



NOTE

## Plant growth-promoting bacterium *Acinetobacter calcoaceticus* P23 increases the chlorophyll content of the monocot *Lemna minor* (duckweed) and the dicot *Lactuca sativa* (lettuce)

Wakako Suzuki,<sup>1</sup> Masayuki Sugawara,<sup>1,3</sup> Kyoko Miwa,<sup>2,3</sup> and Masaaki Morikawa<sup>1,3,\*</sup>

Graduate School of Environmental Science, Hokkaido University, N10-W5, Kita-ku, Sapporo 060-0810, Japan,<sup>1</sup> Creative Research Institution, N21-W10, Kita-ku, Sapporo 001-0021, Japan,<sup>2</sup> and Advanced Low Carbon Technology Research and Development Program (ALCA), Department of Green Innovation, Japan Science and Technology Agency, K's Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan<sup>3</sup>

Received 25 September 2013; accepted 6 December 2013

Available online 24 January 2014

***Acinetobacter calcoaceticus* P23 is a plant growth-promoting bacterium that was isolated from the surface of duckweed (*Lemna aoukikusa*). The bacterium was observed to colonize on the plant surfaces and increase the chlorophyll content of not only the monocotyledon *Lemna minor* but also the dicotyledon *Lactuca sativa* in a hydroponic culture. This effect on the *Lactuca sativa* was significant in nutrient-poor ( $\times 1/100$  dilution of H2 medium) and not nutrient-rich ( $\times 1$  or  $\times 1/10$  dilutions of H2 medium) conditions. Strain P23 has the potential to play a part in the future development of fertilizers and energy-saving hydroponic agricultural technologies.**

© 2013, The Society for Biotechnology, Japan. All rights reserved.

[**Key words:** Plant growth-promoting bacteria; *Acinetobacter calcoaceticus* P23; *Lemna minor*; *Lactuca sativa*; Chlorophyll content]

Food shortage is becoming a critical global issue for humans, and plants are essential primary producers in the biospheric food chain. Because biological elements such as nitrogen and phosphorus are only available in limited amounts on earth, it is clear that the reduction, reuse, and recycling of fertilizers is an important technological trend for saving energy, which is key to sustainable agriculture (1). Future agricultural production may also require vegetables to be grown in barren areas such as deserts and the polar regions, and even in extraterrestrial space. Hydroponic farming of plants and vegetables could be one of the best technologies for achieving this aim (2). Indoor hydroponic farming technology has enabled the cultivation of plants anywhere and at any time. However, hydroponic cultivation has disadvantages, including high initial set-up costs as well as the high costs of the regular energy inputs required under controlled culture conditions. These costs limit the practical application of the hydroponic farming technology to advanced countries such as Japan. To ensure a sustainable food supply, reductions in the use of fertilizers, in the total energy required for food production, and in the costs associated with hydroponic farming, are required.

Replacing chemical fertilizers with biofertilizers that mimic the natural ecosystem is expected to reduce the total energy input. Biofertilizers mainly consists of beneficial plant-associated microorganisms such as plant growth-promoting bacteria (PGPB) (3). The application of beneficial microbes was initiated in agricultural

practice more than 60 years ago, and there is increasing evidence that these beneficial microbial populations can also enhance plant resistance to adverse environmental stresses such as water and nutrient deficiency, heavy metal contamination, and plant pathogens (4–6).

*Acinetobacter calcoaceticus* P23 was isolated from a duckweed species (*Lemna aoukikusa*) in the Hokkaido University Botanical Gardens (7). The bacterium is a PGPB associated with the Lemnaceae family, which includes species such as *Lemna aoukikusa*, *Lemna minor*, *Spirodela polyrrhiza*, and *Wolffia arrhiza* (Dr. T. Toyama, personal communication). It has previously been shown to degrade hydrocarbons including aromatic phenols and aliphatic alkanes, and to promote the growth of *Lemna aoukikusa* (leading to a 2-fold increase in growth rate) (7). It is presently unclear whether the growth-promoting effects of strain P23 are limited to *Lemna aoukikusa*. Therefore, we determined whether strain P23 has a growth-promoting effect on plants other than duckweed, such as vegetables in a hydroponic culture system.

