Molecular Mono-Dikaryon Discrimination of fungi "*Pleurotus sajor-caju*"

Farhat A. Avin, Subha Bhassu, Vikineswary S.

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

Abstract

All 62 single spore isolates of *Pleurotus sajor-caju* (monokaryons) could be clearly discriminated from their parents (dikaryons) by amplification of the partial sequence of nuclear ribosomal DNA. Moreover, by using the sequence data, two different types of monospore were identified that will provide very useful background for further breeding and fusion purpose.

Introduction

Pleurotus sajor-caju as an important edible member of *Basidiomycetes* is heterothallic and produces four uninucleate spores (n) in the sexual life cycle and exchange of the nucleus between two compatible monokaryotic mycelia will end to form the fertile binucleate cells or dikaryon (n+n) (Martnez 1998 and Ramirez et al. 2000). For many years, identification of monokaryons and dikaryons has been carried out through the fruiting trial, estimating the vegetative growth rate, presence or absence of clamp connections, and the mycelia morphology type which are unreliable and time consuming (Fig.1). This novel study recommended a new molecular way to distinguish monokaryons from dikaryons and resolved the ambiguities.

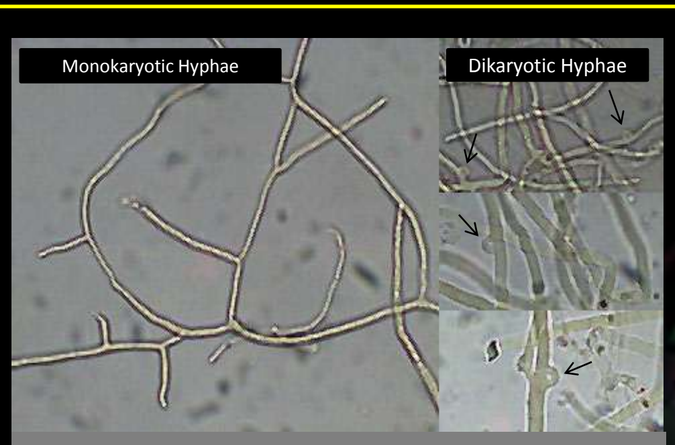


Fig. 1: Representative microscopic photographs of comparison of mono and dikaryotic mycelium. The arrow heads indicate the position of clamp connections forming in dikaryons.

Fig. 2: Different morphology types and growth rates of selected putative monokaryotic cultures.

Results and Discussion

Amplification of nuclear ribosomal DNA produced two distinct bands for dikaryon cultures and one band for monokaryons (Fig. 3). Moreover, the two different monokaryon isolates (Type A and B) were distinguished by amplification on the basis of the length of PCR product. In fact, we found two different types of nuclei in the fungi cells that are able to mate each other and the same nuclei could not produce dikaryon.

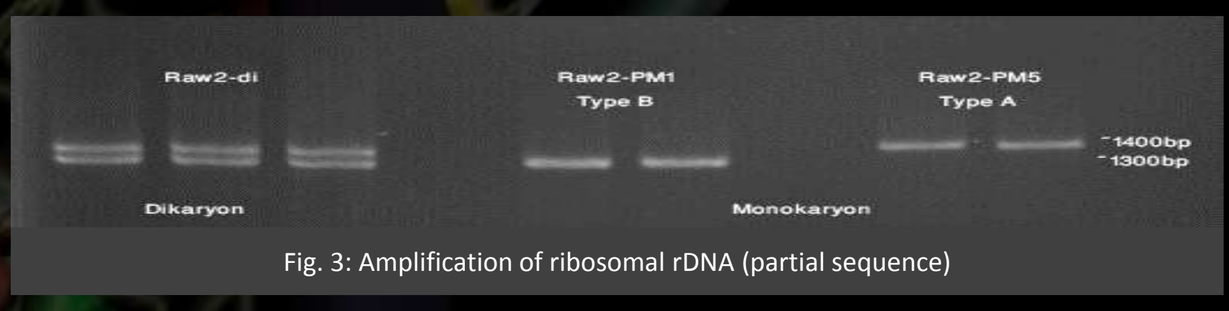


Fig. 3: Amplification of ribosomal rDNA (partial sequence)

The constructed tree based on the sequences data was also proved that the fertile dikaryon hypae receives the nuclei from distinct types of A and B (Fig. 4). Among the 62 examined single spore isolates, 21 were identified as type A and 41 were type B. Hence, the mate calculation showed that this work can prevent 1030 undesirable mates.

