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Genetic diversity of Yeast (*Saccharomyces cerevisiae*) Strains by using RAPD Marker

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Abstract**—** *The term 'wine' is applied to the product made by the alcoholic fermentation by yeast in which the sugars are converted into alcohol and carbon dioxide. Genetic similarity was calculated using Jaccard's similarity coefficient and cluster analysis revealed two major clusters. 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 diversity was analyzed based on data obtained by 11 RAPD primers. Most of the primers were found 85.71 to 100% polymorphic in nature.*

Keywords**— Molecular markers, DNA, Yeast Strains. Genetic diversity. PCR.**

I. INTRODUCTION

The phenotypic diversity of brewing yeasts enables brewers to achieve the flavor, aroma, and other sensory properties that beer consumers seek. Brewers utilize pure strains of yeast for most industrial beer production. The impacts of yeast on beer are numerous and diverse. [7]. the technological differences among yeast strains depend on their intraspecific genetic diversity [4]. The most significant roles of this microbiota are acidification, flavour formation, and leavening of the dough, where yeast produces aroma compounds and CO2 [1]. The heterogeneity between the *S. cerevisiae* populations may provide different functional traits for sourdough yeast [11].

Molecular markers are valuable tools to assess this heterogeneity and analyse the population structure of yeast from different geographical origins. Up to now, several molecular markers such as inter-simple sequence Repeat (ISSR), randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and single-nucleotide polymorphisms (SNPs) have been employed to evaluate the genetic variation and population structure of *S. cerevisiae* strains from sourdough and other food matrixes [3,5,7,10,]. The RAPD method has been widely applied in the genetic fingerprinting of food yeast or bacterial isolates [9]

II. MATERIAL AND METHODS

A. Isolation of Genomic DNA Each Yeast Strains: 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.

B. 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.

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

Sr. No.	Components	Stock	Require	Volume/ µl
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	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

C. 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.

III. RESULTS AND DISCUSSION

A. Genetic diversity analysis

Overall all 11 primers were generated total 498 amplicons with an average of 45.27 amplicons per primer. 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. 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 (Fig 01).

Genetic relationship between 10 strains of wine yeast were determined on the basis of presence of band was scored as (1) and absence as (0) was subjected to NTSYS pc2.02 software to calculate similarity among them and dendrogram was depicted by using Jaccard's similarity coefficient. The genetic similarity matrix obtained by Jaccard's similarity coefficient (Figure 01) 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.

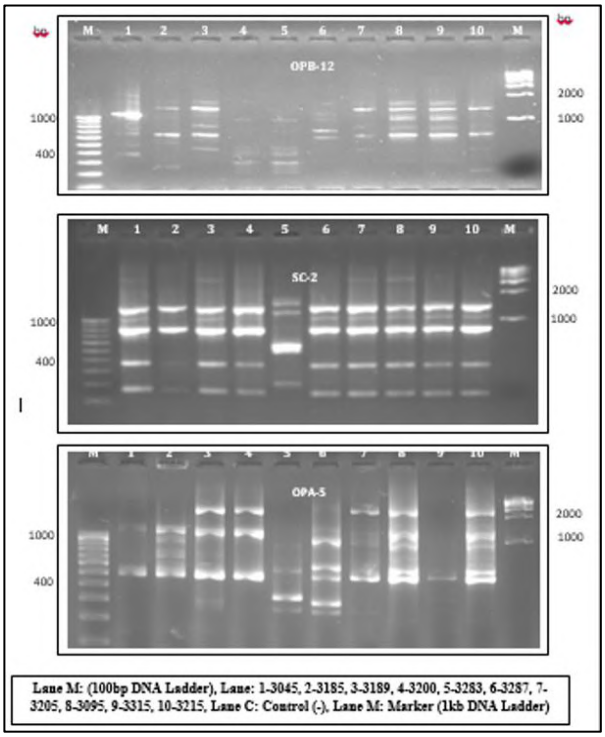

Fig.1: DNA fingerprinting of 10 wine yeast strains by using RAPD primers

B. Dendrogram Analysis

Dendrogram generated based on UPGMA analysis of RAPD data grouped all these strains of *s. cerevisiae* were grouped in to two major clusters A and B. The cluster A contains 09 strains viz. 3045, 3200, 3189, 3287, 3095, 3185, 3215, 3205 and 3315 of wine yeast while cluster B contain 01 strain i.e. 3283 of wine yeast i.e. NCIM 3283 and shared 39% similarity with all other strains (Fig 02).

The cluster I comprised cultures NCIM 3045 and 3200 together showed 100% similarity with each other. The member 3283 maintained its separate cluster.

