氏　　名	**Carla Suzuki Altrao**
学位（専攻分野の名称）	博　士（農芸化学）
学 位 記 番 号	甲　第 851 号
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論 文 審 査 委 員	主査　教　授・博士（獣医）　梶 川 揚 申
	教　授・農 学 博 士　五十君 靜 信
	教　授・博士（農学）　横 田 健 治
	教　授・博士（農学）　篠 原 弘 亮

論 文 内 容 の 要 旨

Background

A number of *Bacillus* strains have been reported to be biological control agents against several kinds of plant diseases. Most of the beneficial strains produce cyclic lipopeptides (cLPs), and cLPs, well-known antimicrobial compounds, are considered to play key roles in the suppression of plant diseases. A few papers have characterized the induced disease resistance elicited by cLPs so far, however, it is still unclear about the specificity to induce the disease resistance among the combinations of cLP molecules and host plants, and the signaling pathways in the innate immune system in planta.

Surfactin and iturin A are *Bacillus* cLPs. Iturin A is composed of the heptapeptide NYNQPNS linked to a β-amino fatty acid, and surfactin is composed of the heptapeptide ELLVDLL linked to a β-hydroxy fatty acid. In the previous studies in my lab, both purified surfactin and iturin A show disease suppression against soil-borne diseases caused by *Fusarium oxysporum* on tatsoi and lettuce, respectively. However, excess amounts of cLP amendments in soil negate the disease suppression for both of surfactin and iturin A.

In my PhD thesis, I aimed to characterize the effect of surfactin and iturin A to suppress disease via induced disease resistance in various edible plants and *Arabidopsis thaliana*.

Comparative study of disease suppression on various edible host plants by *Bacillus* cyclic lipopeptides

To evaluate disease suppression activities of purified cLPs via induced disease resistance, I conducted a bioassay system by hydroponic culture of host plants. *Brassica oleracea* (cabbage), *Solanum lycopersicum* (tomato), *Oryza sativa* (rice), *Glycine max* (soybean) and *Cucumis sativus*

(cucumber) were used as host plants. Seedlings of host plants were treated with purified surfactin or iturin A on their roots by addition of cLP to hydroponic culture. Two-days after cLP-treatment, the bacterial pathogen suspension with sterilized 10 mM $MgSO_4$ was inoculated on the abaxial side of leaves by infiltration method.

Most of the studied host plants were elicited by both cLPs treatment on roots to suppress diseases through induced disease resistance, except tomato and cucumber. On tomato, only surfactin showed significantly disease suppression at a range of 1 to 4 μM, whereas no disease suppressions were observed at a range of 0.25 to 32 μM of iturin A. On cucumber, although significantly disease suppressions were observed by iturin A treatments, surfactin treatments enhanced disease comparing with disease control. It was notable that the effective disease-suppressing concentrations varied by host and cLP, and the negation of disease suppressive activity that was observed at excess concentrations of either surfactin or iturin A for all host plants was confirmed to be through loss of disease suppression.

These findings strongly suggested that cLPs elicit induced disease resistance on a variety of edible host plants.

Insights on conferring of disease suppression by *Bacillus* cyclic lipopeptides via induced disease resistance on *Arabidopsis thaliana*

Induced disease resistance in plants is characterized into two systems; systemic acquired resistance, SAR, is an immune system triggered by pathogen recognition, which uses salicylic acid (SA) as a signaling molecule, whereas induced systemic resistance, ISR, triggered by rhizobacteria recognition, which uses jasmonic acid (JA) and ethylene. These two systems are inducible and strictly regulate the expression of distinct defense genes in planta.

To identify the signaling system in induced disease resistance elicited by cLPs treatments, *A. thaliana* Col-0 and its mutants were used as host plants. As well as described above on edible host plants, I conducted a bioassay system by hydroponic culture.

