Genetic diversity of Pleurotus ostreatus (Jacq.) P. Kumm. strains in Java based on random amplified polymorphic DNA markers

by Nuraeni Ekowati

Submission date: 21-Nov-2022 05:24AM (UTC+0700)
Submission ID: 1959475160
File name: biodiversitas-_Genetik_juni_2021.pdf (317.56K)
Word count: 4206
Character count: 22549

BIODIVERSITAS
Volume 22, Number 6, June 2021
Pages: 3488-3493

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220658

Genetic diversity of *Pleurotus ostreatus* (Jacq.) P. Kumm. strains in Java based on random amplified polymorphic DNA markers

NURAENI EKOWATI[1,♥], ARIS MUMPUNI[1], JUNI SAFITRI MULJOWATI[1], NUNIEK INA RATNANINGTYAS[1], ARDHINI RIN MAHARNING[2]

[1]Mycology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63, Purwokerto, Banyumas 53122, Central Java, Indonesia
[2]Ecology Laboratory Faculty of Biology. Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63, Purwokerto, Banyumas 53122, Central Java, Indonesia.
Tel.: +62-281-638794, Fax.: +62-281-631700, ♥email: nuraeniekowati29@gmail.com

Manuscript received: 30 September 2020. Revision accepted: 30 May 2021.

Abstract. *Ekowati N, Mumpuni A, Muljowati JS, Ratnaningtyas NI, Maharning AR. 2021. Genetic diversity of Pleurotus ostreatus (Jacq.) P. Kumm. strains in Java based on Random Amplified Polymorphic DNA markers. Biodiversitas 22: 3488-3493.* Genetic variation in a fungal population can occur due to mutation and recombination, resulting in changes in the nucleotides that encode specific DNA sequences. Strains with a high genetic distance and good production capabilities can be used to develop genetic breeding. This study aimed to investigate genetic relationship among *Pleurotus ostreatus* strains cultivated in Java (Bogor, Cianjur, Tasikmalaya, Purwokerto, Yogyakarta, Tawangmangu, Malang, and Madiun) based on random amplified polymorphic DNA (RAPD) markers. The research method consisted of DNA isolation and DNA amplification using six primers, i.e. OPA2, OPA3, OPA4, OPA7, OPA9, and OPA10. DNA band data were analyzed using NTSYSpc21 software to determine the level of genetic similarity, based on the Unweighted Pair Group Method with Arithmetic Average Algorithm (UPGMA). In all, 101 amplified DNA bands were obtained, with sizes ranging from 136 to 2320 bp and 96.0% of the bands were polymorphic. Based on cluster analysis, it shows that three clusters were formed. There were genetic variations and relationships among eight *P. ostreatus* strains in Java with a genetic similarity varying from 37-98%.

Keywords: Genetic diversity, *Pleurotus ostreatus*, polymorphism, primers, RAPD

INTRODUCTION

Pleurotus ostreatus (Jacq.) P. Kumm. is a type of edible mushroom with high nutritive value and cultivated in several regions in Indonesia. This fungus is known as a white oyster mushroom because of its white color and shape cap. It can be grown on various agricultural wastes as media (Tsegaye and Tefera 2018). Among *P. ostreatus* cultivation centers in Indonesia, particularly Java including Bogor, Cianjur, Tasikmalaya, Purwokerto, Yogyakarta, Tawangmangu, Malang, and Madiun, it is suspected that cultivation of the fungus has resulted in several strains representing some genetic diversity. *P. ostreatus* has good nutritional value, resulting in increased interest in its cultivation. The determination of genotypic identity is also important for analyses of populations, structure, and evolution within and among species. An important issue is the identification of species beyond morphological and physiological criteria, as this affects fruiting bodies (Khan et al. 2011). This mushroom has low resource and space requirements and can be grown throughout the world and year-round from low-cost starting materials (Chakravarty 2011).

Information regarding genetic variation in *P. ostreatus* strains cultivated in Java is particularly important for selecting superior strains for further development. Inyod et al. (2017) reported that traditional morphological characterization must be supplemented with molecular characterization to obtain more accurate results. Gupta et al. (2011) and Hasan et al. (2018) reported the development of molecular techniques that can genetically differentiate individuals from one another, in addition to fungal populations. Knowing genetic diversity in *P. ostreatus* will assist in the process of selecting parent strains (parental selection) that are genetically superior. One technique that can be used to determine genetic diversity is the random amplified polymorphic DNA (RAPD) technique. This method can be used to analyze whether there are significant genetic differences in isolates from different locations.

