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学 位 論 文 要 旨

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題目: Antifungal Metabolites Produced by *Bacillus amyloliquefaciens* Biocontrol Strain SD-32
（生物農薬*Bacillus amyloliquefaciens* SD-32株の生産する抗菌性代謝産物）

Botrytis cinerea Pers. Fr. is a pathogen that causes gray mold disease in many crops and causes serious crop losses worldwide. Although chemical fungicides have been used for many years to control the pathogen, the ability of *B. cinerea* to adapt quickly to new chemicals by developing resistance creates the need for continuous development of new fungicides. With increasing concern over fungicide resistance and over the environmental impact and food safety of chemical fungicides, biological control has attracted considerable attention.

To develop new and effective biological control agents against gray mold disease, a screening search was performed, and *Bacillus amyloliquefaciens* SD-32 was isolated from soil in Japan as a promising and effective agent against gray mold disease. To gain insight into the mechanisms responsible for the biological control achieved by *B. amyloliquefaciens* strain SD-32, in this study, attempts were made to identify the factors produced by the bacterium that contribute to the control of gray mold disease and to clarify the role of these factors in the biological control system.

Using an in vitro antifungal-activity-guided purification, two new cyclic lipopeptides (**3** and **4**) were isolated from the culture supernatant of *B. amyloliquefaciens* strain SD-32, together with two known metabolites, *iso*-C_{15} and *iso*-C_{16} bacillomycin D (**1** and **2**). Spectroscopic and chemical analyses identified the structures of the new compounds **3** and **4** as *anteiso*-C_{17} bacillomycin D and *iso*-C_{17} bacillomycin D, respectively. The activities of compounds **1-4** were evaluated against 13 plant pathogens: compounds **1-4** inhibited the growth of all fungi tested. The activities of *anteiso*- and *iso*-C_{17}

bacillomycin D (**3** and **4**) were almost the same of each other, and stronger than those of *iso*-C_{15} and *iso*-C_{16} bacillomycin D (**1** and **2**); the activities of *iso*-C_{15} bacillomycin D (**1**) were the weakest. Furthermore, compounds **1**-**4** at concentrations of 80, 40, 30, and 30 μM, respectively, completely inhibited *B. cinerea* infection in cucumber leaves.

The activity of the culture supernatant, however, was not ascribed exclusively to bacillomycin D homologues; therefore, metabolites other than bacillomycin D were also investigated. After purifying the fractions that exhibited synergistic activity with bacillomycin D, two new cyclic lipodepsipeptides (**5** and **6**) were found in the culture supernatant of this strain, together with three known metabolites, *iso*-C_{14}, *anteiso*-C_{15}, and *iso*-C_{15} [Ile^7]surfactin (**7**, **8** and **9**). Spectroscopic and chemical analyses identified the structures of the new compounds **5** and **6** as *anteiso*-C_{13} and *iso*-C_{13} [Ile^7]surfactin, respectively. Interestingly, [Ile^7]surfactin homologues significantly enhanced the suppressive effect of bacillomycin D against *B. cinerea* in an in vivo cucumber leaf-disk assay, although they showed no suppressive effect by themselves, suggesting that synergistic actions between the [Ile^7]surfactin homologues and bacillomycin D were involved in the suppression of gray mold disease by the bacterium. Furthermore, the synergistic effects were not observed in in vitro mycelial growth or conidial germination inhibition assays, implying that these effects might play a role in gray mold infection in cucumber leaves.

Based on these results, I conclude the following.

1) *B. amyloliquefaciens* biocontrol strain SD-32 produces bacillomycin D homologues and [Ile^7]surfactin homologues.

2) Bacillomycin D homologues have antifungal activity and play an important role in the biological control by *B. amyloliquefaciens* SD-32.

3) The strain SD-32 is unique because it produces a higher content of powerful C_{17} bacillomycin D homologues than other strains reported previously.

4) [Ile^7]surfactin homologues enhance the activities of bacillomycin D homologues under actual conditions.