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FULL PAPER

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Genetic differences in wild strains of *Lentinula edodes* collected from a single fallen tree

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Abstract Genetic differences among 18 *Lentinula edodes* strains isolated from a fallen trunk of *Quercus mongolica* var. *grosseserrata* were characterized by mating tests and restriction fragment length polymorphism (RFLP) analyses of mitochondrial DNA (mtDNA). These strains could be divided into six genets of different mating types. Because the mtDNA of the 18 strains showed four different RFLP genotypes, these strains seemed to have originated from at least 4 distinct parental strains. Strains belonging to the same genet were collected from fruiting bodies located not more than about 1 m apart on the fallen tree. Implications of these findings regarding the degree of genetic variation and territory sizes of individual genets of wood-decaying basidiomycetes such as *L. edodes* are discussed.

Key words Genetic difference · *Lentinula edodes* · Mating type · Mitochondrial DNA genotype · Wild strain

Introduction

The development of superior new cultivars of edible mushrooms is important for promoting commercial production. Wild strains are valuable genetic resources for mushroom breeding. Therefore, it is useful to clarify the genetic relations among populations in the wild.

So far, there have been studies on the genetic diversity in natural geographically distant populations of several cultivated mushrooms such as *Agaricus bisporus* (Lange) Imbach (Kerrigan and Ross 1989), *Lentinula edodes* (Berk.) Pegler (Fukuda and Tokimoto 1991; Fukuda et al. 1994; Hibbett et al. 1995), *Pleurotus ostreatus* (Jacq : Fr.) Kummer (Matsumoto and Fukumasa-Nakai 1995; Matsumoto et al. 1995), *Pholiota nameko* (T. Ito) S. Ito and Imai (Obatake et al. 2002), and *Flammulina velutipes* (Curt.: Fr.) Sing. (Nishizawa et al. 2003). On the other hand, it is also important to understand the genetic relatedness among wild strains occurring on the same natural substrate (such as a fallen tree) for using them as breeding materials. Genetic differences and the sizes of inhabited territory of wild strains on the same substrates have been studied in *P. ostreatus* (Kay and Vilgalys 1992), *L. edodes* (Chiu et al. 1999), and *F. velutipes* (Fukuda et al. 2000). However, more information and additional case studies are necessary to further clarify the genetic structure within natural habitats, especially for wood-decaying basidiomycetes used for mushroom cultivation.

We accidentally found a single fallen tree of *Quercus mongolica* Fischer var. *grosseserrata* Rehd. et Wils. on which many fruiting bodies of *L. edodes* were forming at the same time. The purpose of the present study is to examine the genetic differences among these fruiting bodies by mating tests and restriction fragment length polymorphism (RFLP) analyses of mitochondrial DNA (mtDNA).

Materials and methods

Strains of *L. edodes*

Eighteen fruiting bodies of *L. edodes* developing on a fallen tree of *Q. mongolica* var. *grosseserrata* (diameter at breast height about 40 cm, trunk about 10 m long) were found in a forest near Sakae Village in the north of Nagano Prefecture in Japan, on May 25, 1995. They were collected, and their fruiting positions on the fallen tree were recorded (Fig. 1). To make a pure culture from each fruiting body, small tissue blocks were removed aseptically and were transferred to MYG agar (2% malt extract, 0.2% yeast extract, 2% glucose, 2% agar) medium. The samples were incubated at 25°C, and newly grown hyphae were transferred to new MYG agar medium. These 18 dikaryotic strains (SA172–SA189) were maintained in the Laboratory of Applied

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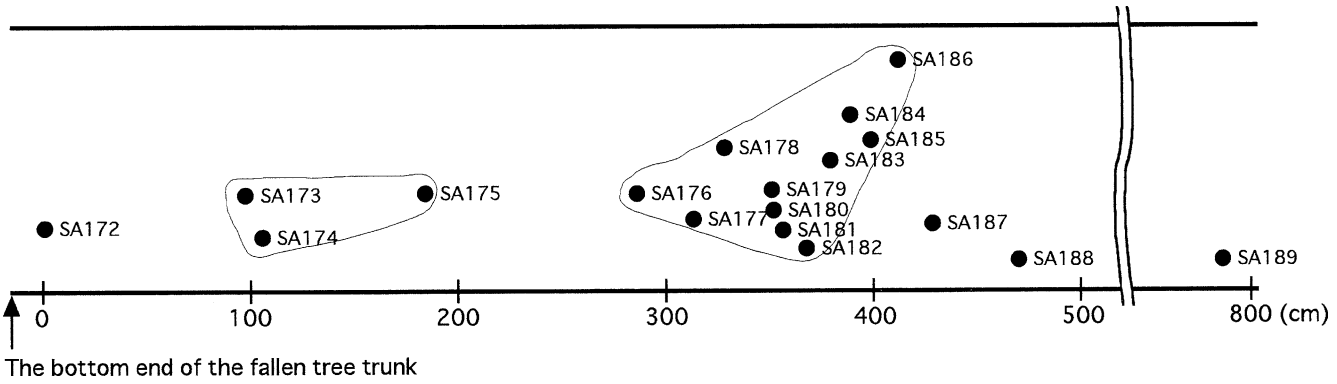