The RAPD technique can easily and quickly detect genetic changes throughout the genome and can be performed with small amounts of DNA. Therefore RAPD-PCR is becoming an extremely popular technique. It has numerous applications such as genetic mapping for fungi, DNA fingerprinting, population genetics studies, epidemiological studies, and the identification of fungal strains (Abdulmalk 2013). Genotyping is particularly important in analyses of population biology, involving the construction of genetic maps and recognizing evolutionary changes. Genetic variation can occur due to changes in the nucleotides that make up an organism's DNA molecules. These changes can in turn then affect phenotypes or reactions to a particular environment. Strains with a high genetic distance and good production ability can be used in genetic breeding (Khan et al. 2011).

The genetic diversity analysis on mushrooms has been using RAPD marker. This technique can determine the genetic similarity of *Pleurotus* strains (Ravash et al. 2010 and Khan et al. 2017). Analysis of the genetic diversity of *Pleurotus* have been conducted on wild or cultivated individual and showed high genetic diversity (Otieno and Onyango 2015). Khan et al. (2017) reported that genetic diversity on local and exotic of *P. ostreatus* using RAPD markers produced 70 bands that could be scored, of which 41 bands were polymorphic, with an average of 2.73 polymorphic bands per primer.

The *P. ostreatus* strains used in this study were commercially cultivated. However, to date, no studies have quantified the genetic diversity of isolates from these various cultivation centers. This study aimed to investigate genetic relationship among *P. ostreatus* strains cultivated in Java (Bogor, Cianjur, Tasikmalaya, Purwokerto, Yogyakarta, Tawangmangu, Malang, and Madiun) based on RAPD markers.

MATERIALS AND METHODS

Research materials

The study was conducted using a survey method, taking two samples of *P. ostreatus* mushrooms from each location. Eight strains were analyzed, one each originating from Bogor (PoB), Cianjur (PoC), Tasikmalaya (PoTs), Purwokerto (PoP), Yogyakarta (PoY), Tawangmangu (PoTw), Malang (PoMl), and Madiun (PoMd). All fungal strains were obtained in the form of pure mycelium culture. Then molecular research was done in Mycology Laboratory, Faculty of Biology, University of Jenderal Soedirman, Purwokerto Indonesia.

DNA extraction

The eight fungal strains were cultured on 50 mL potato dextrose broth (PDB) and incubated in a rotary shaker at 28°C for 15 days at a speed of 100 rpm. Mycelia were harvested using Whatman filter paper no. 41 in a Büchner funnel and used for DNA isolation (Ekowati et al. 2011).

The Nucleon Phytopure fungal DNA extraction RPN-8511/GE (Healthcare, UK) Kit was used to accomplish genomic DNA isolation. Fresh mycelia from 0.1 g to 0.3 g were crushed in liquid nitrogen and placed into 1.5 mL Eppendorf tubes. Then, 500 µL Phytopure I Reagent was added, and the mixture was homogenized by hand or vortex. Next, 200 µL Phytopure II Reagent was added. Samples were incubated at 65°C for 10 min in a water bath, then at 4°C for 20 min. Next, 400 µL cold chloroform that had been stored at 4°C was added to the samples and 20 µL Phytopure III reagent (resin) was added. Centrifugation was performed using a microcentrifuge at a speed of 1,300 × g for 15 min at RT until the upper phase in each sample was clear. The supernatant portions containing DNA in a 200 to 250 µL volume were transferred to new 1.5 mL tubes. Cold isopropanol/propanol with a volume equal to that of the supernatant was added. Then the tubes were closed and inverted to combine the supernatant and isopropanol. Centrifugation was performed at 4,000 × g for 5 min at room temperature. The supernatants were discarded and the precipitates (pellets) were washed by adding 100 µL 70% ethanol. Then the samples were centrifuged three times at 4,000 × g for 5 min, and after each centrifugation, any remaining ethanol was removed by opening and inverting the tubes on tissue paper until the sediments (pellets) were dry. After drying, the pellets were each resuspended in 100 µL Tris-EDTA (TE) buffer. These aqueous DNA samples were stored at -20°C until further analyses. To assess the quality of genomic DNA extracted from each *P. ostreatus* strain, the samples were electrophoresed before RAPD analyses.

