

Molecular Identification and Phylogenetic Relationships of *Pleurotus* spp. Diversity in Malaysia by ITS Marker

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Introduction

Pleurotus species is an edible mushroom in Malaysia which is commonly known as Grey Oyster mushroom and grow by small holder farmers. This species is important for nutraceutical, pharmaceutical and cosmeceutical industries. However, there is some misidentification in the species due to phenotypic variation in which the species shared some similarities due to environmental factors (Ravash, Shiran & Alavi 2009). Knowing the species might help in applying nutrient, moisture and specificity of substrate use for cultivation in which may differ from one another

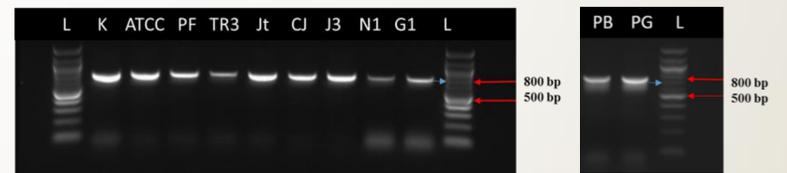
Methodology

11 *Pleurotus* strains including 4 *Pleurotus* species, ATCC 32048 (*P. pulmonarius*) were used for strain and species identification including one of selected *Pleurotus* mutant line. Total genomic DNA was extracted, quantified and amplified by using rDNA-ITS (Ribosomal DNA Internal Transcribed Spacers) ITS8-F: 5'-AGTCGTAACAAGGTTTCCGTAGGTG-3' and ITS6-R: 5'-TTCCCGCTTCACTCGC-AGT-3' primers.



ITS1 and ITS2 region that were used to classified between intra-species and interspecies of mushrooms. The regions are between highly conserved region of small subunit ribosomal DNA (SSU rDNA), 5.8S rDNA and large subunit ribosomal DNA (LSU rDNA). Source of figure is from Avin et al. (2012); Muruke et al., (2002).

Results & Discussion



Gel electrophoresis image for ITS amplification of 11 *Pleurotus* cultivars; 1.5% agarose, 75 V. 11 samples were products of standard PCR programmed for 34 cycle at the annealing temperature of 57.1 °C.

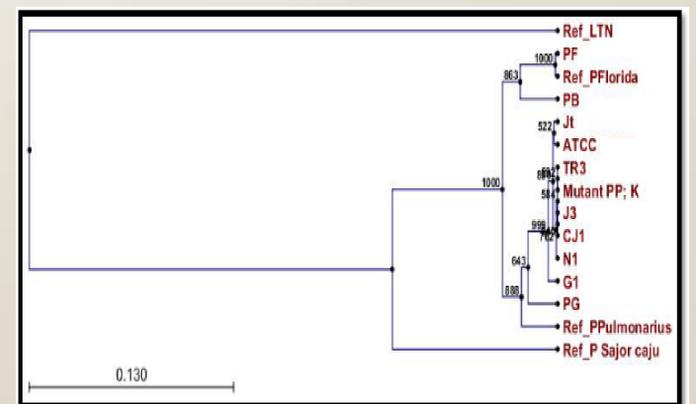
Code and origin of strains and species used in this study

Code	Species	Origin of samples
PP Mutant; K	<i>P. pulmonarius</i>	Malaysia Nuclear Agency
ATCC 32078	<i>P. pulmonarius</i>	American Collection Centre
Tr3	<i>P. pulmonarius</i>	Terong Perak
CJ	<i>P. pulmonarius</i>	Changkat Jering Perak
Jt	<i>P. pulmonarius</i>	Jerantut Pahang
J3	<i>P. pulmonarius</i>	Johor
N1	<i>P. pulmonarius</i>	Selangor
G1	<i>P. pulmonarius</i>	Gading Kelantan
PB	<i>P. columbinus</i>	Kundasang Sabah
PG	<i>P. geesteranus</i>	Selangor
PF	<i>P. florida</i>	Kundasang Sabah

Phylogenetic (UPGMA) was constructed by using CLC Sequence Viewer 6.8.1 based on PCR product sequences. Distinct clades of the *Pleurotus* species and its strains. *Pleurotus pulmonarius* were found to be grouped in one group while *Pleurotus florida*. and *Pleurotus columbinus* were in the other different clade.

ITS marker found to be reliable, rapid, robust and reproducible approach in screening of *Pleurotus* species and its variants for taxonomical purposes and phylogenetic analysis

It is clear that *P. pulmonarius*, *P. florida*, *P. columbinus* and *P. sajor caju* has fallen into four different clades. Collected samples of commercial Grey Oysters from different locations grouped under *P. pulmonarius*.



Phylogenetic tree and molecular variability analysis based on UPGMA algorithm sequence of *Pleurotus* sp. prior to sequence alignment using CLC Sequence Viewer Version 6.8.1

References

- Avin, F. A., Bhassu, S., Shin, T. Y., & Sabaratnam, V. (2012). Molecular classification and phylogenetic relationships of selected edible Basidiomycetes species. *Molecular biology reports*, 39(7), 7355-7364
- Muruke, M. H. S., Kivaisi, A. K., Magingo, F. S. S., & Danell, E. (2002). Identification of mushroom mycelia using DNA techniques. *Tanzania Journal of Science*, 28(1), 115-128.
- Ravash, R., Shiran, B. & Alavi, A. (2009). Genetic variability and molecular phylogeny of *Pleurotus eryngii* species-complex isolates from Iran, and notes on the systematics of Asiatic populations Genetic variability and molecular phylogeny of *Pleurotus eryngii* species-complex isolates from Iran, and notes (March 2016). doi:10.1007/s11557-009-0624-2



P. pulmonarius



P. pulmonarius
(Mutant)



P. florida



P. geesteranus



P. columbinus