Molecular Diversity of commercially available edible oyster mushroom by SSR marker

Abstract

Oyster mushroom (*Pleurotus species*) was commercially grown in the worldwide. Oyster mushroom naturally found in rotten wood or agricultural lignocellulosic residues as saprophytic in nature. Due to the good amount of protein with low carbohydrates people prefer it in their daily diet. It can be alternate option of vegan protein with high lysine content up to 6-8% essential amino acid from fresh and dried sample. Our study aims to evaluated that availability of Simple sequence Repeat (SSR) PCR based marker for genetic diversity analysis on five different oyster mushrooms were also identified on the basis of polymorphism. The dendrogram obtained from UPGMA cluster analysis of Jaccard's similarity values based on 7 SSR markers grouped 5 strains into two clusters species wise (*P. djamor*, *P. florida*, *P. ostreatus*, *P. pulmonarius* and *P. sajor-caju*) which confirm the reliability of PCR based molecular markers. diversity analysis also confirmed the grouping of genotypes as they were present in same group as revealed by SSR markers.

Keyword: *Pleurotus species*, Genetic distance, SSR marker, Genetic diversity, Cluster analysis, Polymorphisms etc.

Introduction

Genetic diversity plays a crucial role in the adaptability and survival of species, including economically and ecologically important fungi such as *Pleurotus species*, commonly known as oyster mushrooms (Tang *et al*., 2010). These species are widely cultivated due to their nutritional value, medicinal properties, and ability to grow on a variety of lignocellulosic materials, making them important for sustainable agricultural practices. In the world, 44 million tons of mushrooms are produced currently (FAOSTAT, 2023) a five-fold increase since 2000. The Asian continent accounts about 95% of global production, followed by European (3%), and American continental (1%) (Bijla and Sharma 2023). India is one of the world's top 5 mushroom-producing nations, with 0.31 million tons produced annually. With an approximate 35.6 thousand tones, production and an 11% share in the entire product, Bihar is the leading state in terms of mushroom production. The next highest producing state is Odisha, with about 34.5 thousand tones, followed by Maharashtra (32.5 thousand tones), Uttar Pradesh (23.4 thousand tones), and Uttarakhand (22.4 thousand tones).

One of the most effective ways to assess genetic diversity is through molecular markers. Among these, Simple Sequence Repeats (SSRs), also known as microsatellites, are widely used due to their high polymorphism, reproducibility, and co-dominant inheritance.

SSR markers target regions of repetitive DNA sequences scattered throughout the genome, making them powerful tools for studying genetic variation within and between populations of *Pleurotus species*. Using SSR markers to evaluate the genetic diversity of *Pleurotus species* provides valuable insights into their population structure, evolutionary relationships, and breeding potential. This information is crucial for conservation efforts, improvement of strains for commercial production, and the identification of new strains with desirable traits such as enhanced yield, disease resistance, or specific bioactive compounds.

Materials and methods

All the materials were collected from mushroom division under department of plant pathology including *P. djamor*, *P. florida*, *P. ostreatus*, *P. pulmonarius* and *P. sajor-caju*. Seven SSR markers were identified and designed primers by using MISA-Web tool (Beier *et al*., 2017) from the downloaded genome sequence of *P. ostreatus* PC15 (AYUK00000000.1) for diversity analysis (Grigoriev *et al*., 2012 and Riley *et al*., 2014).