PCR reaction and electrophoresis

DNA amplification was performed with the PCR-RAPD technique, using six primers 10 nucleotides in length, i.e. OPA2, OPA3, OPA4, OPA7, OPA9, and OPA10. Each sample was prepared for PCR with the following reaction components: 13.0 µL kit Master mix (dNTP, Taq-DNA polymerase, 1X magnesium chloride buffer), 2.5 µL primer (30.0 pmol), and 3.0 µL the appropriate DNA sample (50.0 ng/mL). Nuclease-free water was added to a 25 µL total volume for each sample. The samples were placed in a thermocycler programmed as follows: one initial stage of denaturation at 92°C for 3 min, then a set of 45 cycles were carried out. Each cycle consisted of temperature settings of 92°C for 1 min to denature the DNA, 37°C for 1 min to anneal primers to template and 72°C for 2 min for extension. After 45 cycles, extension at 72°C was carried out for 10 min. Then the samples were cooled to 4°C. The amplified samples were removed from the thermocycler and stored at -20°C for later gel electrophoresis analyses. Each reaction was repeated three times.

Amplified DNA samples were electrophoresed using 2% agarose gel in Tris-Borat-EDTA 1X (TBE). The gels were immersed in a 1X TBE running buffer. A total of 5 µL of each amplified sample was mixed with 1 µL loading dye, pipetted into a separate well in the gel, and then exposed to a 100V field for 30 min. Then the gels were immersed in ethidium bromide (EtBr) for 30 min. DNA bands were visualized with a UV transilluminator (Khan et al. 2017; Familoni et al. 2018).

Data analysis

The amplified DNA band data were analyzed for size and assessed based on the appearance of the bands. The data obtained were analyzed using the Numerical Taxonomy and Multivariate Analysis System Program, Version 2.1. (NTSYSpc21). Genetic similarity among accessions was calculated according to Jaccard's similarity coefficients using SIMQUAL (Similarity for Qualitative Data). The similarity coefficients were then used to construct dendrogram based on the unweighted pair group method with arithmetic average algorithm (UPGMA) (Rohlf 2000).

RESULTS AND DISCUSSION

RAPD profiles

DNA was isolated at concentrations between 210.20–2152.90 μg/mL with a DNA/RNA ratio between 1.86 and 2.10. The samples were of good quality, quantity, and purity (Figure 1). Template DNA purity is important for its use in PCR amplification. According to Sharon (2010), DNA isolation results are ideal if the ratio of DNA to RNA is between 1.8–2. Therefore, the samples possessed sufficient quality for further PCR analyses.

Genomic DNA samples were used for DNA amplification using six primers resulted in different amounts and sizes of amplified DNA band fragments. The results band sizes ranged from 136 to 2,320 base pairs (bp) (Figure 2). Our results are in line with previous studies, which have reported ranges of 200–2,000 bp in *P. nebrodensis* (Alam et al. 2009), 250–3,500 bp in *P. eryngii* (Ravash et al. 2010), and 646–779 bp in *P. sajor-caju* (Avin et al. 2013).

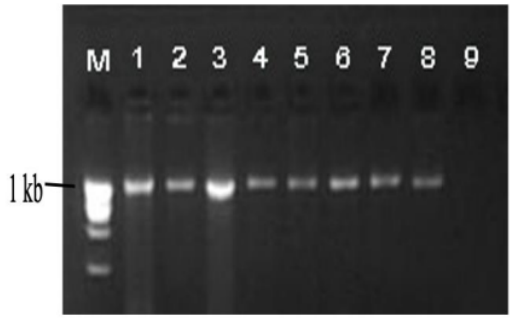

Figure 1. Genomic DNA band isolated from eight *Pleurotus ostreatus* strains. M: DNA marker 1 kb. Strains 1: Bogor, 2: Cianjur, 3: Tasikmalaya, 4: Purwokerto, 5: Yogyakarta, 6: Tawangmangu, 7: Malang, 8: Madiun, 9: Negative control (without DNA).

