(様式６－２)

Student ID : 1631D01

Name : Chihiro OSAKI

Dissertation Title : Evaluation and clarification of the mechanism of the antifungal activity without direct contact by microbial material including new *Bacillus* TM-I-3

Dissertation Abstract

Several recent studies have reported certain bacteria of genus *Bacillus* exhibited antimicrobial activity. Some companies have focused on this property and have already introduced products to the market as “microbial material”. However, clear legislation regarding microbial materials doesn’t exist. Based on the above, we isolated the bacteria designated as TM-I-3 strain belonging to *Bacillus* from the soil in Nagasaki and examined it. Consequently, it was discovered this bacterium was able to inhibit the generation of molds, without coming into direct contact with the subject. In this study, we revealed the bacteriological properties of TM-I-3 strain and evaluated the antifungal activity of the ingredients emitted from this bacterium. Besides, we analyzed the antimicrobial substances released from this bacterium using gas chromatography (GC)-flame ionization detection and GC-mass (MS) spectrometry analysis to elucidate the mechanism of its action. To explore the mechanisms involved in its non-contact antifungal properties, we examined the differences in the amounts of each volatile substance emitted by TM-I-3 and another *Bacillus* sp., *Bacillus subtilis,* which does not exhibit this same type of non-contact antifungal action. We also attempted to identify the stage of fungal growth that TM-I-3 restrains. Regarding the results of the study, it demonstrated possible efficacy in inhibiting the generation of *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Penicillium expansum*, and *Trichosporon cutaneum* which may lead to inhibit the growth of common fungal contaminants of household products and prevent some pulmonary diseases. It also showed that antimicrobial ingredients from TM-I-3 strain identified by GC/MS as acetic acid, butyric acid, propanoic acid, *iso*-butyric acid, *iso*-valeric acid, 2-methyl butanoic acid, benzaldehyde, and benzoic acid which are all reported to have antimicrobial activity. Especially, TM-I-3 was found to release much higher concentrations of benzaldehyde and benzoic acid than *B. subtilis*; thus, this chemical compound was considered to contribute to the non-contact antifungal effect of TM-I-3. Furthermore, the sigmoid curves of *A. fumigatus* and *C. cladosporioides* and the plot of duration of exposure to TM-I-3 related to daily mycelial growth were obtained to clarify the response of these fungi to the antifungal action of TM-I-3. The fungal growth inhibition test over time showed that TM-I-3 inhibited fungal spore germination (the lag phase in the sigmoid curve). The present study demonstrates that TM-I-3 might be an effective fungistatic agent against pathogenic and allergenic fungi.