Fig. 1. Positions of 18 fruiting bodies of *Lentinula edodes* on a fallen trunk of *Quercus mongolica* var. *grosseserrata*. The bark face of the fallen tree is drawn in plane. Locations of fruiting bodies of identical mating type and mtDNA genotype, as identified in pure cultures originating from individual fruiting bodies, are enclosed in *lines*

Table 1. Mating types and mtDNA genotype of *Lentinula edodes* strains used in this study

Strain no.	Mating type	MtDNA genotype[a]	RFLP pattern[a]	
			*Bam*HI	*Eco*RI
SA172	*A1B1*, *A2B2*	J-3	A	B
SA173	*A3B3*, *A4B4*	J-9	A	G
SA174	*A3B3*, *A4B4*	J-9	A	G
SA175	*A3B3*, *A4B4*	J-9	A	G
SA176	*A5B5*, *A6B6*	J-2	B	A
SA177	*A5B5*, *A6B6*	J-2	B	A
SA178	*A5B5*, *A6B6*	J-2	B	A
SA179	*A5B5*, *A6B6*	J-2	B	A
SA180	*A5B5*, *A6B6*	J-2	B	A
SA181	*A5B5*, *A6B6*	J-2	B	A
SA182	*A5B5*, *A6B6*	J-2	B	A
SA183	*A5B5*, *A6B6*	J-2	B	A
SA184	*A5B5*, *A6B6*	J-2	B	A
SA185	*A5B5*, *A6B6*	J-2	B	A
SA186	*A5B5*, *A6B6*	J-2	B	A
SA187	*A5B7*, *A6B6*	J-2	B	A
SA188	*A5B7*, *A7B8*	J-3	A	B
SA189	*A5B7*, *A1B9*	J-13	C	I

RFLP, restruction fragment length polymorphism
[a] Details of mtDNA genotypes and RFLP patterns were published in a previous study (Fukuda et al. 1994)

Mushroom Sciences, Faculty of Agriculture, Shinshu University (Table 1).

Mating tests

Basidiospores were collected from each wild fruiting body and were allowed to germinate on MYG agar plates. Up to 30 germinating basidiospores per fruiting body were randomly isolated, transferred individually to new MYG agar slants, and incubated at 25°C. Also, monokaryons were prepared through artificial dedikaryotization by the protoplast regeneration method (Fukumasa-Nakai et al. 1994) from the 18 dikaryotic strains originated from individual fruiting bodies. The monokaryons regenerated from protoplasts (neohaplonts) were identified by the absence of clamp connections under a light microscope.

Matings among the single-spore isolates and neohaplonts were made by placing small plugs of mycelia (2 mm in diameter) at a distance of 5 mm on MYG agar plates. Dikaryosis and common-*B* heterokaryosis of the paired monokaryons were confirmed by the appearance of clamp connections and pseudoclamps, respectively, under a microscope.

mtDNA isolation and restriction analyses

mtDNA was isolated by the procedure of Fukumasa-Nakai et al. (1992). mtDNA isolated from each of the 18 dikaryotic strains was digested separately with two endonucleases, *Bam*HI and *Eco*RI (Nippon Gene, Tokyo, Japan), following the supplier's specifications. Electrophoresis of all digests was carried out on 1% agarose (Nippon Gene, Type S) gel at 5 V/cm for 4 h, followed by staining with ethidium bromide (0.5 µl/ml). Restriction patterns were recorded by photographing the gels on a UV transilluminator.

Results

Mating tests

A tetrapolar mating system has previously been demonstrated in *L. edodes* (Takemaru 1961). To obtain the monokaryons of all four mating types (mating-type testers), at least 12 single-spore isolates derived from each wild dikaryotic strain were selected randomly and paired in all possible combinations. As the mating-type testers could be obtained from each wild strain, they were paired in interisolate combinations to determine whether the incompatibility factors (*A* and *B*) of the wild strains were identical. One group of three strains (SA173–SA175) and another group of 11 strains (SA176–SA186) were found to possess identical incompatibility factors within each group (data not shown).