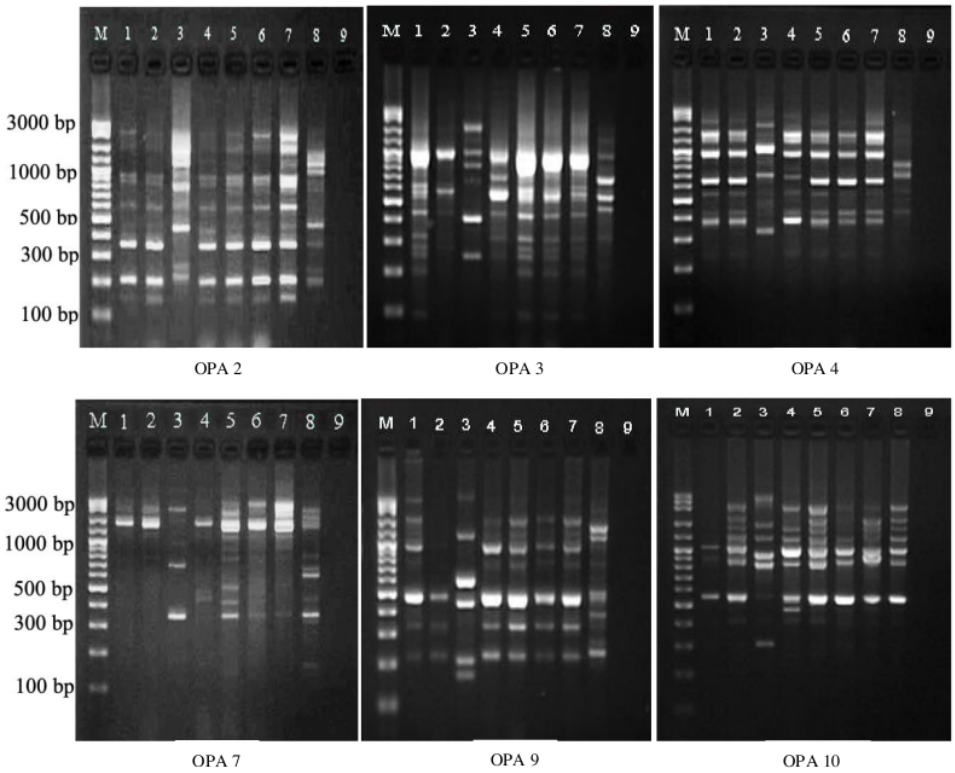

Figure 2. Pattern *Pleurotus ostreatus* DNA bands amplified using OPA2, OPA3, OPA4, OPA7, OPA9 and OPA10 primers. Note : M: 100 bp DNA marker of *P. ostreatus* starins 1: Bogor, 2: Cianjur, 3: Tasikmalaya, 4: Purwokerto, 5: Yogyakarta, 6: Tawangmangu, 7: Malang, 8: Madiun, 9: Negative control (without DNA).

Table 1. DNA size, numbers of amplified and polymorphic bands of eight *Pleurotus ostreatus* strains amplified using six RAPD primers

No.	Primer	Sequence (5'-3')	DNA band size (bp)	Numbers of amplified DNA bands	Numbers of polymorphic DNA bands
1	OPA 2	TGCCGAGCTG	136 - 2098	20	17
2	OPA 3	AGTCAGCCAC	170 - 1640	19	18
3	OPA 4	AATCGGGCTG	332 - 2056	17	17
4	OPA 7	GAAACGGGTG	330 - 2256	15	15
5	OPA 9	GGGTAACGCC	155 - 2288	17	17
6	OPA 10	GTGATCGCAG	276 - 2320	13	13
	Total			101	97
	Average			16.83	16.17

The results of polymorphism analyses indicated different levels of polymorphisms resulting from the use of each primer. Of the six primers, all exhibit the presence of polymorphisms among the eight strains of *P. ostreatus*. Thus, the six primers can be used to distinguish genotypes among these strains. The primer that could produce the most polymorphism patterns was OPA3 of 19 amplified DNA bands, and 18 (94.7%) were polymorphic. Based on the combined results of those six primers, 101 amplified DNA bands were observed, and 97 (96.0%) of the DNA bands were polymorphic. For each primer, the average yield was 16.83 DNA bands per primer (Table 1). Yadav et al. (2017) reported that 79.5% of RAPD bands of *Pleurotus* strains were polymorphic, and Pawlik et al. (2012) reported that 21 strains of *Pleurotus* obtained 90.4% polymorphism. The polymorphic band obtained from this current study is higher than those two previous studies which can be caused by the variety of primers used and the higher genetic variation of the strains used in this study.

