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SUMMARY OF DOCTORAL THESIS

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Title: **Taxonomic studies of *Hypocrea/Trichoderma* species isolated from substrates of shiitake mushroom (*Lentinula edodes*) cultivation in Japan and Korea**

(日本および韓国におけるシイタケ栽培ほだ木および菌床に発生する *Hypocrea/Trichoderma* 属種の分類学的研究)

Hypocrea/Trichoderma is a genus of economically important fungi with many different applications, including plant disease management, plant growth promotion, induction of resistance in plants, and bioremediation. In contrast, in the cultivation of commercial fungi such as button (or champignon; *Agaricus bisporus*), shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*), some species of *Hypocrea/Trichoderma* behave as competitors, mycoparasites or antagonists.

More than 20 species of *Hypocrea/Trichoderma* have been reported as causal agents of green mold and *Hypocrea* diseases, e.g. “*Trichoderma* disease”, on shiitake mushroom cultivation in Japan and Korea. Species isolated from substrates of shiitake mushroom cultivation have been identified based solely on their morphological features. However, the morphology alone is insufficient for accurate identification, and these isolates need to be re-identified and re-evaluated using modern taxonomy concepts.

To better understand the taxonomy of these species, I carried out a investigation of *Hypocrea/Trichoderma* spp. deposited and maintained in the culture collections of the Fungus/Mushroom Resource and Research Center, Tottori University (FMRC), the Tottori Mycological Institute (TMI), in Japan and the culture collection of Chungnam National University, in Korea. In addition, I collected fresh *Hypocrea/Trichoderma* materials (specimens and their isolates) to find new species and re-evaluate East Asian *Hypocrea/Trichoderma* species concepts.

For phylogenetic analyses, a total 85 representative strains of *Hypocrea/Trichoderma* were selected from the cultures of ca. 420 strains (ca. 130 Japanese and ca. 290 Korean isolates) of *Hypocrea/Trichoderma* isolated from mainly substrates of shiitake mushroom cultivation in Japan and Korea. I used the internal transcribed spacer (ITS) regions of ribosomal RNA, and four protein coding gene regions, RNA polymerase II subunit (*rpb2*), translation elongation factor 1- α (*tef1*), chitinase 18-5 (*chi18-5*), and actin (*act*) genes for determination of exact phylogenetic positions.

Phylogenetically, the strains were recognized as 14 phylogenetic species (*H. lutea*/*T. deliquescens*, *H. pachybasioides*/*T. polysporum*, *H. peltata*, *H. pseudogelatinosa*/*T. pseudogelatinosum*, *H. pseudostraminea*/*T. pseudostramineum*, *H. strictipilosa*/*T. strictipile*, *T. atroviride* complex, *T. citrinoviride*, *T. eijii*, *T. harzianum* complex, *T. koningiopsis*, *T. longibrachiatum*, *T. mienum*, and *T. pseudolacteum*; CHAPTER 2). Subsequently, I investigated their morphological features in detail for taxonomic description. As the results, three newly discovered species were created new taxonomic names (*T. eijii*, *T. mienum* and *T. pseudolacteum*). And, I established new combinations of *H. pseudogelatinosa* and *H. pseudostraminea* within the genus *Trichoderma* (*T. pseudogelatinosum* and *T. pseudostramineum*, respectively), and reported a species for new to Japan (*T. koningiopsis*) (CHAPTER 3).

In CHAPTER 4, I was conducted to assay the antagonistic ability of fourteen identified *Hypocrea*/*Trichoderma* species, actual (12 species) and potential (2 species) competitors, to the mycelia of *L. edodes*. For this purpose, 18 representative strains of *Hypocrea*/*Trichoderma* were selected, and competition tests between mycelia of *Hypocrea*/*Trichoderma* species and mycelia of *L. edodes* (commercial strain A817) were performed on potato dextrose agar media (PDA) – it is well known that dual culture assay on Petri dish is a basic method to assess antagonistic properties of microorganisms. I also did the competition test on sawdust media to assay the degree of invasion by *Hypocrea*/*Trichoderma* species into the *L. edodes*-occupied sawdust media. These investigation were to evaluate the antagonistic potential of each *Hypocrea*/*Trichoderma* species, and to clarify the main competitors of shiitake mushroom cultivation against the mycelia of *L. edodes*. The result of dual cultures assay revealed that most strains of *Hypocrea*/*Trichoderma* antagonized *L. edodes* growth to various degrees and that different isolates within the same species, *T. harzianum* complex and *T. atroviride* complex, also showed different degrees of inhibition depend on temperature. *Trichoderma harzianum* subclade IV showed the most severe invasion not only on PDA but also on sawdust media. Although some species of *Hypocrea*/*Trichoderma* weakly invaded into the mycelia of *L. edodes*, *in vitro*, the others *Hypocrea*/*Trichoderma* species also need to caution in shiitake mushroom cultivation.

In CHAPTER 5, I discuss the overall studies of *Hypocrea*/*Trichoderma* species - shortly mentioned that diversity of East Asian *Hypocrea*/*Trichoderma* species, strategies for protection of *Hypocrea*/*Trichoderma* disease in commercial mushroom cultivation, ability of *Hypocrea*/*Trichoderma* species to antagonize and parasitize, and prospect for studies of *Hypocrea*/*Trichoderma* species.