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Mini Research Article

Effect of Freshwater and Marine Photosynthetic Bacteria on Plant Growth

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Abstract

The effects of freshwater and marine purple non-sulfur photosynthetic bacteria (PNSB) on plant growth were compared. The PNSB cells were applied to radish plants (*Raphanus sativus* var. *sativus*) for 20 days using foliar spray and it was observed that the freshwater PNSB, *Rhodobacter sphaeroides*, significantly promoted the growth of radish, whereas the marine PNSB, *Rhodovulum* sp. OKHT16, displayed no promotive effects on plant growth.

Keywords: Plant Growth Promotion; Photosynthetic Bacteria; Purple Non-sulfur Bacteria

Introduction

Chemical nitrogen and phosphate fertilizers are used worldwide to increase the yield of crops. Chemical fertilizers have been successful in improving crop production, but these compounds induce some environmental problems, such as eutrophication of water, and contribute to many human and animal health problems. Scientists have been trying to eradicate the negative effects of the enormous use of chemical fertilizers by substituting them with biofertilizers without negative effects. Purple non-sulfur photosynthetic bacteria (PNSB) are one of the best candidates for biofertilizer.

PNSB are anoxygenic photosynthetic bacteria that live in various environments, such as soil, rivers, ponds, and seas. The plant growth-promoting effects of PNSB are well investigated. PNSB promote plant growth by fixing nitrogen, degrading toxic hydrogen sulfide, and producing several chemicals, including plant hormones, 5-aminolevulinic acid (ALA), siderophores, riboflavin, extracellular polymeric substances, and bacterial acyl-homoserine lactone [1]. Among the plant growth-promoting chemicals, ALA is well-known as a precursor of tetrapyrroles such as vitamin B12, heme, and chlorophyll.

All previous studies on plant growth-promoting effects of PNSB have been performed using freshwater (terrestrial) PNSB strains, such as those from the genera *Rhodobacter* [2,3] and *Rhodopseudomonas* [4,5], and there is no information on whether marine PNSB strains have such effects on plants. In this study, we compared the plant growth-promoting effects of a freshwater PNSB strain, *Rhodobacter sphaeroides*, and a marine strain, *Rhodovulum* sp. OKHT16 [6].

Materials and Methods

Bacterial strains and culture condition

The most widely used commercial PNSB *R. sphaeroides* (Rbs) strain in Japan, with a 16S rRNA sequence that shows 100% similarity to *R. sphaeroides* ATCC 17025 (GenBank accession number CP000661; data not shown), was utilized. The marine PNSB strain *Rhodovulum* sp. OKHT16 (Rvs), isolated from seawater in Osaka Bay, Japan [6], was also used. Rbs and Rvs were cultured in gluta-

mate–malate (GM) medium [7] and GM medium with 30 g/L NaCl, respectively.

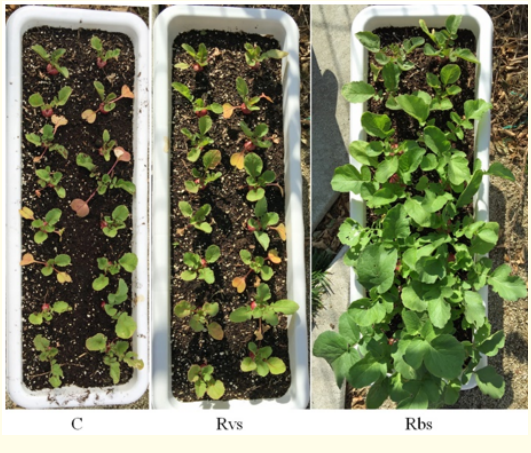
Pot experiments with radish (*Raphanus sativus var. sativus*)

Radish seeds were purchased from Atariya Noen Co., Ltd, Chiba, Japan. The seeds were sown in commercial soil (Protoleaf, Hana Yasai-yo karui baiyodo) in planters (64 cm × 23 cm × 18.5 cm, Iris Ohyama, 650E) and kept outdoors.

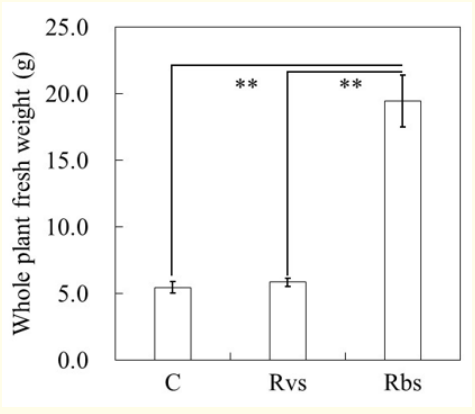
For foliar spray, PNSB strains Rbs and Rvs were suspended in tap water at a concentration of 1×10^9 cells/mL. From the seventh day after the start of cultivation, the PNSB suspension was sprayed onto the plants (100 μL per plant) once a day. The same amount of tap water was sprayed onto the control plants. About 30 days after the start of cultivation, plants were harvested and whole plant fresh weights were individually measured.

Results and Discussion

The effects of freshwater and marine PNSB on plant growth were compared by outdoor 30-day pot experiments using radish plants. For 24 days (7^{th}–30^{th} day), the plants were treated with either Rbs or Rvs by foliar spray. Figure 1a shows the growth of the radish plants 30 days after the start of cultivation. Rbs exhibited a clear growth-promoting effect, whereas Rvs displayed no effect. The average fresh weights of the radish plants cultivated under each condition are shown in figure 1b. The Rbs-treated plants exhibited approximately 4 times higher fresh weight values compared to those of the control ($P < 0.01$), whereas no difference was observed between Rvs-treated and control plants.



(A)



(B)

Figure 1: (a) Radish plants before harvesting (after 30-day cultivation), (b) average fresh weight of radish plants under different treatments. Error bars show standard errors (n = 18). C: control, Rvs: Rhodovulum sp. OKHT16 (marine PNSB), Rbs: Rhodobacter sphaeroides (freshwater PNSB). **: $P < 0.01$ in Student's t-test.

The present study clearly indicates that the plant growth-promoting ability is limited to freshwater PNSB, while it is absent from marine PNSB. There seem to be two possible explanations for these findings. First, freshwater PNSB support plant growth by degrading toxic chemicals in the soil such as hydrogen sulfide, by fixing nitrogen, or both. However, these bioactivities may be suppressed in marine PNSB under freshwater conditions because marine bacterial cells are adapted to seawater. Second, with respect to the co-evolution of plants and rhizobacteria, freshwater PNSB and plants may have developed a symbiotic (mutualistic) relationship in which PNSB support plant growth and, in return, PNSB receive benefits from plants, such as carbohydrates released from plant roots. This relationship might have led freshwater PNSB to produce several plant growth-promoting chemicals, such as plant growth hormones and ALA, whereas marine PNSB may have not developed any symbiotic relationship with plants; therefore, they do not produce these chemicals. To elucidate this point, the content of plant growth-promoting chemicals, such as indole acetic acid (IAA) and ALA, in freshwater and marine PNSB are being investigated in our laboratory.

Conclusion

The plant growth-promoting effects of PNSB have been intensively investigated, but previous studies are limited to freshwater

(terrestrial) PNSB strains. In the present study, we compared, for the first time, the effects of freshwater and marine PNSB on plant growth and clearly indicated that the plant growth-promoting ability is limited to freshwater PNSB. This finding seems to be reasonable with respect to both the physiological and evolutionary characteristics of marine PNSB.

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