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SHORT COMMUNICATION

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Intersterility between populations of *Lentinula edodes* from Papua New Guinea

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Abstract Mating tests among strains of *Lentinula edodes* distributed in Asia-Australasia were conducted. As a result, 26 strains were classified into three groups: 2 strains from Mt. Wilhelm in Papua New Guinea (PN1 group) showed intersterility with 7 strains from Mt. Albert Edward and Mt. Kaisenik in Papua New Guinea (PN2 group) and semi-compatibility (clamp formation restricted to contact zone between paired monokaryons) with 17 strains from Asia-Australasia (AA group), whereas the strains of the PN2 group showed compatibility with the AA group. These results suggest that the shiitake populations distributed in Asia-Australasia including Papua New Guinea are in the process of speciation.

Key words Geographic lineage · Intersterility · *Lentinula edodes* · Mating compatibility

Shiitake, *Lentinula edodes* (Berk.) Pegler, is the major edible mushroom in Asia, particularly in China and Japan, and many excellent cultivars have been developed by cross-breeding in Japan. Development of superior new cultivars of this mushroom is very important for promoting commercial production, and wild strains are considered to be potential genetic resources for breeding. Shiitake is distributed in Japan, China, Southeast Asia, and Australasia (Kobayashi and Shimizu 1951). Significant variations have been observed in the shape of fruiting bodies (Pegler 1983), agronomic characteristics (Hasebe 1992), isozyme patterns (Fukuda and Tokimoto 1991), and restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA (Fukuda et al. 1994) among wild strains from geographically distinct regions. Furthermore, Hibbett et al. (1995) described four independent shiitake lineages in Asia-Australasia that were closely correlated with their geographic origins. Based on the differences in fruiting-body morphology, Pegler (1983) established three shiitake species: *L. edodes*, which is distributed in the region from temperate to northern tropical Asia; *L. lateritia* (Berk.) Pegler, from Australia, India, Papua New Guinea, and Borneo; and *L. novaezelandieae* (Stev.) Pegler, from New Zealand. On the other hand, Shimomura et al. (1992) demonstrated interfertility between geographically distinct strains and proposed that shiitake distributed in these regions was considered to be *L. edodes*. However, only a few strains from Papua New Guinea were tested, and dikaryosis between paired monokaryons was not examined. In this study, we examined mating relationships for a relatively large sample of wild-collected strains, particularly from Papua New Guinea, with particular emphasis on observations of dikaryotic hyphae formation by mating tests.

As shown in Table 1, 26 wild dikaryotic strains of *L. edodes* and 1 wild strain of *L. boryana* (Berk. & Mont.) Pegler from Brazil as an outgroup were used in this study. The strains have been deposited in the Tottori Mycological Institute Culture Collection, and some of the strains had been used by Hibbett et al. (1995) in their phylogenetic study. The strains were maintained on malt extract agar (MA) medium (20 g malt extract, 20 g agar, 1 l tap water). For each of the strains, fruiting-bodies were produced on sawdust medium prepared with 400 g air-dried hardwood sawdust and 80 g rice bran wetted with 1 l tap water (Shimomura et al. 2007). Basidiospores from the fruiting-bodies were then released on an MA plate and then incubated at 23°C for 2 days. The resulting basidiospore germlings were individually isolated using the method described by Miles at al. (1966) and cultured on MA medium at approximately 23°C. From the isolates, four representative testers with different mating types were selected on the base of the pattern of mating compatibility between the isolates. Mating tests were performed in all interstrain combinations using all four representative mating testers. Matings were performed by placing two mycelial plugs (about 2 mm in diam-

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Table 1. Sample cross-matrix showing mating relationships among monosporous isolates of *Lentinula edodes* from Asia-Australasia and *L. boryana* from Brazil

