Abstract of Thesis		
氏	名	Toshiyuki Morita
題	Ħ	Fungal growth inhibition by volatiles produced by bacterial isolates from soil and environments (土壌および環境から分離した細菌が生産する揮発性抗菌物質による糸状菌の生育 抑制)

To obtain biocontrol agents for the use of agricultural and environmental purposes, bacteria that produce volatile organic compounds (VOCs) with strong antifungal activity were screened and isolated from various environmental samples. Among 136 bacterial isolates, strain TM-R showed the strongest and broadest antifungal activity. Based on physiological and genetical characterization, the bacterium was identified as *Bacillus pumilus*. The effects of VOCs produced by the bacterium, which was grown on four types of agar media (nutrient, Trypto-Soya, Luria-Bertani, and TM Enterprise), were examined against six species of fungi (*Alternaria alternata* NBRC31188, *Aspergillus niger* NBRC33023, *Cladosporium cladosporioides* NBRC4459, *Curvularia lunata* NBRC100182, *Fusarium oxysporum* NBRC30701, and *Penicillium italicum* NBRC32032) in both small- and large-scale tests (plate and 12-L tests, respectively).

Twenty-two of 32 VOCs detected by GC-MS were identified using three databases (NIST 2011, AromaOffice, and AroChemBase). To identify causative antifungal VOCs, the correlation between growth inhibition of *P. italicum* NBRC32032 by the bacterium grown on each of the four media vs. the relative abundance of individual VOCs was estimated. Four VOCs (methyl isobutyl ketone, ethanol, 5-methyl-2-heptanone, and  $S^{-}(-)-2$ -methylbutylamine) were determined to be the predominant antifungal VOCs.

As a possible biocontrol agent, *B. pumilus* TM-R was examined in bathrooms and on house mandarin orange fruits. The number of fungi (mostly *Cladsporium* spp.) developed on the walls in the treated bathrooms in which the biocontrol agent containing *B. pumilus* TM-R was placed was markedly lower than those of control for nine months. In the house mandarin test, the growth of *C. cladosporioides* on the surface of mandarin orange was strongly inhibited.

To investigate the distribution of bacteria that have ability to produce antifungal volatiles in the environments, bacteria were randomly isolated from soil and compost. Among 208 bacterial isolates, 184 (88%) isolates produced volatiles that inhibit the growth of one or more of test fungi (*A. niger* RR, *C. cladosporioides* SK-1, and/or *P. italicum* 2) in the dual plate test. In the 12-L test, only one strain (SNB2) absolutely inhibited the growth of *C. cladosporioides* SK-1. *B. pumilus* TM-R still revealed, however, the strongest antifungal activity.