(Form No. 13)

SUMMARY OF DOCTORAL THESIS

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Title: Comparative molecular biological study on mating type locus in bipolar mushrooms
(二極性きのこの交配型遺伝子に関する比較分子生物学的研究)

Most of the cultivated edible basidiomycetous mushrooms are heterothallic. In the basidiomycetous mushroom, mating compatibility is controlled by one or two sets of allelomorphic gene which are defined as mating type locus. *A* locus and *A* and *B* loci can control in bipolar and tetrapolar mating system, respectively. *A* mating type locus and *B* mating type locus was carrying at least one pair of the homeodomain (*HD*) protein genes which are designated as *HD1* and *HD2*, and pheromone (*Phb*) and pheromone receptor (*Rcb*) genes, respectively. In the previous reports, *HD* protein controlled and Phb and Rcb protein regulate gene expression for the nuclear migration and reciprocal nuclear exchange. Moreover, clamp formation required high expression levels of *HD* protein genes and that altered expression of only the *A*-mating-type genes were sufficient to drive true clamp formation. With the development of high-throughput sequencing technologies, the genomes of many common edible mushrooms have been published in recent years, which has allowed a more detailed analysis of the molecular genetic structure of different types of the mating locus. However, there are still many blanks in the study of mating locus in bipolar mushrooms, such as the relationship between homeodomain and nuclear localization signals in both HD proteins, and their function in clamp connections formation. In this study, mating system and mating type genes of the important cultivated mushroom *G. frondosa* was determined in Chapter 2. In Chapter 3, a genomic structure of mating type genes of the cherished mushroom *R. roseolus* was presented. In Chapter 4, we described the structure of mating-type locus and the association between homeodomain and nuclear localization signals in putative proteins and their contribution in clamp cells formation in *M. aitchisonii*.

In chapter 2, we crossed 31 basidiospore isolates from the fruit bodies of dikaryotic strain *Grifola frondosa* IM-BM21 to analyze mating compatibility groupings. Two incompatibility groups (*A1* and *A2*) were identified, identifying *G. frondosa* as a bipolar mushroom. Based on nucleotide sequences of whole genomic DNA of *G. frondosa* WM1-25 from next-generation sequencing, we designed oligonucleotide primers to amplify the homeodomain protein gene located on the *A* locus (*A1hox2*) and pheromone genes (*A1ph1*, *A1Ph5* and *A1Ph6*) located on the *B* locus in the tetrapolar mushroom. The DNA fragment of the *A1hox2* gene was amplified only from *A1* monokaryotic strains and the *A1phb1*, *A1Ph5* and *A1Ph6* genes were amplified in all *A1* and *A2* monokaryotic strains. Therefore, the linkage groups of the analyzed genes were defined as *A1hox2* in linkage group I, which is closely linked to *A* mating-type locus, and *A1ph1*, *A1Ph5* and *A1Ph6*, which has no relationship with mating type.

In chapter 3, by next-generation sequencing analysis, 27 mating-type and mating-type-related genes were identified in the bipolar basidiomycete *Rhizopogon roseolus*, including two homeodomain protein genes (*hox1* and *hox2*), four pheromone receptor genes (*rcb1*, *rcb2*, *rcb3* and *rcb4*), four pheromone genes (*phb1*, *phb2*, *phb3*, *phb6, phb7*) and genes (*glydh*, *up1*, *sec61*, *up8*, *up2*, *up11*, *mip*, *β-fg*, *hp1*, *glgen*, *up12*, *snx*, *mad, rpb2*, *pk*, *fruk*) around the *A* mating-type

locus that are conserved among mushrooms. The 27 genes were clustered in three different nodes (assembled contig nucleotide sequences). Two pheromone receptor genes, *A2-Rcb1* and *A2-Rcb2*, were closely linked with the *Hox* genes on node 8,230, and the distance between *A2-Rcb1* and *A2-hox2* was only 875 bp. The other two *Rcb* genes (*A2-Rcb3* and *A2-Rcb4*) were located on node 11,781. Six genes (*glydh*, *up1*, *sec61*, *up8*, *up2*, *up11*) that flanked the *A* mating-type locus and are conserved with other mushrooms were found in node 880. Linkage analysis using single-nucleotide polymorphism (SNP) analysis revealed that the 27 genes located in the three nodes were genetically linked, and that the three nodes comprised a partial nucleotide sequence of a single chromosome. We hypothesized that the chromosome on which the *A* mating-type locus is located might have resulted from a recombination event between the *A* mating-type gene cluster and the pheromone and pheromone receptor cluster in ancient species of this bipolar mushroom.

In chapter 4, *Mycoleptodonoides aitchisonii* is a bipolar mushroom with monokaryotic strains that could form clamp connections and are independent of the type of mating. Only homeodomain 2 (*HD2*) gene was detected in *A* mating-type locus of *A1 M. aitchisonii*. In this study, two *HD* genes (*Mahd1-7* and *Mahd2-7*) and nine *A* loci flanking genes (*glydh*, *sec61*, *up8*, *up2*, *up11*, *mip*, *β-fg*, *glgen*, *RPB2*) were structured in comparison with other mushrooms. *MAhd1-7* and *Mahd2-7* were characterized in strain 50005-7. After phylogenetic and putative protein structure analysis, there was no homeodomain but three NLSs and one coiled-coil at the C- terminus in Mahd1-7, one homeodomain in Mahd2-7; a homeodomain and two NLSs in Mahd2-18. Combining the RT-PCR result of HD genes, both HD genes could be transcribed in all *A2* monokaryon of *M. aitchisonii*. In short, we hypothesize that the homeodomain of HD2 and NLS were required for the efficient formation of clamp cells and the heterodimer formation of Mahd1-7 and Mahd2-18 would promote the frequency of clamp cells. And during the evolution of this species, *A* locus had a chromosomal recombination event in ancient this mushroom.

In Chapter 5, the structural characteristics of the mating type locus of three different bipolar edible mushrooms were analyzed, and the importance of the mating type *A* and *B* locus for each edible mating type and molecular breeding were elucidated by genetic linkage analysis. The *A* locus determined the mating type in all three bipolar edible mushrooms, although genes of the *B* locus could be detected, and transcriptional processes occurred. Evidence of genetic recombination was showed at the *A* locus during the evolution of the species in all three bipolar edibles. Furthermore, we concluded that in some bipolar edible mushrooms, such as *M. aitchisonii*, HD2 is the gene that mainly impacts on clamp connections formation, and the presence of a homeodomain in HD1 is not essential. A minor level of clamp connections was formed by having a homeodomain and an NLS, where the NLS and homeodomain might be distributed in the same or different HD proteins. Heterodimerization of HD protein in different mating type could produce more clamp connections. This study could be used to understand the evolutionary pathways of sexual reproduction and mating types, and can be widely used in strain identification, varieties protection, parentage tracing and molecular breeding. Finally, the possibility of controlling clamp formation by regulating the expression of homeodomain proteins at the *A* locus was discussed.

(Note: When part of the summary is published in a simplified manner for certain reasons, please specify as follows.)

"* In addition, some of the figures, etc., have been omitted."