Group[a]	Culture no.	Origin	PN1		PN2							AA																	LB
			TMIC-1500	TMIC-1502	TMIC-1460	TMIC-1474	TMIC-1479	TMIC-1483	TMIC-1485	TMIC-1489	TMIC-1504	TMIC-818	TMIC-1734	TMIC-1732	TMIC-1742	TMIC-1744	TMIC-1774	TMIC-1358	TMIC-1706	TMIC-1507	TMIC-1519	TMIC-1529	TMIC-1631	TMIC-1632	TMIC-1633	TMIC-1706	TMIC-1448	TMIC-1563	TMIC-1071
PN1	TMIC-1500	Mt. Wilhelm, Papua New Guinea	+	+	–	–	–	–	–	–	–	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	–
PN1	TMIC-1502	Mt. Wilhelm, Papua New Guinea	+	+	–	–	–	–	–	–	–	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	–
PN2	TMIC-1460	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1474	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1479	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1483	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1485	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1489	Mt. Albert Edward, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
PN2	TMIC-1504	Mt. Kaisenik, Papua New Guinea	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-818	Okinawa, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1734	Miyazaki, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1732	Tottori, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1742	Tottori, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1744	Tottori, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1774	Ehime, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1358	Hokkaido, Japan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1706	China	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1507	Kubei, China	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1519	Guangdong, China	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1529	Zhejiang, China	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1631	Thailand	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1632	Thailand	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1633	Thailand	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1706	Taiwan	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1448	New Zealand	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
AA	TMIC-1563	Nepal	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
LB	TMIC-1071	Brazil	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

LB, an outgroup, *L. boryana*

[a] Based on the interstrain mating reactions, the 26 *L. edodes* strains were classified into three groups: PN1, PN2, and AA

+, dikaryosis throughout mating colony: (+), dikaryosis limited to contact zone between paired monokaryons; –, no clamp connections observed

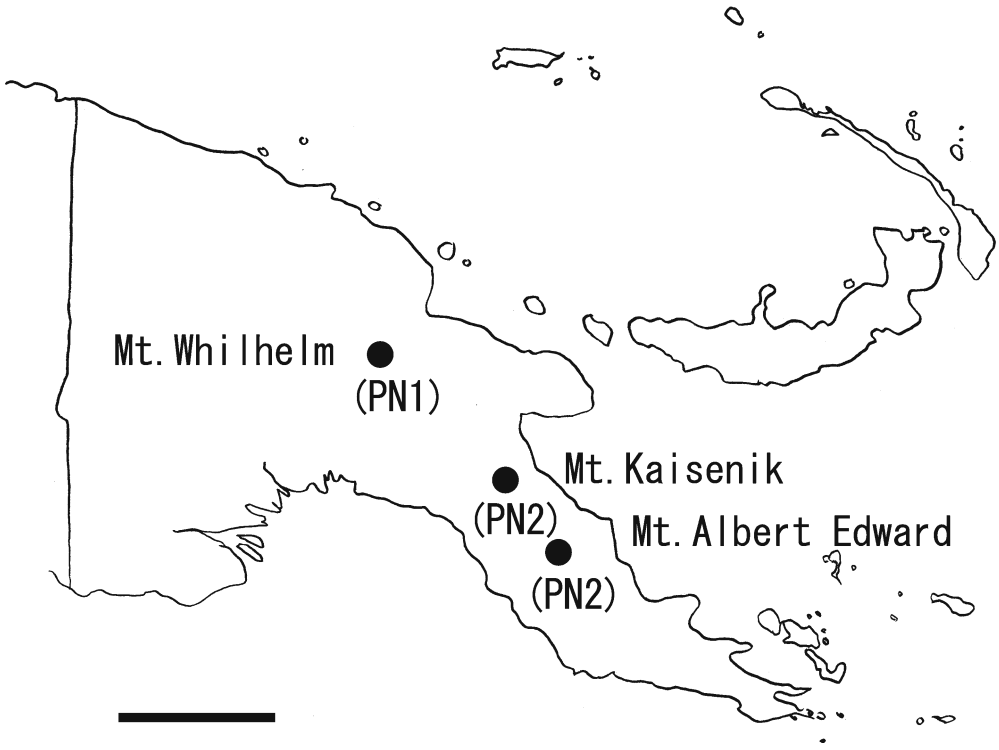

Fig. 1. Localities of two groups of *Lentinula edodes* in Papua New Guinea. *Bar* 500 km

eter) on an MA plate about 2–5 mm apart. After a week of incubation at 23°C, colony peripheries were examined microscopically for the occurrence of dikaryotic hyphae bearing clamp connections. When true clamp connections were abundantly observed around a colony, sexual compatibility of the crossed pair was scored as "compatible." When true clamp connection formation was restricted to the contact zone between paired monokaryons, sexual compatibility of the crossed pair was scored as "semicompatible."