Figure 2 shows the result of mating test carried out in all intra- and interisolate combinations among 6 representative

		SA172				SA173				SA176				SA187				SA188				SA189			
		1[1]	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
SA172	1	2[2]	+	−	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−
	2	+	−	B	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	B	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−
	4	B	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
SA173	1					−	+	−	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2					+	−	B	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3					−	B	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4					B	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA176	1									−	+	−	B	−	+	−	+	−	+	−	+	−	+	−	+
	2									+	−	B	−	+	−	B	−	+	+	+	+	+	+	+	+
	3									−	B	−	+	−	B	−	+	−	+	−	+	−	+	−	+
	4									B	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+
SA187	1													−	+	−	B	−	+	−	B	−	+	−	B
	2													+	−	B	−	+	+	+	+	+	+	+	+
	3													−	B	−	+	−	+	−	+	−	+	−	+
	4													B	−	+	−	B	+	+	B	B	+	+	B
SA188	1																	−	+	−	B	−	+	−	B
	2																	+	−	B	−	+	+	+	+
	3																	−	B	−	+	−	+	−	+
	4																	B	−	+	−	B	+	+	B
SA189	1																					−	+	−	B
	2																					+	−	B	−
	3																					−	B	−	+
	4																					B	−	+	−

[1] Number of mating-type tester.
[2] −: no clump connections, +: dikaryosis, B: common-*B* heterokaryosis.

Fig. 2. Mating compatibility patterns among mating-type testers from six representative wild strains of *Lentinula edodes*

Table 2. Mating types of six representative strains of *Lentinula edodes*

Tester no.	Mating type					
	SA172	SA173	SA176	SA187	SA188	SA189
1	*A1B1*	*A3B3*	*A5B5*	*A5B7*	*A5B7*	*A5B7*
2	*A2B2*	*A4B4*	*A6B6*	*A6B6*	*A7B8*	*A1B9*
3	*A1B2*	*A3B4*	*A5B6*	*A5B6*	*A5B8*	*A5B9*
4	*A2B1*	*A4B3*	*A6B5*	*A6B7*	*A7B7*	*A1B7*

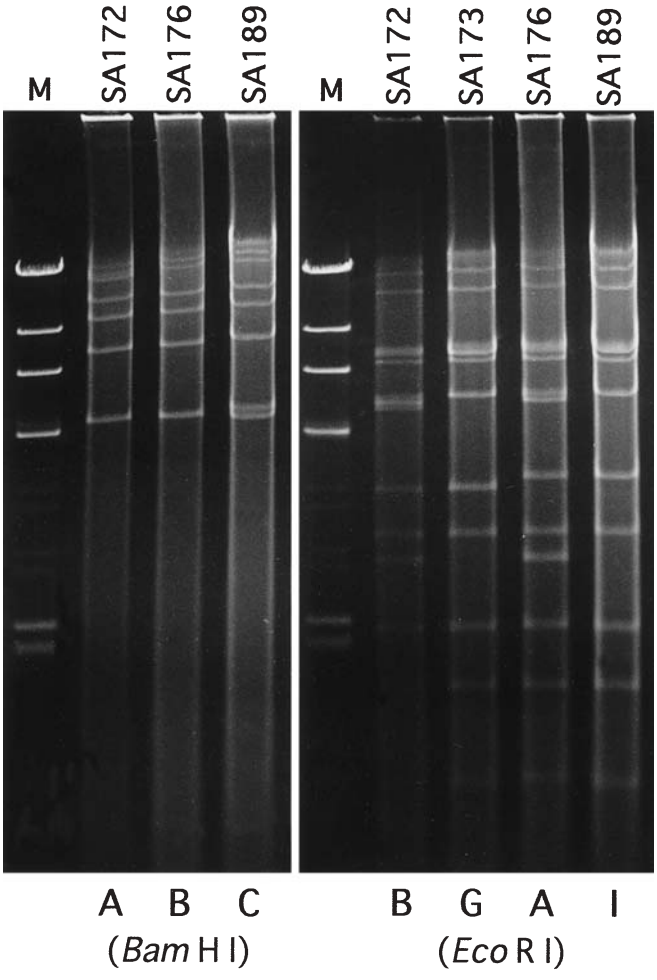

Fig. 3. Restriction fragment length polymorphism (RFLP) patterns of mtDNA produced from representative strains (indicated on *top*) with *Bam*HI and *Eco*RI. The strains associated with particular RFLP patterns are presented in Table 1. Types of RFLP patterns shown at the bottom of each gel track were defined previously (Fukuda et al. 1994)

strains (SA172, SA173, SA176, and SA187–SA189) carrying different *A* and/or *B* factors. On the basis of these mating reactions, mating types of four testers (testers no. 1 to 4 in Fig. 2) from each representative strain were designated as shown in Table 2; seven different *A* factors and nine different *B* factors were present in the 18 wild strains.