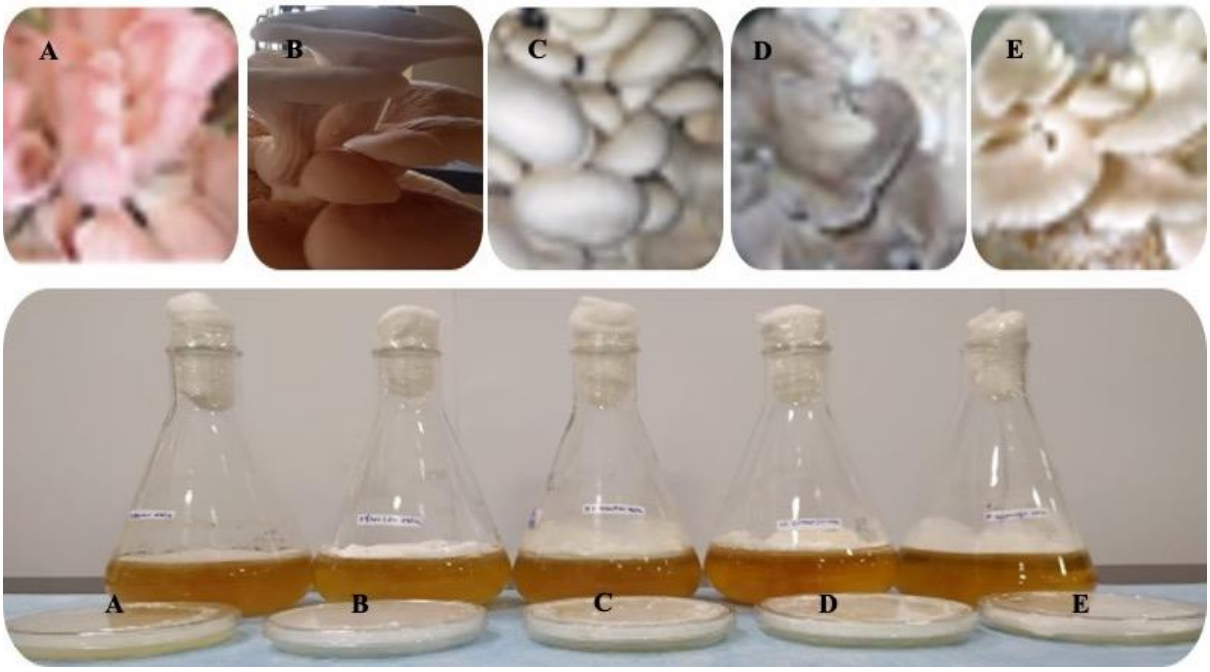

Figure 1: Culture of different species of oyster mushroom: A. *P. djamor,* B. *P. florida*, C. *P. ostreatus*, D. *P.pulmonarius and* E. *P. sajor-caju*

DNA extraction

Genomic DNA was extracted from the all mushroom samples by using Cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1990) with some modifications. CTAB buffer was prepared 2% CTAB, 100 mM Tris-HCl, 1.4 M

NaCl, 20 mM EDTA. 100 mg of fruiting body samples were prepared by grinding in a mortar and pestle. Ground samples was transferred in 2ml centrifuge tube homogenized each samples by adding 500 µl of CTAB Buffer. Mixed and thoroughly vortex for preparation of homogenized sample. And placed the tube in a 60°C water bath for 20 minutes. Then added an equal volume of chloroform/isoamyl alcohol (24:1). Samples were centrifuged for 15 minutes at 14,000 x g to separate the phases. The aqueous upper phase was transferred in 1.5 ml of centrifuge tubes and repeat thrice this extraction until the upper phase is cleared. Added equal volume of pre chilled isopropanol and incubate at 4°C for overnight to precipitate the DNA. Second day centrifuged the samples at 14,000 x g for 15 minutes and decant the supernatant without disturbing the pellet and subsequently washed with 500 µl 75% ethanol and dried it upto removal of ethanol residues. Pellet was dissolved in 20 µl TE buffer (10 mM Tris, pH 8, 1 mM EDTA).

SSR analysis

A SSR primer set of oligoes (Bioserve Pvt. Ltd. Bangalore) were screened purchased for the Molecular diversity analysis (Williams *et al.* 1990) using 5 diverse strain of oyster mushrooms. Out of these, 2 which gave sufficient polymorphism were used for assessing genetic diversity among genotypes.

The amplification reactions were carried out in a 25 µl volume containing 10X PCR buffer with $MgCl_2$, 10mM dNTPs (dATP, dCTP, dGTP and dTTP), 10 p mol/µl primer, 3 U/µl*Taq* DNA Polymerase (Bangalore Genei, India) and 50 ng/µl genomic DNA template. DNA amplifications were performed in duplicate in a DNA thermal cycler (Applied Biosystem, USA) programmed for a preliminary step of SSR amplification. DNA was denatured at 94°C for 5 minutes followed by 35 amplification cycles (94°C for 30 seconds; 35°C for 45 seconds; 72°C for 45 seconds) and a final extension step at 72°C for 7 minutes. The amplified products were resolved by electrophoresis in 2% Polyacrylamide gel run in 1X TBE buffer and amplified fragments were visualized by ethidium bromide staining. The resolved product was photographed under UV light using gel documentation Gel Doc^{TM} 5500 (Alpha InfoTech, USA.).

Table 1. List of SSR primers for diversity analysis of *Pleurotus species*.