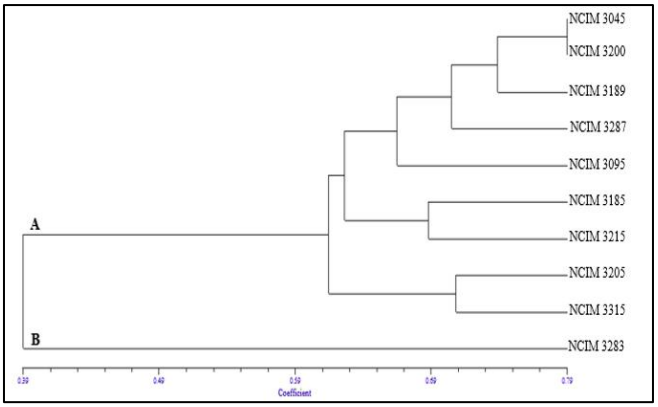

Fig.2: Dendrogram for 10 wine yeast strains based on NTSYS-pc UPGMA clustering method with generic similarity from DNA based RAPD markers analysis

IV. CONCLUSIONS

The RAPD molecular marker system found efficient to discriminate diverse population of *Saccharomyces cerevisiae.* 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.

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REFERENCES

[1] De Vuyst L, Harth H, Van, S and Leroy F. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. Int J Food Microbiol.2016. 239:26–34.

[2] Erfianti, T. Wijanarka, W and Kusumaningrum, H. Molecular identification of inulinolytic yeast isolated from cherry fruit (*Muntingia calabura* L.) based on internal transcribed spacer sequence. Journal of Physics: Conference Series.2021. 943. 012059.

[3] Gallego, F. Perez, M. Núñez, Y and Hidalgo P. Comparison of RAPDs, AFLPs and SSR markers for the genetic analysis of yeast strains of *Saccharomyces cerevisiae.* Food Microbiol. . 2005. 22(6):561– 568.

[4] Huy, G. Daniel, M. and De Vuyst L. Taxonomy and biodiversity of sourdough yeasts and lactic acid bacteria. In: Gobbetti M, Gänzle M (eds) Handbook on sourdough biotechnology. Springer, US, Boston, MA.2013.105–154.

[5] Legras, J. Erny C and Charpentier C. Population structure and comparative genome hybridization of European for yeast reveal a unique group of *Saccharomyces cerevisiae* strains with few gene duplications in their genome. PLoS ONE.2014. 9 (10):e108089.

[6] Leite, L. Lelis, F.Xavier, M. Santos, J. Cardoso, L. Barbosa, F. Santos, R. Dias ,S. and Xavier, A. Molecular identification and characterization of filamentous fungi and yeasts isolated in a pharmaceutical industry environment. J Appl Pharm Sci. 2020. 10(07): 027–036.

[7] Liu, S. Impact of Yeast and Bacteria on Beer Appearance and Flavour. In: Brewing Microbiology; Elsevier: Amsterdam. 2015. pp 357–374.

[8] Matthewm, T. Fingerprinting *Saccharomyces cerevisiae* Strains Using Next Generation Sequencing of PCR Amplicons Generated from Delta Elements, Journal of the American Society of Brewing Chemists.2023. 81:3, 374-382.

[9] Nisiotou, A. Gyftogianni, E and Banilas, G. Evaluation of Different Molecular Markers for Genotyping Non-Saccharomyces Wine Yeast Species. Microbiol. Research. 2022 (13). 643–654.

[10] Nisiotou, A. Parlapani, F. Kormas, K. and Boziaris, I. Old Targets, New Weapons: Food Microbial Communities Revealed with Molecular Tools. In Novel Food Preservation and Microbial Assessment Techniques; CRC Press: Boca Raton, FL, USA, 2014. pp. 277–312.

[11] Palla M, Cristani C, Giovannetti M and Agnolucci M. Large genetic intraspecifc diversity of autochthonous lactic acid bacteria and yeasts isolated from PDO Tuscan bread sourdough. Appl Sci .2020.10(3):1043.

[12] Salah, E., Alla, M. and Seada, M. Molecular and biochemical studies of some yeast strains. *African J. Biotech*.2011. 10(8): 1309-1319.

[13] Skelin, A., Sanja, S. Sandi, O. Lejla, D and Sulejman, R. Genetic diversity of indigenous saccharomyces sensustricto yeasts isolated from southern croatia. *Agriculturae Conspectus Scientificus.*2008. 73(2): 89-94

[14] Valero, E., Brigitte, C. Dorit, S. Margarida, C. and Sylvie, D. Biodiversity of *Saccharomyces* yeast strains from grape berries of wine producing areas using starter commercial yeasts. *FEMS Yeast Res.* 2006. 317–329.

[15] Xufre, A. Simoes, F. Girio, F. Clemente, A. and Amaral-Collaço, M. Use of RAPD analysis fordifferentiation among six enological *Saccharomyces* spp. strains. *Food Tech. Biotech.*2000. 38: 53-58.

[16] Zahara, J., Guiamal, A. and Cynthia, T. Microsatellite and RAPD analysis of six local wine strains of *saccharomyces cerevisiae. Philippine Sci. Letters*. 2011. 4 (2): 122-130.