SHORT COMMUNICATION

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A distinctive phenotype in the common-*A* heterokaryon of *Coprinus cinereus*

Abstract The homobasidiomycete *Coprinus cinereus*, unlike *Schizophyllum commune*, is not known to exhibit an obvious heterokaryotic phenotype in common-*A* matings. In the present study we found that progeny isolated from a fruit-body collected in the field exhibit a distinctive mycelial development in common-*A* matings. Genetic analysis suggested that the common-*A* heterokaryotic phenotype is brought about by a nuclear factor(s) other than the mating type genes.

Key words Basidiomycete · *Coprinus cinereus* · Mating reaction

Sexual compatibility is regulated via a tetrapolar mating system in about 55% of homobasidiomycetes (Whitehouse 1949), which include the model mushrooms *Coprinus cinereus* and *Schizophyllum commune* (see Raper 1966; Casselton and Olesnicky 1998) and the cultivated edible species *Lentinus edodes, Pleurotus ostreatus*, and *Flammulina velutipes* (see Takemaru 1961). In these fungi, mating is under the control of genes at the two unlinked mating-type loci, *A* and *B*. Therefore, four mating types of homokaryons occur in F_1 progeny from a cross between two homokaryons with different alleles in both *A* and *B* mating-type genes. When the F_1 progeny are mated with each other, four types of heterokaryons could occur in terms of combinations of alleles of the mating type genes (Raper 1966): the dikaryon ($A \neq B \neq$), the common-*A* heterokaryon ($A = B \neq$), the common-*B* heterokaryon ($A \neq B =$), and the common-*AB* heterokaryon ($A = B =$).
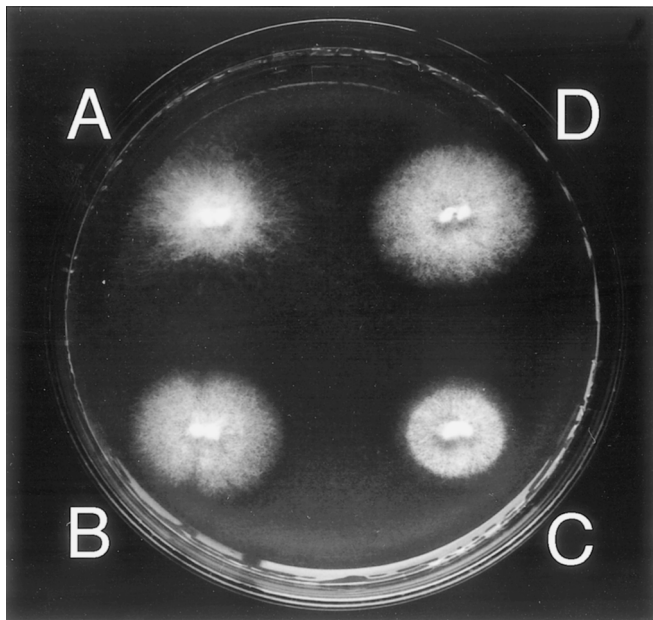
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Morphology of the established common-*A* heterokaryon varies from species to species. In *S. commune*, common-*A* matings result in a distinctive mycelium in which aerial growth is reduced, the hyphae branch frequently, and nuclear migration associated with septal disruption occurs continuously (Papazian 1950; Raper and San Antonio 1954; Raper 1966). In *C. cinereus*, however, no obvious phenotype of the common-*A* heterokaryon has been reported, although nuclear migration leading to a heterokaryon was confirmed by complementation of auxotrophy (Swiezynski and Day 1960). We report here *C. cinereus* strains that display a distinctive mycelial phenotype in common-*A* matings. Genetic analysis suggested that the mycelial phenotype is brought about by dominant nuclear factor(s) other than the mating-type genes.

We made matings between progeny from a *C. cinereus* fruit-body collected in the field (Muraguchi and Kamada 1998), and mating reactions were observed after incubation at 37°C for 3 days. Mating, fruiting, and single-spore isolation were performed as described previously (Kamada et al. 1984). Figure 1 shows representative mating reactions in the four types of matings. A distinctive mycelial development was recognizable in the common-*A* mating (Fig. 1C). The colony of the common-*A* heterokaryon was smaller than those of the other heterokaryons. Aerial hyphae were well developed in the central region of the colony but reduced in the margin. In addition, aerial hyphae distal to the center of colony appeared to be powdery, making a white boundary between central and marginal regions.

To examine whether the distinctive mycelial development always occurs in common-*A* matings, we isolated 16 progeny from a cross between KF_2#1 (*A91B92*) and KF_2#11 (*A92B91*), F_2 progeny from the fruit-body, mated them in all combinations except selfings, and examined the mating reactions. The mating type of each progeny was identified by the four testers, KF_1#2 (*A91B91*), KF_1#9 (*A92B92*), KF_2#1 (*A91B92*), and KF_2#11 (*A92B91*). The *A* and *B* mating-type genes segregated independently as expected (data not shown), and 32 of the 120 ($16 \times 15 \div 2$) matings were common-*A* associations. All the common-*A* matings displayed the distinctive mycelial development, which sug-