S.No.	**Primers**	**Sequence (5'-3')**	**TNB**	**TPB**	**PIC(%)**

1	PoM908	$(C)_{15}$	7	5	71.4
3	PoM909	$(AT)_{11}$	6	0	0
5	PoM910	$(ACG)_{6}$	17	5	29.4
7	PoM911	$(AG)_{6}$	16	5	31.2
9	PoM912	$(G)_{13}$	8	4	50
11	PoM913	$(G)_{13}$	12	5	41.6
13	PoM914	$(GGA)_{7}$	12	1	8.3

Computation analysis

Genetic diversity analysis was done using NTSYS-pc Version 2.1 (Numerical Taxonomic System) software (Rohlf 2000) and similarity matrix was created to the cluster analysis of Unweighted Paired Group method using Arithmetic Averages (UPGMA) and dendrogram was constructed.

Result and Discussion

The primary objective of this study was to assess the genetic diversity and group *Pleurotus* spp based on molecular markers. These markers, which evaluate genome sequence composition, help to detect genetic variations among genotypes, enabling the effective use of genetic variability for crop improvement. Previous studies, such as those by Davierwala et al. (2000), Neeraja et al. (2002), and Saker et al. (2005), have highlighted the use of molecular markers to study genetic diversity and relationships among different crop cultivars. The use of SSR markers for species identification and molecular characterization has been well-documented, including in recent research on *Pleurotus* species in Seoul, Korea (Ryu et al., 2024). The genetic distances among five commercially cultivated oyster mushroom species are presented in Table 2.

Molecular diversity and polymorphism studies in five Pleurotus species were carried out through seven SSR primers.

The highest Nei's genetic distance (0.73) was observed in *P. ostreatus, P. florida* and *P. pumlonarius*with all species except *P. djamor* and *P. sajor-caju*.The lowest genetic distance (0.44). The results revealed varying levels of genetic distance among the five Pleurotus species, ranging from low to high. Primers PoM912, PoM913, and PoM914 exhibited high genetic diversity, particularly between P. florida, P. sajor-caju, and P. florida. In contrast, primers PoM910 and PoM911 demonstrated a high degree of similarity between P. ostreatus and P. pulmonarius, followed by P. florida. Notably, primer PoM908 failed to produce any bands in the gel electrophoresis for all species (Fig. 2).

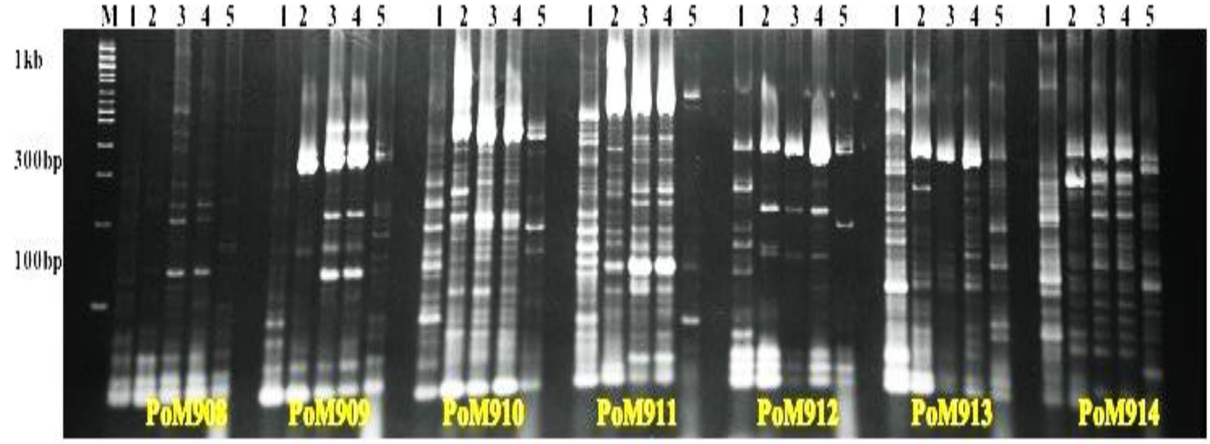

Figure 2: Gel image of amplified product M: Ladder, 1. *P. djamor*, 2. *P. florida*, 3. *P. ostreatus*,4. *P. pulmonarius*, 5. *P. sajor-caju*and SSR markers PoM908, PoM909, PoM910, PoM911, PoM912, PoM913 and PoM914.