Three to nine neohaplonts, respectively, were obtained through artificial dedikaryotization by the protoplast regeneration method from each of the 18 wild strains. Because the neohaplont has a mating type identical with one of the two component nuclei of the original dikaryotic strain (Fukumasa-Nakai et al. 1994), mating tests between the neohaplonts and mating-type testers were carried out to determine the mating types of each wild strain. Because each of the neohaplonts obtained was compatible with only one of four testers derived from each original strain, the mating types of the 18 wild strains could be determined as shown in Table 1. These 18 wild strains were divided into six mating-type groups, each of which consisted of 1 to 11 strains (see Table 1).

mtDNA RFLP analyses

*Bam*HI and *Eco*RI digests of mtDNAs from 18 wild strains produced three and four distinct RFLP patterns, respectively (Fig. 3). By combining the RFLP patterns obtained, mtDNA from the 18 strains could be assigned to four mtDNA genotypes (see Table 1). The same assignments could be made based on the *Eco*RI RFLP patterns alone. Grouping of the wild strains by mtDNA genotypes correlated well with that by the mating types, although some strains that had different mating types (SA172 and SA188; SA187 and SA176–SA186) showed an identical mtDNA genotype.

Discussion

The mating system of *L. edodes* is a multiple allelomorph heterothallism controlled by two incompatibility factors *A*

and *B* (Takemaru 1961). Because 40 to 65 different *A* factors and 63 to 100 different *B* factors appear to exist in this fungus (Tokimoto et al. 1973), mating-type identity provides a useful parameter for the characterization of genetic differences among wild strains of *L. edodes*. Seven different *A* factors and nine different *B* factors were contained in the 18 wild strains used in this study, and they could be divided into six distinct genets by the differences of their mating types. This result implies that multiple strains of *L. edodes* of different genetic compositions existed around the fallen tree studied.

Because uniparental inheritance of mtDNA in sexual crosses has been demonstrated for *L. edodes* (Fukuda et al. 1995), it is possible to estimate the derivation of each strain by analysis of mtDNA genotypes. The 18 wild strains examined in this study seemed to be derived from at least 4 distinct strains, judging from the number of mtDNA RFLP genotypes detected among them. In the 3 strains SA187–SA189, one of the two nuclei had the same mating type, *A5B7*. However, the others had different mating types. Hence, every one of the two parental nuclei of the three wild strains probably has a different origin, because their mtDNA genotypes were distinct. On the other hand, the group consisting of 11 strains (SA176–SA186) and SA187 may be derived from common parental strains, as their mtDNA genotypes are identical and only one of the *B* factors was different between them (see Table 1).

Distance values between the mtDNA genotypes of *L. edodes* based on the presence or absence of comigrating restriction fragments of *Bam*HI and *Eco*RI digests were calculated in a previous study (Fukuda et al. 1994). The distance values between all pairs of the four mtDNA genotypes detected in this study (J-2, -3, -9, and -13) varied between 0.077 (J-3 and J-9) to 0.231 (J-3 and J-13), a slightly smaller range than that previously reported for 38 wild strains in the Japanese natural population of *L. edodes* (0.026–0.300; Fukuda et al. 1994). In this fungus, mtDNA variation presumably correlates well with nuclear variation (Fukuda et al. 1994; Hibbett et al. 1995). It is, therefore, very interesting that a range of mtDNA variation similar to that recognized in the Japanese natural population was detected in a small number of wild strains derived from a single decayed log.

Individual genets of root-infecting basidiomycetes such as species of *Armillaria* (Fr.: Fr.) Staude might inhabit large territories (Smith et al. 1992; Kile 1993; Worrall 1994), as they can spread by vegetative growth from tree to tree. In contrast, basidiospore dispersal is probably the main means of spread of wood-decaying basidiomycetes without root-infecting ability, and small territory sizes of single genets within a fallen tree have been reported for *P. ostreatus* (Kay and Vilgalys 1992), *L. edodes* (Chiu et al. 1999), and *F. velutipes* (Fukuda et al. 2000). From the results of these studies, we estimate that the territories of individual genets of these wood-decaying basidiomycetes are not more than about 1 m in diameter. The present study supports these estimates. Thus, relatively small territory sizes of single genets appear common among wood-decaying basidiomycetes used in mushroom cultivation, which should be considered when collecting genetic resources for breeding in the wild.

In this study, mating tests and mtDNA analyses proved useful for examining genetic difference and variation among *L. edodes* wild strains from a single substrate. However, it will be necessary to employ additional indicators such as nuclear DNA fingerprinting to clarify the genetic relation between the wild strains in more detail.

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