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# Identification and Functional Analysis of Mating Type Loci in the *Pleurotus eryngii*

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*Pleurotus eryngii* has recently become a major cultivated mushroom; it uses tetrapolar heterothallism as a part of its reproductive process. Sexual development progresses only when the A and B mating types are compatible. Such mating incompatibility occasionally limits the efficiency of breeding programs in which crossing within loci-shared strains or backcrossing strategies are employed. Therefore, understanding the mating system in edible mushroom fungi will help provide a short cut in the development of new strains. We isolated and identified pheromone and receptor genes in the B3 locus of *P. eryngii* and performed a functional analysis of the genes in the mating process by transformation. A genomic DNA library was constructed to map the entire mating-type locus. The B3 locus was found to contain four pheromone precursor genes and four receptor genes. Remarkably, receptor PESTE3.3.1 has just 34 amino acid residues in its C-terminal cytoplasmic region; therefore, it seems likely to be a receptor-like gene. Real-time quantitative RT-PCR (real-time qRT-PCR) revealed that most pheromone and receptor genes showed significantly higher expression in monokaryotic cells than dikaryotic cells. The pheromone genes PEphb3.1 and PEphb3.3 and the receptor gene PESTE3.3.1 were transformed into P5 (A3B4). The transformants were mated with a tester strain (A4B4), and the progeny showed clamp connections and a normal fruiting body, which indicates the proposed role of these genes in mating and fruiting processes. This result also confirms that PESTE3.3.1 is a receptor gene. In this study, we identified pheromone and receptor genes in the B3 locus of *P. eryngii* and found that some of those genes appear to play a role in the mating and fruiting processes. These results might help elucidate the mechanism of fruiting differentiation and improve breeding efficiency.