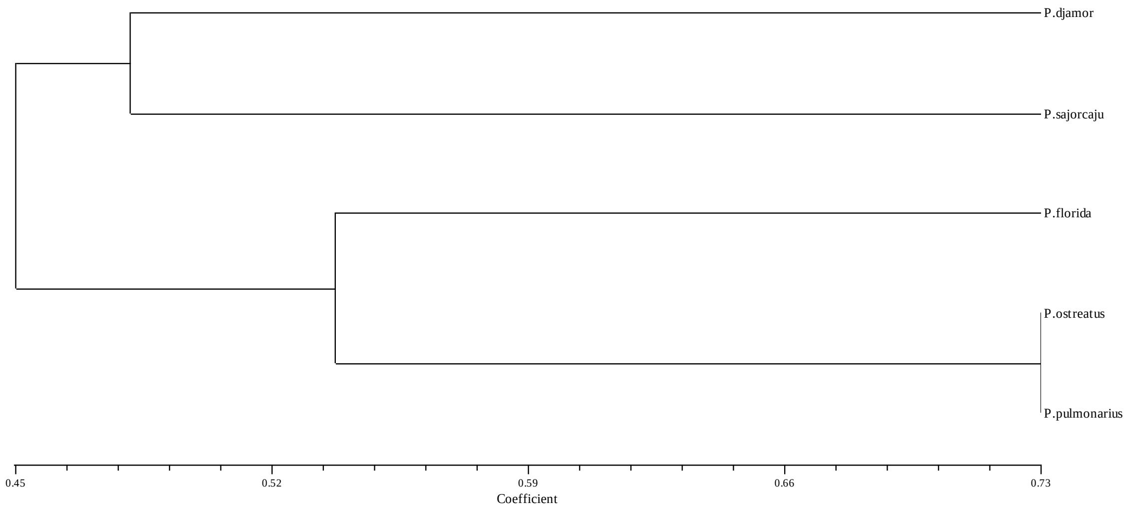

Figure 3: Genetic diversity of species in the genus *Pleurotus* by UPGMA dendrogram. All data were analysed by using the NTSYS.

3.1 Similarity coefficient analysis and Clustering

The relationships among Pleurotus species were estimated by a UPGMA cluster analysis of genetic similarity matrices. Similarity coefficient between different genotypes ranged from 0.392 to 1.00. Table is given below:

Table 2. Similarity matrix of all five Pleurotus species based on SSR results

	P. djamor	***P. florida***	***P. ostreatus***	***P. pulmonarius***	***P. sajor-caju***
P. djamor	1.000				

P. florida	0.456	1.000			
P. ostreatus	0.392	0.582	1.000		
P. pulmonarius	0.430	0.494	0.734	1.000	
P. sajor-caju	0.481	0.519	0.481	0.418	1.000

Two major clusters were identified in the analysis. The first cluster comprised two genotypes, while the second cluster included three *Pleurotus species* (Fig.1). Genetically similar accessions grouped together, with Group I exhibiting a 50% similarity coefficient across all five species, including two genotypes. Group II consisted of three genotypes with a 54% similarity. The similarity index revealed a high degree of similarity (73%) between *P. ostreatus* and *P. pulmonarius*, followed by *P. florida* and *P. ostreatus* (58.2%), and *P. florida* and *P. sajor-caju* (51.8%). Equal similarity (48.1%) was observed between *P. djamor* & *P. sajor-caju*. Various molecular markers, including RAPD, ISSR, and SRAP, have been used in Pleurotus diversity studies, but SSR markers were found to produce a higher frequency of polymorphisms due to their long repetitive loci. SSRs are therefore an efficient tool for genetic diversity analysis. Previous data has been described that *P. ostreatus*, *P. pulmonarius* and *P. florida* was phenotypically different but our results strongly supported that they have a close genetic relationship, compare to *P. djamor* an *P. sajor-caju* (Lin *et al*., 2022). The results will also aid in the identification and functional validation of hybrid strains, particularly those involving PoM912, PoM913, and PoM914 SSR markers.

Conclusion:

The study highlights the effectiveness of SSR markers in assessing genetic diversity and clustering among *Pleurotus species*, revealing significant genetic variation and polymorphism. These findings provide valuable insights for molecular characterization and the development of improved hybrid strains. Based on the results, it is recommended to focus on further exploration of SSR markers (PoM912, PoM913, and PoM914) for their potential in identifying genetic variability and supporting hybrid strain development in *Pleurotus species*. Expanding the analysis to include more genotypes and additional molecular markers could enhance understanding of genetic relationships. Incorporating these findings into breeding programs may aid in developing high-performing, biotic and abiotic tolerant Pleurotus hybrids.

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