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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^{0}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 mitochondria1 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^{0}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^{0}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 94º C for 5 min. Final denaturation at 94 ºC for 30 seconds, Annealing temperature was 35 ºC for 30 seconds and Extension was at 72 ºC for 30 seconds. This cycle was repeated for 36 cycles. The final elongation was carried out at 72 ºC 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).

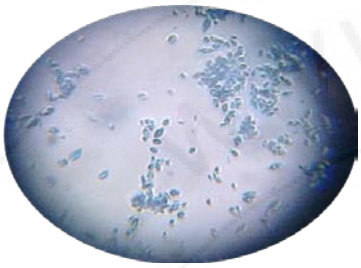

Figure 1: Microscopic view of Yeast

Figure 2: Qualitative Analysis of the Genomic DNA

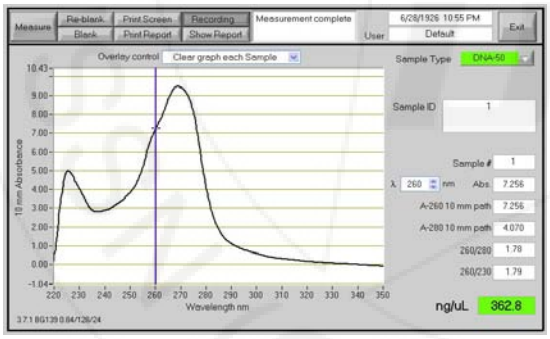

Figure 3: Quantitative Analysis of the DNA

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 king 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).

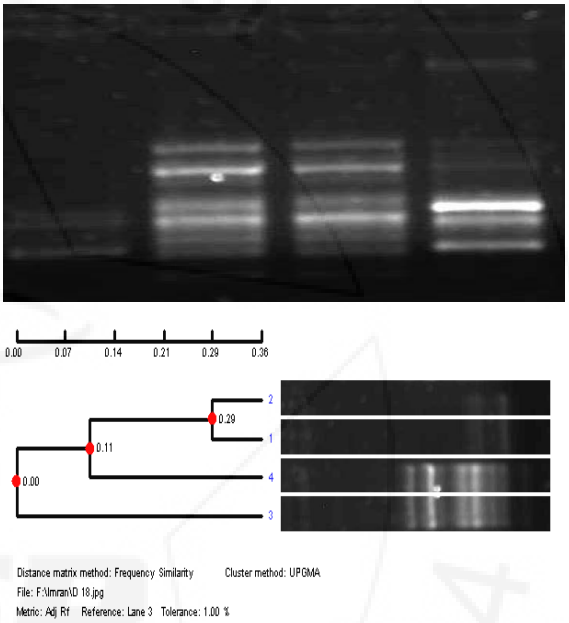

Figure 4: DNA fingerprint and Dendrogram for D18

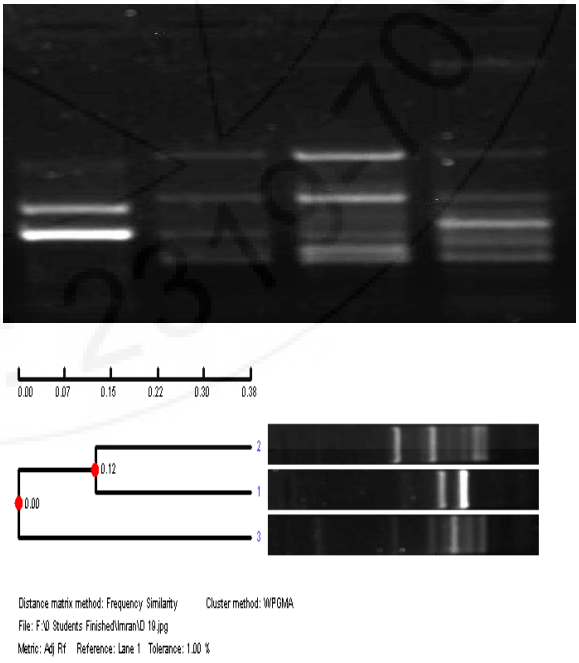

Figure 5: DNA fingerprint and Dendrogram for D19

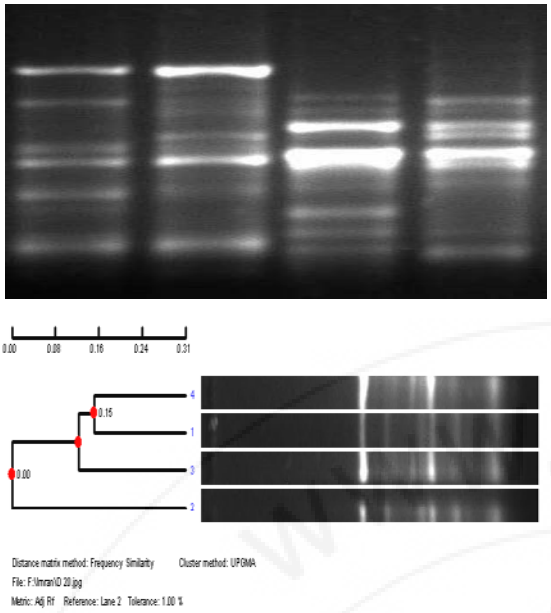

Figure 6: DNA fingerprint and Dendrogram for D20

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 (AFLPTm) 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 pro¢les 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.

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