Mating patterns among single-spore isolates from each of wild strains (intrastrain crosses) were consistent with a bifactorial mating incompatibility system in every case. The results of interstrain matings among the monosporous isolates from geographically distinct strains are shown in Table 1. All 16 matings between testers (4 testers from one strain × 4 testers from another strain) formed dikaryotic colonies in all compatible interstrain combinations except 2 (TMIC-1460:1485 and TMIC-1483:1485), in which 4 of 16 tester matings failed to form dikaryotic colonies. The result of interstrain matings led to the recognition that the geographically distinct strains used in this study were grouped into three in *L. edodes*, and one *L. boryana* strain was incompatible with all *L. edodes* strains. We designated three groups in *L. edodes* as PN1, PN2, and AA: the PN1 consisted of 2 strains from Mt. Wilhelm in Papua New Guinea, the PN2 group consisted of 7 strains from Mt. Albert Edward and Mt. Kaisenik in Papua New Guinea, and the AA group consisted of 17 strains from Japan, China, Thailand, Taiwan, Nepal, and New Zealand. Interestingly, the PN1 group showed intersterility with the PN2 group. In compatible matings between the PN1 and the AA groups, true clamp formation occurred only in the contact zones, and dikaryotic isolates from the contact zones revealed very slow growth. On the other hand, the PN2 group showed a compatible interaction with the AA group.

The strains of the PN1 group were collected above 3000 m above sea level (ASL) in the areas of Mt. Wilhelm, whereas those of the PN2 group were collected at sites less than 2100 m ASL on either Mt. Albert Edward or Mt. Kaisenik; the distributions of these two intersterility groups were thus relatively different from each other (Fig. 1). A previous report using DNA sequences from the internal transcribed spacers of nuclear ribosomal DNA (Hibbett et al. 1995, 1998) also showed the presence of two distinct lineages in the shiitake population from Papua New Guinea. In our study, intersterility was recognized between the two strains (TMIC 1485 and TMIC 1502) that belong to each of the two lineages in their ribosomal DNA study (Hibbett et al. 1995, 1998). These results suggest that the shiitake population distributed in Papua New Guinea comprises different indigenous gene pools, and our mating tests reveal evidence that the reproductive isolation between the two groups has been maintained. This is the first report for demonstrating the intersterility between the two groups from Papua New Guinea.

In the compatible matings, the AA group showed limited dikaryotization ("semicompatible") with the PN1 group and normal dikaryotization ("compatible") with the PN2 group. It has been reported in some basidiomycetous fungi that when specimens from widely separated geographic areas or different substrates were mated in vitro, partial compatibility was observed, even between specimens that are morphologically similar or indistinguishable from each other (Macrae 1967; Duncan 1972; Kemp 1974; Boidin 1986; Bresinsky et al. 1987; Wells and Wong 1989; Chamuris 1991; Hallenberg 1991; Vilgalys 1991; Gordon and Petersen 1992;

Hallenberg and Larsson 1992; Petersen 1992, 1995; Fischer 1994; Murakami and Tsuneda 1995). Several of these sources also reported that limited dikaryotization frequently occurred in the confrontation area of compatible matings. Therefore, it is considered that a mating test is one of the most useful tools for determining the progress and recognizing the results of microevolution in the Basidiomycetes. We recognized different three shiitake populations based on mating reactions, and two of the three populations are distributed in Papua New Guinea. These results indicate that the shiitake populations distributed in Papua New Guinea are not only diverse ribosomal DNA sequences (Hibbett et al. 1995, 1998), but also diverse reproductive systems, and are in the process of speciation.

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