Cluster analysis

To determine the genetic relationship of the eight *P. ostreatus* strains, the combined data from the six primers were analyzed based on the clustering method of UPGMA to obtain genetic similarity matrix (Table 2) and construct a dendrogram (Figure 3). Based on cluster analysis, it shows that three clusters were formed, the first cluster (I) being a group consisting of PoY, PoMl, PoTw, PoB, PoC, and PoP. Cluster I was divided into four sub-clusters; namely, the first sub-cluster consisted of PoY, PoMl and PoTw which were grouped with high genetic similarity between 0.96-0.98. The second sub-cluster consisted of one strain, PoB, the third sub-cluster consisted of one strain, PoC, and the fourth sub-cluster consisted of one strain, PoP. The second cluster consisted of one strain, PoMd, and the third cluster consisted of one strain, PoTs.

The different strains show variation in genetic similarity. However, the similarity of six of those strains was very high due to the number of amplicons and the percentage of polymorphic DNA bands determined by the number of selective nucleotides in the strain. In addition, it can be caused by the ancestor of the same strain so that there is only a slight genetic change at some locations. Khan et al. (2011) examined genetic variation in seven *Pleurotus* species using the RAPD technique and reported a similarity index of 0.76–0.86. Chandra et al. (2010) tested eight strains of *P. ostreatus* in India and reported a similarity index between 0.111 and 0.727. Gupta et al. (2011) performed molecular characterization of *Pleurotus* spp. using the RAPD technique showed genetic similarity between 0.17-0.95. Vieira et al. (2013) carried out the molecular characterization of *P. ostreatus* in Brazil the reported a genetic similarity of 0.69. Windarsih et al. (2019) reported genetic similarity for four samples of *Gracilaria coronopifolia* ranged between 0.45-0.57.

In our study, *P. ostreatus* strains from Yogyakarta, Malang, and Tawangmangu showed very high genetic similarity while strain from Malang and Madiun showed closer genetic similarities as well as strain from Tasikmalaya and Cianjur (Figure 3). Geographically, *P. ostreatus* originating from Malang is closer to the strain from Madiun than to the strain from Yogyakarta. However, the Malang strain showed a closer genetic relationship with the Yogyakarta strain than the Madiun strain. Our UPGMA clustering results show that these genetic similarities are not only influenced by geographic distance but are based on genetic factors and other factors. Al-Tufaili et al. (2019) reported that the genetic convergence or divergence among studied strains may be due to many reasons, such as source of origin, different environmental conditions, and breeding or hybridization for these strains. The variation of cultivated strains depends on mutation and hybridization, range of spread, agricultural processes, domestication, or other factors, such as ecological, geographical, breeding system, and anthropogenic effects. Zhao et al. (2013) reported that the genetic distances among geographical populations of *P. eryngii* var. *tuoliensis* were positively correlated with geographic distance and longitudinal distances. This indicates that geographic isolation is an important factor for the observed genetic differentiation. Zhao et al. (2016) reported that adaptation to stress and a temporarily heterogeneous environment are two important factors that influence intraspecies genetic diversity of *P. ferulae* in China. Phylogenetic analyses have also shown that genetic variation in Chinese samples is not geographically linked to their origin. These results indicate that gene flow is not hindered by distance. The present study reveals the abundant diversity for *P. ferulae* in China although its habitat was restricted. Samples from China and Italy were separated according to their geographical origins, but they belonged to the same genetic group.

