Prospective roles of endophytic actinomycetes in protection of host plants against pathogens

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1. INTRODUCTION

Microorganisms that grow within asymptomatic plants are defined as endophytes. Among endophytes are fungi and bacteria. It is noteworthy that, of the nearly 300,000 species of plants that distribute on the earth, every individual plant harbor one or more endophytes. As for endophytic relationship, however, only a few species of plants have been completely studied. Fungal endophytes are famous for their antibiotic substances produced against herbivores, and thereby protect their host plants. Recently, however, several actinomycetes have been discovered as endophytic microbes. Endophytic actinomycetes are also reported to protect their host plants from the attack of pathogen and/or herbivores.

Actynomycetes are important soil microorganisms and are well known for their antibiotics production. Actynomycetes have been detected not only from the soil of plant rhizosphere but also from the tissues of roots, stem, and/or leaves. Many scientists have been focusing their interests on a genus of actynomycete, *Frankia*, because of its ability of nitrogen fixation. This bacterium deform host roots, thereby facilitate their symbiotic relationships. There are also other actynomycete endophytes which do not have nitrogen fixation ability, but have host-protection ability against pathogens.

We have found various fungal endophytes in pine needles, but have not yet discovered actynomycete ones. We assumed that pine trees could not harbor actynomycete endophytes, because pine species are famous ectomycorrhizal plants. If pine trees harbor actynomycetes, mycorrhizal relationship could be suppressed by their antibiotic ability. To confirm this idea we examined some pine seedlings, and got unexpected facts.

2. MATERIALS AND METHODS

Isolation of actinomycetes from *Pinus thunbergii*

Isolation of actinomycetes was performed as described by Shimizu *et al.* Young plants of *Pinus thunbergii* (2 years old) were harvested from Kitashirakawa experimental station (Kyoto-city, Kyoto pref., Japan). They were separated into leaves and roots and then washed with running tap water, and were cut further into small pieces of ca. 1 ~ 2 mm^2. After surface-sterilization, each piece was placed on IMA-2 agar medium, followed by incubation at 30 C for 1 month. The isolates were identified on the basis of their 16S rDNA gene sequences, which were compared to the GenBank databases.

Protection of the pine disease by actinomycetes

The actinomycetes isolated served inoculation test to confirm their ability to protect host pine seedling from pathogens. The vermicurite moistened by the actinomycetes medium was added to the test tube. After autoclaving, germinated pine seeds were transplanted to a test tube. Then the test tube received 200 μl of hyphal suspension of either of the actinomycetes. Two weeks after planting, the pathogen was inoculated to the test tubes. All seedlings were uprooted and were examined for their infection by pathogen.

3. RESULTS AND DISCUSSION

Isolation of actinomycetes from *Pinus thunbergii*

We discovered two species of actinomycetes from a potted pine seedling. As a result of the sequence analysis, these actinomycetes isolated were revealed to be *Streptomyces* sp. and *Microbispora* sp. Inoculation test showed that *Streptomyces* sp. and *Microbispora* sp. distributed in roots and leaves, respectively.

Protection of the pine disease by actinomycetes.

One week after cultivation, the seedlings pretreated with *Streptomyces* sp. suppressed the diseases development, whereas control seedlings were killed by pathogen. The seedlings pretreated with *Microbispora* sp. also delayed in symptom development. Thus, seedlings infected with either of the actinomycetes delayed in the appearance of symptoms, though *Streptomyces* sp. seemed to be more effective than *Microbispora* sp. as for the biological control agent. This study, however, is at the very beginning, so intensive studies are needed now on.

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Dr. Futai is a Professor of the Laboratory of Environmental Mycoscience and Nematology, Graduate school of Agriculture, Kyoto University. He has been studying on the Pine Wood Disease (PWD) from the viewpoints of microbial ecology. When he started his master course studies, he became interested in the PWD issues, especially in the difference in resistance among pine species. He tried to reveal the mechanism of host resistance using simple apparatus, and succeeded to some extent. He got his doctoral degree from Kyoto University in 1980, then continued his study as post doctoral fellow until 1983. He became an assistant professor in 1983, and was promoted to associate professor in 1995, then full professor in 2003. He was Monbusho Visiting Fellow (1987-88) at PFC, Canadian Forestry Service, Canada, and studied on the resistance of Canadian and European coniferous trees to the pine wood nematode. Nowadays, Dr. Futai and his students are concentrating their efforts on another forest epidemics, the Japanese Oak Wilt, which is caused by a fungus vectored by a tiny beetle. His group are also interested in mycorrhizal relationship between fungi. trees, and its cohabiting microbes. Thus, Dr. Futai has been focusing his interests on the interrelationships between microbes and tree plants. Dr. Futai has been a member of the Japanese Society of Forestry and won the Award of the Society in 2005. He has also been a member of Japanese Society of Nematologists, and has chaired it since three years before..

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