学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称：応用生物科学専攻　　氏名：　Mengcen Wang

学 位 論 文 題 名

Studies on screening of paddy-rhizospherous microbes against rice seedling blight disease and characterization of their physiological traits

（イネ苗立枯細菌病に拮抗性を示すイネ根圏微生物のスクリーニングと
その生理学的特性の解明に関する研究）

1. Introduction

Burkholderia plantarii, a rice bacterial pathogen, produces tropolone as a phytotoxin and a virulence factor that causes rice seedling blight. As a seed-borne pathogen, *B. plantarii* infects rice during either pre-emergence or post-emergence; therefore, chemical bactericides are rarely effective for prevention, and a practical measure against this rice seedling blight remains unknown. Aiming to develop novel methods for biocontrol of this disease, we screened catechol-tolerant microorganisms by means of chemical-stress selection using catechol—a compound analogous to tropolone. Based on 186 morphologically distinguishable microbial isolates from a paddy rhizosphere, 16 microbial isolates were selected as tolerant to 10 mM catechol and were then subjected to biocontrol assay-guided isolation of biocontrol agents. In this study, metabolic and morphological traits of two representative biocontrol agents *Trichoderma virens* PS1-7 and *Burkholderia heleia* PAK1-2 were investigated with respect to the pathogen *B. plantarii*. In particular, we studied the interaction between *B. plantarii* and the antagonistic microorganisms that is mediated by diverse chemical signaling molecules.

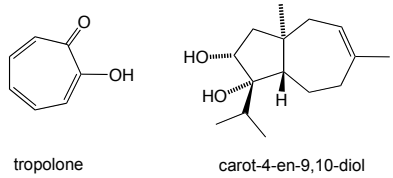
2. Induction of conidiation of *Trichoderma virens* PS1-7 by means of an autoregulatory signal responsive to chemical stress caused by highly active iron chelators

During our studies of the metabolic traits of *T. virens* PS1-7 exposed to catechol, we found a secondary metabolite that was released into the extracellular environment and that was accumulated selectively in the culture medium. This compound is induced by the chemical stress from catechol, and we isolated and identified it as carot-4-en-9,10-diol by spectroscopic analysis. The production of carot-4-en-9,10-diol, a metabolic response of *T. virens* PS1-7 to catechol, was enhanced in a dose-dependent manner from 0.1 to 1.0 mM. Similarly, *T. virens* PS1-7 also produced carot-4-en-9,10-diol in the range of 0.05 to 0.2 mM in response to tropolone. Some iron chelators such as pyrogallol, gallic acid, salicylic acid, and citric acid at 0.5 mM also induced production of carot-4-en-9,10-diol by *T. virens* PS1-7. This chemical stress-responsive compound caused a morphological response in the form of conidiation in *T. virens* PS1-7 mycelia, suggesting that the secreted sesquiterpene diol is involved in the autoregulatory signaling system. In a bioassay for metabolic and morphological responses of *T. virens* PS1-7, the conidiation in the mycelium grown on potato dextrose agar plates was promoted or induced by 20 μM carot-4-en-9,10-diol, regardless of nutritional conditions. Carot-4-en-9,10-diol thus exhibits intracellular signaling properties linked with regulative conidiation of *T. virens*.

3. Repression of tropolone production by *T. virens* PS1-7, leading to induction of pseudobiofilm in *B. plantarii* by a disrupter of its cell-to-cell signaling system

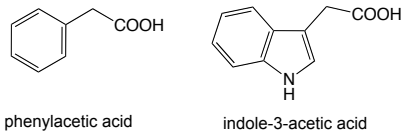
To demonstrate an antagonistic action by *T. virens* PS1-7 on *B. plantarii* leading to

repression of tropolone production in the coculture system, we conducted a bioassay-guided screening for active ingredients in a 3-day shake culture of *T. virens* PS1-7. Carot-4-en-9,10-diol was isolated and characterized as a component that repressed tropolone production of *B. plantarii* from 10 to 200 μM dose-dependently, and attenuated the virulence of *B. plantarii* against rice seedlings. Quantitative reverse transcriptase PCR analysis revealed that suppression of transcription of the *N*-acyl-homoserine-lactone synthase gene *plaI* of *B. plantarii* was the main mode of action of carot-4-en-9,10-diol in inhibiting the tropolone production. Conversely, tropolone was shown to be an autoinducing signaling compound of *B. plantarii*. Besides, we observed a unique response of *B. plantarii* to carot-4-en-9,10-diol: biofilm formation in the static culture system. The initial stage in the biofilm formation of *B. plantarii* was actively induced by tropolone and carot-4-en-9,10-diol. Furthermore, the biofilm of *B. plantarii* induced by carot-4-en-9,10-diol at the late stage showed defects not only in the matrix structure but also in cell viability. These findings demonstrate that carot-4-en-9,10-diol released by *T. virens* PS1-7 is a quorum-sensing inhibitor that suppresses tropolone production and induces the pseudobiofilm of *B. plantarii*, which shortens the lifespan of the pathogen's cells.



3. Blockage of tropolone biosynthesis in *Burkholderia plantarii* by an indolic auxin from *Burkholderia heleia* PAK1-2

Among the catechol-tolerant microorganisms, the bacterium PAK1-2 was identified as a strain of *Burkholderia heleia* that acted as a plant growth-promoting rhizobacterium (PGPR) on rice seedlings and as a potent antagonist of *B. plantarii*. *B. heleia* PAK1-2 induced both the reduction of pigmentation and colony wrinkle of *B. plantarii* in the coculture system, suggesting that some diffusible metabolites produced by *B. heleia* PAK1-2 suppress tropolone production. Particularly, a non-antibacterial metabolite produced by the bioassay-guided isolation was structurally identified as indole-3-acetic acid (IAA) and was found to suppress tropolone production of *B. plantarii* in a dose-dependent manner from 25 to 500 μM. Besides, spectroscopic analysis of the metabolic profile of the *B. plantarii* cultures led to identification of phenylacetic acid (PAA) as a precursor of tropolone. When *B. plantarii* was exposed to 25 μM or more of IAA, PAA production was noticeably inhibited. These data suggest that the disruption of PAA biosynthesis from L-phenylalanine is the main mode of action of IAA in the suppression of tropolone production. In summary, our findings show that IAA, an interspecies signaling chemical produced by *B. heleia* PAK1-2, attenuates the virulence of *B. plantarii* against rice seedlings via blockage of the integrated tropolone biosynthesis from L-phenylalanine.



4. Conclusion

This study demonstrates that an understanding of microbial interactions in the rhizosphere ecosystem suggests alternative ways to control plant pathogens. The results of this study can serve as a basis for the development of novel biopesticides to control bacterial rice seedling blight caused by *B. plantarii*. Characterization of the *N*-acyl homoserine lactone-type quorum-sensing regulatory system and the tropolone biosynthesis as well as their physiological roles are some of our new findings that shed light on the ecological behavior of *B. plantarii* and on microbial harmonization in the rice nursery system. These results may lead to the discovery of other natural products that target virulence-associated molecular systems of pathogens.