Table 2. Similarity matrix of eight *Pleurotus ostreatus* strains obtained through RAPD markers

Strains	PoB	PoC	PoTs	PoP	PoY	PoTw	PoMl	PoMd
PoB	1.00							
PoC	0.78	1.00						
PoTs	0.48	0.48	1.00					
PoP	0.70	0.72	0.41	1.00				
PoY	0.78	0.76	0.37	0.74	1.00			
PoTw	0.80	0.72	0.38	0.72	0.96	1.00		
PoMl	0.78	0.76	0.37	0.74	0.98	0.96	1.00	
PoMd	0.50	0.64	0.46	0.52	0.52	0.50	0.54	1.00

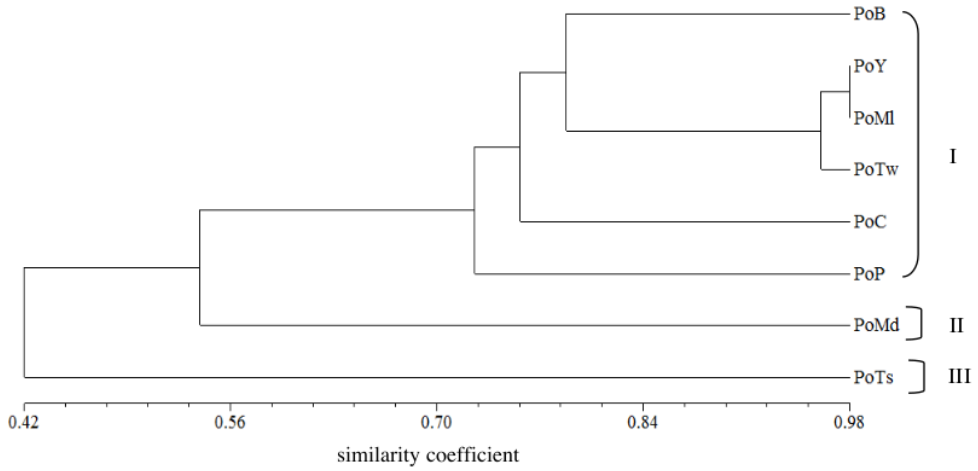

Figure 3. Dendrogram of genetic relationship among the eight strains of *Pleurotus ostreatus*

P. ostreatus strains from Madiun and Tasikmalaya showed quite high genetic differences versus the other six strains, which means that both strains can be used for genetic breeding development. Khan et al. (2011) stated that strains that have a high genetic distance and good production ability can be used in genetic breeding. Barh et al. (2019) reported that the genetic improvement in *Pleurotus* started with a simple selection technique, which later utilized hybridization (intraspecific, interspecific and intergeneric) and mutation breeding. The traits such as productivity, sporelessness, and quality improvement are important objectives on which most of the works have been done so far. However, new generation approaches such as molecular breeding, genetic transformation and genome editing techniques also added pace to the present improvement process. The improvement of *Pleurotus* mushroom primarily utilizes selection. Selection may be a direct selection from germplasm collected during fungal forays or from cultures made by multispore germination. Selection and mating of genetically diverse parents is another approach to exploit heterosis through hybridization. Avin et al. (2016) reported that the genetic variance components and heritability analyses indicated that the bulk of the *P. pulmonarius* genetic variations observed for most traits were associated with genes that were dominant in their effects. The newly generated hybrids were identified by both morphological and molecular fingerprinting. Familoni et al. (2018) reported that the outcome of phylogenetic relationships based on the molecular analysis between different or similar populations of *Pleurotus* species provides a better resolution and understanding of their biogeography and speciation. RAPD is a valuable method in distinguishing the different genotypes in mushrooms and evaluating their genetic similarities

In conclusion, there was variation in the genetic relationship among the eight strains of *P. ostreatus* in Java (Bogor, Cianjur, Tasikmalaya, Purwokerto, Yogyakarta, Tawangmangu, Malang, and Madiun) based on random amplified polymorphic DNA (RAPD) markers, with a genetic similarity varying from 37-98%. Based on cluster analysis, it shows that three clusters were formed.

ACKNOWLEDGEMENTS

We thank the Ministry of Research Technology and Higher Education for providing research funds through the

Strategic National Institution Scheme Project. Our gratitude also goes to LPPM Jenderal Soedirman University, Purwokerto, Banyumas, Indonesia for the sharing of information and collaboration; and the Dean of the Faculty of Biology, Jenderal Soedirman University, Purwokerto, for giving the research permission and supporting this research.

