Inter-Subspecies Hybrid Dikaryons of Oyster Mushroom Independently Isolated in Vietnam and Japan

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Two strains of the mushroom Pleurotus, isolated from nature in Vietnam and Japan, contained a similar combination of two distinct rDNA internal transcribed spacer (ITS) sequences. They were perhaps hybrid dikaryons between P. cystidiosus subsp. abalonus and a novel P. cystidiosus subspecies. These mushrooms produce dikaryotic arthroconidia. This unique asexual reproduction might allow stable maintenance of a particular pair of nuclei.

Key words: asexual reproduction; internal transcribed spacer (ITS) sequence; hybrid dikaryon; edible mushroom

The Basidiomycota group of fungi is best known for those members that produce large fruiting bodies, such as puffballs and mushrooms. Sexual conjugation in basidiomycetes fungi does not result in the rapid formation of a diploid cells as it does in most organisms. Instead, dikaryons form in which the haploid nuclei contributed by each mating partner are prevented from fusing. The dikaryotic cells proliferate to develop a fruitbody. The original pair of nuclei is precisely maintained during proliferation by a special mode of cell division, so-called “conjugate division” using the clamp connection, a bypass for migration of one of the daughter nucleus.1) The nuclei fuse just before meiosis, which produces haploid basidiospores during the terminal development of the fruitbody. Besides spores for sexual reproduction (basidiospores), basidiomycetes fungi also produce asexual spores called conidia. Conidia are produced either by segmentation of hyphae (arthroconidia) or by budding (blastoconidia). Both the arthroconidia and the blastoconidia produced by dikaryons of many basidiomycetes are primarily monokaryotic, for example, the arthroconidia (oidia) and the blastoconidia (chlamydospores) of Coprinus cinereus.1) Therefore the monokaryotic hyphae from the germinated conidia usually must conjugate again to become dikaryons, and consequently to develop fruitbodies.

Among the mushrooms, Pleurotus cystidiosus and allied species have a unique asexual reproduction system. These fungi form coremium (plural coremia), a small fruitbody-like bundle of hyphae that produces a remarkable number of arthroconidia from its upper surface. Coremia-forming Pleurotus mushrooms are grouped in the subgenus Coremiopleurotus. Either a monokaryotic or a dikaryotic mycelium develops into coremia. The coremia of the dikaryon produces exclusively dikaryotic arthroconidia that contain the remnant of a clamp connection.2) Some Coremiopleurotus strains have been isolated as coremia-forming microfungi without identification of fruitbodies. They have been placed in the anamorphic genus Antromycopsis.3)

We isolated a Coremiopleurotus strain (strain name, Blao) in Vietnam in 1999.2) We expected the strain to be a new species or subspecies because its fruitbodies were larger than the other Coremiopleurotus strains. For species identification of Blao, we sequenced its rDNA internal transcribed spacer (ITS) to compare it with those of a large number of Coremiopleurotus strains isolated from all over the world.4) DNA was purified from the dikaryotic mycelium of Blao, and the region covering the ITS1-5.8S rDNA-ITS2 sequence was am-
numbers of nucleotide substitutions and insertion/deletion events within ITS1-5.8S rDNA-ITS2 regions.

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<th>Blao A</th>
<th>S396 B</th>
<th>Blao B</th>
<th>UP174</th>
<th>CBS61580</th>
<th>D419</th>
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The phylogenetic tree of ITS sequences from *Pleurotus* sp. Blao (accession no for allele A, DQ882570; allele B, DQ882571), *Pleurotus* sp. S396 (allele A, DQ882572; allele B, DQ882573), *Pleurotus cystidiosus* subsp. *abalonus* strain CBS61580 (AY315810), *P. cystidiosus* subsp. *fuscosquamulosus* Strain UP174 (AY315788), and *Pleurotus cystidiosus* subsp. *cystidiosus* Strain D419 (AY315774). The primers for PCR and sequencing were described in Martin et al. The sequences of 615 nucleotides of ITS1-5.8S rDNA-ITS2 regions including gaps were aligned, and the numbers of substitutions and insertion/deletion events were counted (Table 1). The tree was drawn by the neighbor-joining method.