On *A. thaliana* Col-0 wild type, significant disease suppression against the bacterial disease on leaves was observed following root-treatments of purified surfactin and iturin A, respectively. However, the ranges of concentration to show disease suppressions differed between cLPs. Surfactin conferred significant disease suppression at a range of 4 to 16 μM in hydroponic culture, whereas iturin A conferred suppression at a range of 0.5 to 2 μM. Moreover, the disease suppression was negated at 32 μM surfactin and 4 μM iturin A, respectively, whereas no abnormalities were observed at 32 μM surfactin and 4 μM iturin A without bacterial pathogen inoculations.

To evaluate the signaling pathways in *A. thaliana* conferring disease suppression by cLP

treatments, a series of *A. thaliana* Col-0 mutants.

NPR1, nonexpressor of pathogenesis-related protein 1, has been identified as a mediator for the expression in both of SA signaling and JA signaling pathways in *A. thaliana*. In npr1 mutants, no disease suppression was observed using either surfactin- or iturin A-treatments, suggesting that the induced disease resistance elicited by cLPs was conferred through NPR1-dependent pathway.

ICS1, isochorismate synthase 1, is a biosynthetic enzyme for SA via isochorismate pathway. Generated SA is an important plant hormone, and acts as a regulatory component during SA signaling of induced disease resistance in *Arabidopsis*. In an *Arabidopsis ics1* mutant, no disease suppression was observed following treatment with surfactin or iturin A, suggesting that SA biosynthesis through the ICS pathway is important in the induced disease resistance elicited by surfactin and iturin A.

JAR1, jasmonate resistant 1, is a jasmonate-amido synthetase in the biosynthesis of jasmonate-isoleucine conjugate which activates the JA signaling pathway in *Arabidopsis*. In *jar1* mutants, significant disease suppression was observed following treatments of 8 μM surfactin and 1 μM iturin A, similar to as was observed in wild type Col-0, suggesting that the JA signaling pathway does not confer the disease suppression by induced disease resistance elicited by cLPs.

Those findings revealed that SA acid signaling pathway via isochorismate pathway was the major signaling pathway in the induced disease resistance elicited by both cLPs. Moreover, negations of disease suppression were observed by excess amount of surfactin or iturin A treatment, and the negation of disease suppression was not correlated to the antagonistic effect by induction of JA signaling pathway in host plants.

Conclusion

In my PhD thesis, I characterized disease suppression activities of *Bacillus* cLPs depended on induced disease resistance. The cLPs, surfactin and iturin A, showed disease suppressive activities against bacterial leaf diseases by eliciting of induced disease resistance on a variety of edible host plants. On the other hand, the specificity to induce the disease resistance among the combinations of cLP molecules and host plants were observed on a particular host plants, tomato and cucumber. Moreover, under the excess amount of cLP treatments, the disease suppression were negated for both of cLPs whereas no abnormalities were observed without bacterial pathogen inoculations.

On *A. thaliana*, the signaling pathway in induced disease resistance elicited by *Bacillus* cLPs was SA acid signaling pathway via isochorismate pathway. Moreover, negations of disease suppression were observed by excess amount of surfactin or iturin A treatment, and the negation of disease suppression was not correlated to the antagonistic effect by induction of JA signaling pathway in host

plants.

審 査 報 告 概 要

本博士論文は, 枯草菌を利用した植物病害の生物防除において主体的な役割を示す環状リポペプチドについて, 宿主植物の病害抵抗性誘導に依存した病害抑制効果の検証とその機構の解明を目的として次の成果を得た。①試験対象とした 2 種類の環状リポペプチドは, 各種宿主植物において, 病害抵抗性誘導に依存した顕著な病害抑制効果が確認された。②両環状リポペプチドは共に過剰処理濃度では病害抑制効果が消失し, 病害抑制効果を示す適正な処理濃度域が存在すること, そしてその濃度域は環状リポペプチド分子種と宿主植物との組合せにより異なることを明らかした。③シロイヌナズナ変異体を宿主とした評価系において, 両リポペプチドの病害抑制効果には, イソコリスミ酸生合成経路によるサリチル酸シグナル経路による病害抵抗性誘導経路が主要な病害抵抗性誘導経路となることを明らかにした。これらの研究成果などを詳細に検討した結果, 審査委員一同は博士（農芸化学）の学位を授与する価値があると判断した。