25] Lieckfeldt, E., Meyer, W. and Borner, T. (1993) Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. *Journal of Basic Microbiology* 6, 413–426.
- [26] Bart Theelen, Massimiliano Silvestri, Eveline Gueho, Alex van Belkum, Teun Boekhout, "Identification and typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLPTm), random amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE)", *FEMS Yeast Research* 1 (2001) 79-86.
- [27] Fausto Gardini, Giovanna Suzzi, Angioletta Lombardi, Fernanda Galgano, Maria Antonietta Crudele, Christian Andrighetto, Maria Schirone, Rosanna Tofalo, "A survey of yeasts in traditional sausages of southern Italy", *FEMS Yeast Research* 1 (2001) 161-167.
- [28] Hennequin, C., A. Thierry, G. F. Richard, G. Lecointre, H. V. Nguyen, C. Gaillardin, and B. Dujon, "Microsatellite Typing as a New Tool for Identification of *Saccharomyces cerevisiae* Strains", *JOURNAL OF CLINICAL MICROBIOLOGY*, Feb. 2001, Vol. 39, No. 2, p. 551–559.