![Phylogenetic Tree of the ITS Sequences from Pleurotus sp.](image)

**Fig. 1.** Phylogenetic Tree of the ITS Sequences from *Pleurotus* sp. Blao (accession no for allele A, DQ882570; allele B, DQ882571), *Pleurotus* sp. S396 (allele A, DQ882572; allele B, DQ882573), *Pleurotus cystidiosus* subsp. *abalonus* strain CBS61580 (AY315810), *P. cystidiosus* subsp. *fuscosquamulosus* Strain UP174 (AY315788), and *Pleurotus cystidiosus* subsp. *cystidiosus* Strain D419 (AY315774).

The sequences of 615 nucleotides of ITS1-5.8S rDNA-ITS2 regions including gaps were aligned, and the numbers of substitutions and insertion/deletion events were counted (Table 1). The tree was drawn by the neighbor-joining method.

Our results raise three questions. First, what species does the A allele come from? Second, how are the hybrid dikaryons produced and maintained? Third, why do the two strains, independently isolated from distant places, contain a similar combination of nuclei?

As for the first question, we note that available sequences are presently limited to strains that form a fruitbody. The A allele might come from an as yet unsequenced *Antromycopsis*, the anamorph of *Coremio-
pleurotus. It is difficult to test whether the putative parental subspecies that contributed to the A allele can develop a fruitbody or not, because Blao and S396 produce no monokaryotic conidia, and the monokaryons derived from the basidiospores have chromosomes that are already shuffled.

As for the second question, the mating capability between P. cystidiosus subspecies in the laboratory might explain the formation of the hybrid. Six taxa of Coremiopleurotus are distributed worldwide, but the subspecies/species are geographically separated each other. Natural mating between the different subspecies might have become more frequent after modern human activities that accelerated the immigration of the alien species. In addition, these mushrooms produce a tremendous amount of dikaryotic arthroconidia by forming coremia. This unique asexual reproduction might allow stable maintenance of a particular pair of nuclei through a large number of generations. Furthermore, two properties unique to Blao and S396 might be involved in preventing their reduction to monokaryons. One is devolution of the blastoconidium to the nematode-attacking structure toxocyst, which no longer contains a nucleus. The other is extremely poor survival of the basidiospores or germinated monokaryons (unpublished results), which suggests a distance between the two subspecies that contributed to the hybrid dikaryons.

As for the third question, we do not have an adequate explanation. It is not known whether Blao and S396 originated independently or derived from a common dikaryotic ancestor. The ITS sequences of Blao and S396 are not identical (three and four differences in A and B alleles respectively, Table 1). If they derived from a common dikaryotic ancestor, they must have maintained the nuclear pair over the ages sufficiently to alter the nucleotide sequence. It might be suspected that Blao and S396 are recent commercial products that escaped from nature, but the ITS sequences of four agricultural strains of P. cystidiosus subsp. abalonus (Japanese name, kuroawabitake) matched only sequences within P. cystidiosus subsp. abalonus (maximal four-base differences with that of strain CBS61580, unpublished data). P. cystidiosus subsp. abalonus is a tropical mushroom. Hence we assume that S396 originated in a tropical land and then migrated to Ehime, Japan. The other subspecies of P. cystidiosus are not restricted to tropical areas. The climate for the putative parental subspecies that contributed to the A allele is also unknown. Therefore the possibility that S396 has long survived for in nature of Ehime Prefecture cannot be excluded. To answer this question, the collection of additional hybrid dikaryons from various areas is necessary.

Blao produces large edible fruitbodies. Generally, maintenance of a mushroom strain is difficult due to its complex mating system, but the unique reproduction of Coremiopleurotus by dikaryotic conidia provides an exceptionally stable maintenance of the hybrid dikaryon. The dikaryotic arthroconidia are dormant spores and appear to be suitable for storage. Maintaining good mushroom breeds by dikaryotic conidia might be worthy of consideration.

Acknowledgments

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References