REFERENCES

Abdulmalk HW. 2013. Rapid differentiation of *Pleurotus ostreatus* from *Pleurotus sapidus* using PCR technique. Curr Res J Biol Sci 5: 157-160. DOI: 10.19026/crjbs.5.5482

Alam N, Shim MJ, Lee MW, Shin PG, Yoo YB, Lee TS. 2009. Phylogenetic relationship in different commercial strains of *Pleurotus nebrodensis* Based on ITS sequence and RAPD. Mycobiol 37: 183-188. DOI: 10.4489/MYCO.2009.37.3.183

Al-Tufaili AKH, Aboohanah MA, Salman FA, Abdel-Hassan NM. 2019. Analysis of genetic distance and similarity in some radish cultivars (*Raphanus sativus* L.) by random amplified polymorphic DNA (RAPD) markers. J Glob Pharm Technol 11: 464-470.

Avin FA, Bhassu S, Sabaratnam V. 2013. A simple and low-cost technique of DNA extraction from edible mushrooms examined by molecular phylogenetics. Res Crops 14:897-901.

Avin FA, Bhassu S, Rameeh V, Tan YS, Vikineswary S. 2016. Genetics and hybrid breeding of *Pleurotus pulmonarius*: Heterosis, heritability and combining ability. Euphytica 209: 85-102. DOI: 10.1007/s10681-016-1638-x

Barh A, Sharma VP, Annepu SK, Kamal S, Sharma S, Bhatt P. 2019. Genetic improvement in *Pleurotus* (oyster mushroom): A review. Biotech 9: 322. DOI: 10.1007/s13205-019-1854-x

Chakravarty B. 2011. Trends in mushroom cultivation and breeding. Aus J Agric Eng 2: 102-109.

Chandra S, Ghosh K, Acharya K. 2010. Comparative studies on the Indian cultivated *Pleurotus* species by RAPD fingerprinting. Nat Sci 8: 90-94.

Ekowati N, Kasiamdari RS, Pusposendjojo N, Soegihardjo CJ. 2011. Genetic variation base on random amplified polymorphic DNA molecular markers on shiitake mushrooms (*Lentinula edodes*). Biota 16: 178-186. DOI: 10.24002/biota.v16i2.97

Familoni TV, Ogidi CO, Akinyele BJ, Onifade AK. 2018. Genetic diversity, microbiological study and composition of soil associated with wild *Pleurotus ostreatus* from different locations in Ondo and Ekiti States, Nigeria. Chem Biol Technol Agric 5: 7. DOI: 10.1186/s40538-018-0119-y

Gupta B, Reddy PN, Kotasthane AS. 2011. Molecular characterization and mating type analysis of oyster mushroom (*Pleurotus* spp.) using single basidiospores for strain improvement. World J Microbiol Biotechnol 27: 1-9. DOI: 10.1007/s11274-010-0419-2

Hasan HA, Almomany AM, Hasan S, Al-Abdallat AM. 2018. Assessment of genetic diversity among *Pleurotus* spp. isolates from Jordan. J Fungi 4: 52. DOI: 10.3390/jof4020052

Inyod T, Sassanarakit S, Payapanon A, Keawsompong S. 2017. Morphological characteristics and molecular identification of a wild Thai isolate of the tropical mushroom Hed Taen Rad (*Macrocybe crassa*). Biodiversitas 18: 221-228. DOI: 10.13057/biodiv/d180128

Khan SM, Nawaz A, Malik W, Javed N, Yasmin T, Rehman M, Qayyum A, Iqbal Q, Ahmad T, Khan AA. 2011. Morphological and molecular characterization of Oyster mushroom (*Pleurotus* spp.). Afr J Biotechnol 10: 2638-2643. DOI: 10.5897/AJB10.499

Khan NA, Binyamin R, Awan FS, Khan AI, Waseem M. 2017. Genetic diversity of edible mushroom *Pleurotus* spp. revealed by randomly amplified polymorphic DNA fingerprinting. Pak J Bot 49: 1517-1521.

Otieno OD, Onyango C. 2015. Genetic diversity of Kenyan native oyster mushroom (*Pleurotus*). Mycologia 107: 32-38. DOI: 10.3852/13-186.