Fig. 1. Representative mating reactions in four types of matings between progeny from a *Coprinus cinereus* fruit-body collected in the field. **A** Compatible ($A{\neq}B{\neq}$). **B** Common *B* ($A{\neq}B{=}$). **C** Common *A* ($A{=}B{\neq}$). **D** Incompatible ($A{=}B{=}$)

Table 1. Mating pattern for eight progeny from the cross $5302 \times KF_0\#2$

A Cytoplasmic parent: $KF_0\#2$

F_1 # (Mating type)	1	2	3	4	5	6	7	8
1 (*A91B91*)		F	F	B	+	**F**	B	−
2 (*A91B2*)			−	+	B	−	+	F
3 (*A91B2*)				+	B	−	+	F
4 (*A2B91*)					**F**	+	−	B
5 (*A2B2*)						B	**F**	+
6 (*A91B2*)							+	F
7 (*A2B91*)								B
8 (*A91B91*)								

B Cytoplasmic parent: 5302

F_1 # (Mating type)	1	2	3	4	5	6	7	8
1 (*A2B91*)		+	B	−	−	+	B	B
2 (*A91B2*)			F	+	+	−	F	F
3 (*A91B91*)				B	B	**F**	−	−
4 (*A2B91*)					−	+	−	B
5 (*A2B91*)						+	B	B
6 (*A91B2*)							**F**	**F**
7 (*A91B91*)								−
8 (*A91B91*)								

+, $A{\neq}B{\neq}$; F, $A{=}B{\neq}$; B, $A{\neq}B{=}$; −, $A{=}B{=}$; bold Fs indicate the development of the common-*A* phenotype

gests that one or both of the parental strains harbor a nuclear or cytoplasmic factor(s) that brings about the distinctive mycelial development in common-*A* matings.

To examine whether the cause of the common-*A* phenotype is cytoplasmic, reciprocal matings were made between $KF_0\#2$ (*A91B91*), a basidiospore derivative from the fruit-body collected in the field, and 5302 (*A2B2*), a standard wild-type homokaryon maintained in the laboratory. The latter has been observed not to exhibit the distinctive mycelial development in common-*A* matings with other strains in the laboratory: no difference is recognizable in mycelial development between common-*A* and common-*AB* matings. For reciprocal matings, one mating partner was inoculated in the middle of a plate and cultured for 2 days, then the other partner was inoculated at a point along the margin of the mycelial colony formed, and the mated culture was grown until a dikaryon emerged from the periphery of the colony of the precultured mycelium. Both arrangements of the strains were used, and in this way two dikaryons between $KF_0\#2$ and 5302 with different cytoplasm were produced (see Casselton and Economou 1985). Each dikaryon was transferred to a slant and left in conditions that promote fruiting. Eight progeny from each dikaryon were mated to each other in all combinations and examined for mating reactions. The progeny exhibited the distinctive mycelial development in some of the common-*A* associations, regardless of which cytoplasm they had (Table 1). These results suggest that the distinctive colony development in common-*A* matings is caused not by a cytoplasmic factor but by a nuclear factor(s). Also, because the distinctive common-*A* phenotype occurred with different *A* and *B* alleles (Table 1), the presumptive nuclear factor should be neither the mating-type gene *A* nor *B*.

In the common-*A* matings between the progeny from the cross $KF_0\#2 \times 5302$ with the cytoplasm of 5302, strain #6 (*A91B92*) gave the distinctive mycelial phenotype in all three common-*A* matings (Table 1B). We mated strain #6 with a further eight *A91B91* strains, which were identified among 26 progeny newly isolated from the cross $KF_0\#2 \times 5302$, and found that strain #6 gave the distinctive colony phenotype in all the eight matings. This result suggests that strain #6 carries a dominant factor(s) that gives the distinctive colony phenotype in common-*A* matings. Based on this hypothesis, the mating between strains 6 and 8 in Table 1A is expected to exhibit the common-*A* phenotype. However, the common-*A* phenotype in this mating was too weak to be identified unambiguously. Some unidentified factor(s) may have suppressed expression of the phenotype.

In common-*A* matings, in which *B* mating-type genes are compatible between the two mating partners but *A* mating-type genes are not, only the *B*-regulated developmental pathway is activated in the heterokaryon formed. It has been considered that the *B* mating-type genes regulate nuclear migration for dikaryosis and the cellular fusion that complete clamp connections during growth of the dikaryon because these processes do not occur unless the *B*-regulated developmental pathway is activated (Raper 1966; Casselton and Olesnicky 1998). In *S. commune*, it is also known that another phenotype, the distinctive mycelial phenotype, accompanies the common-*A* mating. In the present study, we found that a distinctive colony phenotype accompanies common-*A* matings in *C. cinereus*. We also suggested that a dominant nuclear factor(s) is responsible for the common-*A* phenotype. The *B* mating-type genes have been demonstrated to encode mating type-specific pheromones and their receptors in *S. commune* and *C. cinereus* (Wendland et al. 1995; Vaillancourt et al. 1997; O'Shea et al. 1998). How-

ever, little is known about downstream genes acting in the *B*-regulated developmental pathway, although the *C. cinereus* pheromone receptor has been shown to be a G-protein-coupled receptor (Olesnicky et al. 1999). The nuclear factor(s) for the distinctive colony development should be a member of the gene network, and future characterization of the factor should help to define the downstream pathway of the pheromone signaling.

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