

科学研究費補助金研究成果報告書

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研究課題名(和文) 菌根菌生長促進微生物が孢子生産と果樹園における土壌病原菌の生物防除に及ぼす影響

研究課題名(英文) Effect of mycorrhizal growth stimulatory compounds on spore production and biocontrol of soil-borne plant pathogens in fruit orchards

研究代表者

クルス A. F. (Cruz Andre Freire)

京都府立大学・生命環境科学研究科・講師

研究者番号：80405240

研究成果の概要(和文):3種の細菌がアバスキュラー菌根菌の孢子から分離され、*Bacillus* sp.、*Bacillus thuringiensis*、*Paenibacillus rhizosphaerae* と同定された。これらの細菌は、土壌病原菌 *Fusarium oxysporum*、*Rosellinia necatrix*、*Rhizoctonia solani* および *Pythium ultimum* による病害の防除に有効であった。また、それらがリンの可溶化、エチレン生産、ニトロゲナーゼ活性を示すとともに *in vitro* の菌根菌の菌糸成長を促進することがわかった。さらに、孢子および菌糸の新鮮な試料を、*in situ* 走査型電子顕微鏡を用いて観察したところ、いくつかの細菌性スライムが菌糸および孢子で確認された。

研究成果の概要(英文): Three kinds of bacteria were isolated from arbuscular mycorrhizal spores and identified as *Bacillus* sp., *Bacillus thuringiensis*, and *Paenibacillus rhizosphaerae*. These bacteria also functioned on biocontrol of soil-borne plant pathogens *Fusarium oxysporum*, *Rosellinia necatrix*, *Rhizoctonia solani* and *Pythium ultimum*. Moreover, they were effective on phosphorus solubilization, ethylene production, nitrogenase activity, and stimulation of mycorrhizae hyphal growth *in vitro*. In addition, fresh samples of spores and hyphae were photographed using an *in situ* scanning electron microscope, where some bacterial slimes were detected on hyphae and spores.

交付決定額

(金額単位：円)

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2007年度	1,600,000	0	1,600,000
2008年度	500,000	150,000	650,000
2009年度	500,000	150,000	650,000
年度			
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総計	2,600,000	300,000	2,900,000

研究分野：バイオテク、持続栽培、エチレン

科研費の分科・細目：農業生命科学・果樹園芸

キーワード：菌根菌、細菌、物質、生物防除、果樹栽培

科学研究費補助金研究成果報告書

1. 研究開始当初の背景

The internal bacteria located in AMF spores has been found by many authors in electronic microscope photos. However, it is necessary to identify them by molecular approaches and to select the best strain to promote plant growth.

Sod culture system with *Vulpia myuros* (L.) C. C. Gmel. has been used for the establishment of a low input and sustainable pomology and environmental conservation. *V. myuros*, an annual Poaceae (Gramineae) is often used as sod culture system for their vigorous growing properties between spring and the beginning of summer.

2. 研究の目的

This research aimed to carry applied and fundamental studies on partner bacteria of mycorrhizal spores. More specifically: 1- To isolate and identify these bacterial from spores, following the verification of effects on biocontrol and phosphorus solubilization; 2- Evaluate the synergetic effect of AMF on fruit tree growth and AMF colonization.

3. 研究の方法

The bacteria were isolate from 500 spores of *G. margarita*, whose identification was proceeded by their morphology and DNA techniques. The DNA was extracted followed by PCR with the 27f/1492r primers and sequencing of the resulting fragments.

Phosphorus solubilization was determined Pikovskaya medium. The ethylene production was analyzed in glass flasks with Gas chromatograph. Nitrogenase

activity, an indicator of N-fixation, was determined by an acetylene reduction assay.

The disease suppression was carried by insert both pathogenic fungus and bacteria in petri dishes with PDA media. Similarly, the effect of bacteria on hyphal growth was investigated by placing disks of bacteria grown on Nissui media in petri dishes containing 10 mL of 1% IH base media together with AMF spores.

For SEM observation, the spores were grown and inserted the bacteria *in vitro*. Hyphae, and bacteria were imaged using an *in situ* SEM Quanta 250FEG (FEI Co., Japan), without prior fixation, dehydration, embedding, or staining.

4. 研究成果

Morphologically, all isolated bacteria were gram positive, 0.5 μm wide, between 2 and 5 μm in length, and rod shaped. The molecular identification indicated similarity of KTCIGM01 to *Bacillus* sp., KTCIGM02 and KTCIGM03 to *Bacillus thuringiensis* and *Paenibacillus rhizosphaerae*, respectively.

All of parameters analyzed (antagonism to pathogens, hyphal growth, ethylene production, nitrogenase activity, P solubilization on agar media, and P solubilization on liquid media) were influenced by some of bacterial strains.

P. ultimum was strongly suppressed by KTCIGM01, whereas KTCIGM02 had an antagonistic effect on *R. solani*. *R. necatrix* was suppressed by both KTCIGM01 and KTCIGM02. All bacterial strains were able

to form a halo around colonies on solid Pikovskaya medium, indicating their ability to solubilize P in this medium.

KTCIGM01 and KTCIGM02 both produced ethylene. KTCIGM02 produced more ethylene than KTCIGM01, while KTCIGM03 produced no more than the control (no bacteria). Only KTCIGM02 showed significant nitrogenase activity and for the other strains the values were not significantly different from the control. The *in situ* SEM images showed absence of bacterial growth around the AMF spores and hyphae in the control petri dish.

5. 主な発表論文等

(研究代表者、研究分担者及び連携研究者には下線)

[雑誌論文] (計 0 件)

[学会発表] (計 5 件)

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2. Cruz, A.F., K. Takemori, Y. Toda, T. Ishii. Antagonism of bacteria living inside or on the surface of arbuscular mycorrhizal fungus, *Gigaspora margarita* spores to the growth of *Roselinia necatrix*: a serious soil-borne pathogen in Japanese fruit orchards. 日本園芸学会-秋田県秋田市-秋田大学—2009年9月26日～27日
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4. Takemori, K., Y. Toda, K. Hayashida, D. Yu, A.F. Cruz, T. Ishii. Isolation of bacteria living inside or on surface of the spores of an arbuscular mycorrhizal fungus, *Gigaspora margarita*, and their antagonistic effects on soil-borne plant diseases. 日本園芸学会-東京都千代田区-明治大学 2009年3月19日～20日
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[図書] (計 件)

[産業財産権]

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〔その他〕
ホームページ等
<http://eureka.kpu.ac.jp/~andre/2017/index.html>

6. 研究組織

(1) 研究代表者

クルス A. F. (Cruz Andre Freire)
京都府立大学・生命環境科学研究科・講師
研究者番号：80405240

(2) 研究分担者

()

研究者番号：

(3) 連携研究者

()

研究者番号：