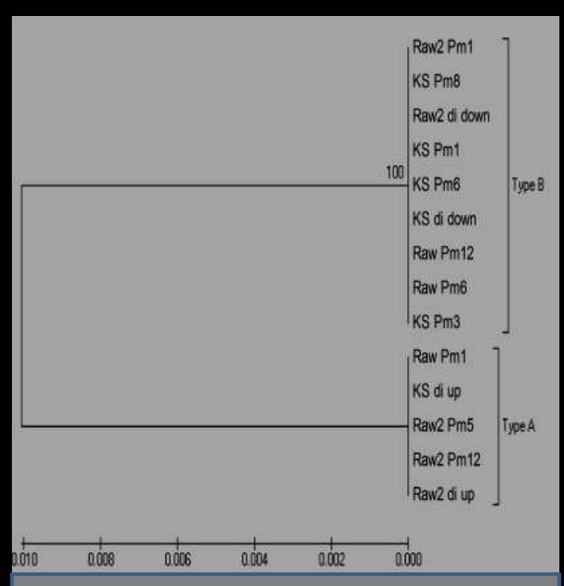
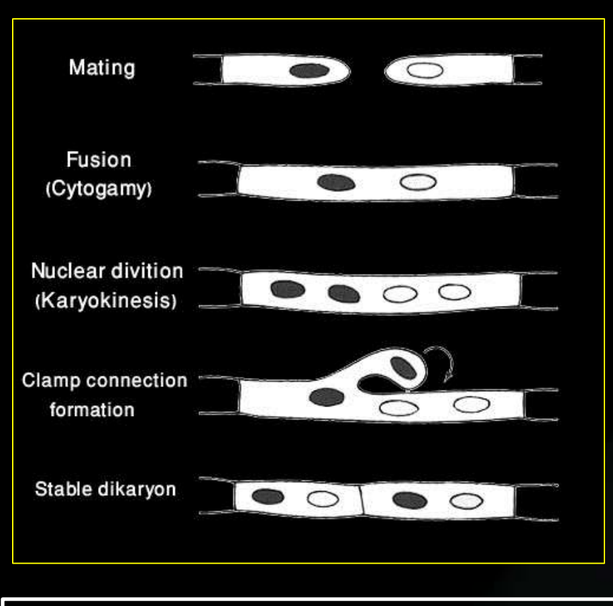
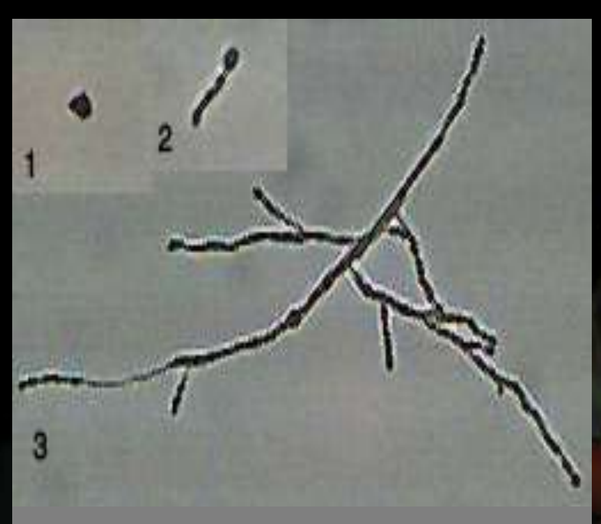


Fig 4. Denrogram based on the mono and dikaryon sequences results.





1: Single spore 2: Spore germination 3: Spore expanding

Objectives

- ✓ To discriminate mono and dikaryon fungi cultures via molecular techniques
- ✓ To discover a new molecular way to screen the monokaryons compatibility before real mating to reduce the number of undesirable fusions

Material and Methods

The final number of 62 putative monokaryon cultures were successfully cultured (Fig. 2)

↓

Mycelium was directly scraped from the surface of agar plate

↓

DNA extraction was followed by the protocol of Weiland, 1997 with some modifications

↓

PCR amplification

Loading in the gel, staining with ethidium bromide (Fig. 3)

↓

Purification of amplicons

Sequencing

↓

Data analysis (Construction of dendrogram) (Fig. 4)

↓

Designing the new pair of primer

Conclusion

This novel study has shown the ability of the molecular markers to discriminate mono and dikaryons. Moreover, it was shown that the number of undesirable matings will be significantly decreased and this marker will be diagnostic marker for new fungi hybrids.

Acknowledgment

Authors would like to thank University of Malaya for the research grant (PS291-2009C) from Institute of Pengurusan dan Pemantauan Penyelidikan (IPPP) and members of Genetics and Molecular biology, and Mycology Laboratories.

References

Ramirez L., Larraya L.M., Pisabarro A.G. 2000. Molecular tools for breeding basidiomycetes. Environmental Microbiol. 3: 147-152.

Martnez-Carrera D. 1998. Oyster mushrooms. *McGraw-Hill Yearbook of Science & Technology 1999.* Ed.: M. D. Licker . McGraw-Hill, Inc., New