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Molecular Characterization of Yeast (*Saccharomyces cerevisiae)* Strains by Using Molecular Marker

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Abstract— *Molecular markers reveals polymorphism at the DNA level. Molecular characterization of Yeast (Saccharomyces cerevisiae) Strains with RAPD Molecular markers. The genetic diversity allelic variations among these strains were estimated by using RAPD molecular markers. Twenty Eight RAPD primers were screened out of them eleven primers had shown amplification of which AB1-15, 1283, OPB12, OPO4, SC1 and OPB01 these six primers showed 100% polymorphism. Out of ten wine yeast strains studied at molecular level, two strains showed maximum similarity i.e. 79 % between them viz. NCIM 3045 and 3200. Genetic diversity was analyzed based on data obtained by 11 RAPD primers. Most of the primers were found 85.71 to 100% polymorphic in nature. The diversity at molecular level was analyzed with elucidian distance of 0.40. molecular markers hold great promise due to their high efficiency, adequate accuracy, and good reproducibility*

Keywords— ***DNA, Genetic variation, Molecular markers, PCR and Yeast.***

I. INTRODUCTION

Recent advances in DNA sequencing have identified many nucleotide polymorphisms in the human genome, but it has been challenging to associate this genetic variation to specific phenotypic differences among individuals for complex traits [4, 8, 14]. Different yeast species or strains may also confer to the quality of wines through the direct production of a variety of sensory compounds. For instance, Starmerella bacillaris improves the analytical profile of wines by increasing glycerol and succinic acid or total ester content and diminishing acetic acid and higher alcohols [5, 17]. Various traditional fermented food products of western Himalayas have been documented [13]. DNA fingerprinting method provides simultaneous detection of highly variable DNA fragments. Initially RFLP was used for the fingerprinting, but with the advent of PCR technology, a number of techniques have become available for fingerprinting of a variety of microbes including yeasts [18]. The application of marker technologies is not affected by climatic and environmental conditions, unlike agricultural practices. Some of the molecular markers include Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Sequence-Related Amplified Polymorphism (SRAP), Inter Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSRs), Sequence Characterized Amplified Region (SCAR), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP), Diversity Array Technologies (DArT), and Next Generation Sequencing (NGS) [1].

II. MATERIAL AND METHODS

1. Isolation DNA: Ten Yeast Strains (NCIM-3045, NCIM- 3185, NCIM-3189, NCIM-3200, NCIM-3283, NCIM-3287, NCIM-3205, NCIM-3095, NCIM- 3315, and NCIM-3215). Determination of quantity and quality of isolated DNA.

2. PCR Amplification: Isolated Yeast strains DNA Optimization with RAPD Primers for Analysis genetic diversity. PCR reaction component and PCR cycle as shown in (Table No. 01 and 02).

3. Agarose gel electrophoresis: DNA sample was diluted with appropriate quantity of sterilized distilled water to yield a working concentration of 25ng/µl for RAPD markers analysis. Used for screening 28 RAPD primers. The technique uses the repeat anchored primers of short oligonucleotide (16-17 bp) for DNA amplification by PCR. The amplified products were resolved on 1.5% agarose gel at 100 V for 1.5 hour. The gel was stained with ethidium bromide (5µl/100ml). Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02). The SIMQUAL programme was used to calculate the Jaccard's coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on Jaccard's coefficient.

Table No. 01: PCR components and stock solutions for RAPD

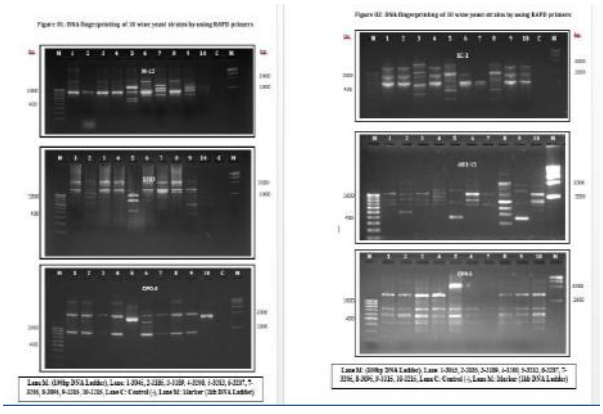
Sr. No.	Components	Stock	Require	Volume/ µl Reaction
1.	D/W	---	---	18.5
2.	PCR buffer	10X	1X	2.5
3.	Primer	10 pm/ µl	10 pm	1.0
4.	dNTPs	25 mM	0.2 mM	0.2
5.	$MgCl_2$	25 Mm	1.5 mM	1.5
6.	*Taq* DNA polymerase	5 U/µl	1U/ µl	0.3
7.	DNA	50ng/µl	30ng	1.0
			Total	**25 µl**

Table No.02 Cyclic parameter of thermal cycler for RAPD

Step	Temp (°C)	Duration	Cycles	Function
1.	94	2 min	1	Initial denaturation
2.	94	30 sec	40	Denaturation
3.	36	45 sec		Annealing
4.	72	2 min		Extension
5.	72	10 min	1	Final extension
6.	4	∞	1	Hold

III. RESULT AND DISCUSSION

Analysis of Genetic variation: All these primers had produced maximum percent polymorphism i.e. 100 % except primer M-13, OPB-10, SC-02 and OPA-04 which showed the minimum percent polymorphism Overall all 11 primers were generated total 498 amplicons with an average of 45.27 amplicons per primer as shown (Fig 01 & 02). Out of 498 amplicons, 438 amplicons were found polymorphic, it showed 93.56 % polymorphism. Similarly, out of the total amplicons, 60 amplicons were found monomorphic. It showed 6.44 % monomorphism and the average number of monomorphic amplicons per primer were 0.55. The genetic similarity matrix obtained by Jaccard's similarity coefficient ranged from 0.39 to 0.79 among ten wine producing strains of *Saccharomyces cerevisiae* based on RAPD profiling.



The diversity at molecular level was analyzed with elucidian distance of 0.40. Out of ten wine yeast strains studied at molecular level, two strains showed maximum

similarity i.e. 79 % between them viz. NCIM 3045 and 3200. Genetic relationship between 10 strains of wine yeast were determined by NTSYS pc2.02 software to calculate similarity among them and dendrogram was depicted by using Jaccard's similarity coefficient.

IV. CONCLUSION

Yeast culture was maintained on YEPD medium. Further, DNA was extracted by modified protocol and subjected for genetic diversity analysis by using RAPD primers. Initially 28 random 10 bp primers were screened, among which 11 primers has generated reproducible and scorable banding pattern. Hence, genetic diversity was analyzed based on data obtained by 11 RAPD primers. Twenty Eight RAPD primers were screened out of them eleven primers had shown amplification of which AB1-15, 1283, OPB12, OPO4, SC1 and OPB01 these six primers showed 100% polymorphism. RAPD OPA 05, M-13, OPB 10, SC2 and OPA04 had shown 80%, 85.71%, 88.89%, 88.89 % and 85.71% polymorphism respectively. The RAPD molecular marker system found efficient to discriminate diverse population of *Saccharomyces cerevisiae.*

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