Pawlik A, Janusz G, Koszerny J, Małek W, Rogalski J. 2012. Genetic diversity of the edible mushroom *Pleurotus* sp. by amplified fragment length polymorphism. Curr Microbiol 65: 438-445. DOI: 10.1007/s00284-012-0175-7

Ravash R, Shiran B, Alavi A, Bayat F, Rajaee S, Zervakis GI. 2010. Genetic variability and molecular phylogeny of *Pleurotus eryngii* species-complex isolates from Iran, and notes on the systematics of Asiatic populations. Mycol Progress 9: 181-194. DOI: 10.1007/s11557-009-0624-2

Rohlf FJ. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Applied Biostatistics, New York.

Sharon A. 2010. Molecular and Cell Biology Methods for Fungi. Department of Plant Sciences. Humana Press, New York.

Tsegaye Z, Tefera G. 2018. Cultivation of oyster mushroom (*Pleurotus ostreatus* Kumm, 1871) using agro-industrial residues. J Appl Microb Res 1: 1-6.

Vieira FR, Pereira DM, Nogueira de Andrade MC, Minhoni MT. 2013. Molecular characterization of *Pleurotus ostreatus* commercial strains by random amplified polymorphic DNA (RAPD). Afr J Agric Res 8: 3146-3150.

Windarsih G, Utami DW, Yuriyah S. 2019. Genetic diversity and productivity of *Gracilaria coronopifolia* as alternative for food resource based on RAPD marker. Biodiversitas 20: 3758-3765. DOI: 10.13057/biodiv/d201239

Zhao M, Huang C, Chen Q, Wu X, Qu J. 2013. Genetic variability and population structure of the mushroom *Pleurotus eryngii* var. *tuoliensis*. PLoS One 8: e83253. DOI: 10.1371/journal.pone.0083253

Zhao M, Huang C, Wu X, Chen H, Qu J, Li Y, Gao W, Zhang J. 2016. Genetic variation and population structure of the mushroom *Pleurotus ferulae* in China inferred from nuclear DNA analysis. J Integr Agr 15: 2237-2246. DOI: 10.1016/S2095-3119(16)61383-7

Yadav MK, Chandra R, Singh HB, Yadav SK, Yadav SK, Naik S, Dhakad PK. 2017. Genetic diversity characterization of *Pleurotus* strains by random amplified polymorphic DNA fingerprinting. Intl J Curr Microbiol App Sci 6: 1260-1267. DOI: 10.20546/ijcmas.2017.605.136.

Genetic diversity of Pleurotus ostreatus (Jacq.) P. Kumm. strains in Java based on random amplified polymorphic DNA markers

ORIGINALITY REPORT

9%	7%	4%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.ung.ac.id Internet Source	1%
2	academic-accelerator.com Internet Source	1%
3	repo.unand.ac.id Internet Source	1%
4	www.scirp.org Internet Source	1%
5	Mahmood Khan Samiya, Nawaz Aamir, Malikshy Waqas, Javed Nazir, Yasmin Tahira, ur Rehman Mehboob, Qayyum Abdul, Iqbal Qumer, Ahmad Tanveer, Ali Khan Azhar. "Morphological and molecular characterization of Oyster mushroom (Pleurotus spp.)", African Journal of Biotechnology, 2011 Publication	1%
6	Moorhead, G.. "Phosphorylated nitrate reductase from spinach leaves is inhibited by	1%

14-3-3 proteins and activated by fusicoccin", Current Biology, 199609
Publication

7	pure.sruc.ac.uk Internet Source	1 %
8	unsworks.unsw.edu.au Internet Source	1 %
9	Huichun Yu, Jun Wang, Cong Yao, Hongmei Zhang, Yong Yu. "Quality grade identification of green tea using E-nose by CA and ANN", LWT - Food Science and Technology, 2008 Publication	1 %
10	Yonggang Yin, Yu Liu, Shouxian Wang, Shuang Zhao, Feng Xu. "Examining genetic relationships of Chinese Pleurotus ostreatus cultivars by combined RAPD and SRAP markers", Mycoscience, 2013 Publication	1 %
11	mdpi-res.com Internet Source	1 %
12	pakbs.org Internet Source	1 %
13	www.univ-soukahras.dz Internet Source	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		