

STUDIES ON PLANT-ASSOCIATED ACTINOMYCETES AND THEIR SECONDARY METABOLITES (3)

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ABSTRACT

Effect of endophytic actinomycetes on plant growth was investigated. Crop seeds were bacterized with the spores of endophytic actinomycetes and grown in a green house. Of the tested microorganisms, *Streptomyces hygroscopicus* S-17 induced the significant growth promotion of tomato *ca* 2 times in height and *ca* 8 times in fresh weight compared to the control. One of the secondary metabolites, pteridic acid A showed growth promotion in the root formation test of kidney bean hypocotyls and the tobacco BY-2 cell culture.

INTRODUCTION

Actinomycetes are widely distributed in association with plant in natural environments. These plant-associated (endophytic) microorganisms are opportunistic and/or saprophytic but do not show pathogenicity in general. Endophytic actinomycete is the new entity for the screening of novel bioactive compounds. We have isolated several new bioactive compounds from endophytic actinomycetes in search for lead molecules for pharmaceutical and agrochemical usages. Although the endophytic actinomycetes produce various secondary metabolites, biological significance of such metabolites in plant-microbe community is open to question. Previously, we identified that endophytic actinomycetes are producing plant hormone-like substances; toyocamycin is a potent cytokinin-like callus growth promoter, and pteridic acid is an auxin-like adventitious roots formation promoter. In this study, effects of endophytic actinomycetes on plant growth was investigated in view of agricultural application.

MATERIALS AND METHODS

Bacterial strains. Endophytic actinomycetes were isolated from plant samples according to the procedure previously reported (Igarashi, 2002). Actinomycete strains used in this study are as follows: *Streptomyces hygroscopicus* S-17 (isolated from *Pteridium aquilinum*), *S. hygroscopicus* S-346 (isolated from *Clethra barbinervis*), *S. sp.* S-231 (isolated from *Leucothe granaya*), *S. sp.* (isolated from *Allium chinese*).

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Seed bacterization. To a mature slant culture of an actinomycete was added a solution of 10% DMSO/10% glycerol. The slant tube was sonicated for 5 min and the spore suspension was taken into a sterilized tube. Crop seeds were soaked in the suspension for 30 min at room temperature. Then, the seeds were planted in a plastic pot filled with commercially available culture soil, and grown in a green house.

Plant growth promotion activity of secondary metabolites. Isolation of pteridic acid and its root formation activity has been described previously (Igarashi, 2002). Tobacco BY-2 cells were cultured in LS medium in dark at 25°C. After the addition of pteridic acid or benzyladenine, the cells were cultured for 7 days and the fresh weight of the cells was measured. Isolation of 6-prenylindole has been reported previously (Sasaki, 2002). Five mm length slices of barley sprouts were incubated with 6-prenylindole in a buffer solution (pH 7) for 48 hr in dark, and the length of the slices was measured.

RESULTS AND DISCUSSION

Previously we reported that the crop growth is promoted by seed bacterization with actinomycetes that are isolated from a wide variety of plants (Igarashi, 2003; Igarashi, 2004). Although it is uncertain whether these actinomycetes are plant-specific or not, they are considered endophytes because they can be reisolated from the crops inoculated to.

Seed bacterization of crops was carried out by immersing the seeds in the spore suspension. The seeds were planted in a pot and grown in a green house. After four weeks of cultivation, growth of bacterized crops was compared with that of untreated ones. Among the tested microorganisms, the actinomycete *Streptomyces hygroscopicus* S-17 showed significant growth promotion of tomato *ca* 2 times in height and *ca* 8 times in fresh weight compared to the control (Fig 1, Fig. 2). Several factors are possibly involved in this growth activating effect: firstly, the microorganism induces the systemic acquired resistance or phytoalexin production; secondly, the actinomycete produces antimicrobial substances effective against plant-deleterious soil microorganisms; thirdly, the actinomycete produces bioactive compounds that have plant growth promoting activity.



Cont S-17 S-231 S-328 S-346
Fig. 1. Growth of tomato seedlings after bacterization.

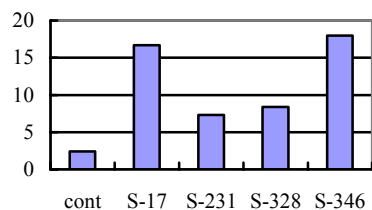


Fig. 2. Growth of tomato in fresh weight (g/plant).

Although actinomycetes produce numerous kinds of secondary metabolites, their plant bioactivity is known very little. We investigated the plant growth promoting activity of the secondary metabolites of strain S-17 which induces significant growth promotion by seed bacterization. By using HPLC, MS and NMR, we have identified that at least 10 chemically different classes of secondary metabolites are produced by strain S-17. Of these compounds, pteridic acid A induced the adventitious root formation of kidney bean hypocotyls (Table 1) and growth promotion of tobacco BY-2 cells (Table 2), suggesting the possible involvement of secondary metabolites in plant growth promotion. Further analysis of the mechanism of plant growth promotion by seed bacterization is currently investigated.

Table 1. Adventitious root formation induced by pteridic acid A.

Conc (μ M)	Number of roots/hypocotyl	
	Pteridic acid A	IAA
0	30.5 \pm 5.9	
0.001	39.7 \pm 4.8	34.7 \pm 4.8
0.01	42.3 \pm 12.9	42.3 \pm 4.6
0.1	60.0 \pm 8.5	51.0 \pm 5.1
1	66.3 \pm 8.5	86.7 \pm 1.1

Table 2. Growth promotion of tobacco BY-2 cells induced by pteridic acid A.

Cell fresh weight (mg)/flask	
Initial	55 \pm 5
Control	447 \pm 4
0.1 μ M Pteridic acid A	936 \pm 88
3.0 μ M Pteridic acid A	702 \pm 48
1.0 μ M Benzyladenine	820 \pm 33

LITERATURE CITED

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