Strain P23 was grown in L broth (5 g/L Bacto Yeast extract, 5 g/L NaCl, and 10 g/L Bacto Tryptone; pH 7.2; BD Difco, NJ, USA) overnight at 30°C. *Escherichia coli* DH5 $\alpha$  was grown in L broth at 37°C and used as a reference for comparison. *Lemna minor* (sourced from Hokkaido University Botanical Gardens) and vegetable seeds were surface-sterilized in 0.5% hypochloric acid and 0.02% Triton X-100 for 3–5 min, then washed five times with sterilized water. While majority of *Lemna minor* were killed after this treatment, one frond of bacteria-free *Lemna minor* survived and grew normally. Hoagland medium 1 (H1) (pH 7.0) was used for *Lemna minor* culture. The medium contains 36.1 mg/L KNO<sub>3</sub>, 293 mg/L K<sub>2</sub>SO<sub>4</sub>, 147 mg/L CaCl<sub>2</sub>·2H<sub>2</sub>O, 103 mg/L MgSO<sub>4</sub>·7H<sub>2</sub>O,

\* Corresponding author at: Graduate School of Environmental Science, Hokkaido University, N10-W5, Kita-ku, Sapporo 060-0810 Japan. Tel./fax: +81 11 706 2253.

E-mail address: [morikawa@ees.hokudai.ac.jp](mailto:morikawa@ees.hokudai.ac.jp) (M. Morikawa).

5.03 mg/L  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 3.33 mg/L  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.95 mg/L  $\text{H}_3\text{BO}_3$ , 0.39 mg/L  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.08 mg/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.39 mg/L  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.03 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and 0.23 mg/L  $\text{H}_2\text{MoO}_4$ . The culture conditions for the plants were as follows: 25°C, 70% humidity, 5000 lux, and 16-h light/8-h dark cycle, unless indicated otherwise. Bacterial cells were washed twice by centrifugation and suspended with the appropriate medium at a concentration of  $\text{OD}_{600} = 0.3$ . This bacterial suspension was used for inoculating the plants. In order to minimize nutrient leakage from dead bacterial cells, we transferred the hydroponic plants in their bacterial suspension medium to a fresh bacteria-free medium at the start of the experiments. This enabled us to evaluate the effects of only the bacteria that attached to the plants. Plant growth was measured by the increase in wet and dry (48 h after drying at 60°C) weight. Chlorophylls were extracted by 1 mL of cold ethanol saturated with  $\text{Ca}(\text{CO}_3)_2$  using 0.5 g glass beads ( $\phi$  0.1 mm) and a multibead shocker [MB755U(S), Yasui Kikai, Japan] at 2500 rpm for 60 s (4°C). Chlorophylls present in the centrifuged clear extracts were quantified by measuring photometric absorption at 649 nm and 665 nm (8). The chlorophyll content was determined by mg chlorophylls/100 g wet weight of frond or leaf specimens.

We first compared the growth and chlorophyll content of *Lemna minor* with and without bacteria. Ten fronds of sterilized *Lemna minor* were incubated for 72 h in 50 mL H1 medium with and without strain P23. Further, two fronds with colonized bacteria were rinsed in sterilized water and transferred to a 100-mL flask containing 50 mL of fresh H1 medium and cultured for a further 10 days. Three sets of the flask cultures were prepared for each. The growth of *Lemna minor* plants inoculated with strain P23 (Lemna/P23, 60.5 mg wet weight [SD = 5.75 mg], 3.34 mg dry weight [SD = 0.182 mg], 164 fronds) was 2-fold that of sterilized *Lemna minor* plants only (31.3 mg wet weight [SD = 15.4 mg], 1.69 mg dry

weight [SD = 0.848 mg], 102 fronds) (Fig. 1A–C), indicating that the strain P23 has apparently the growth-promoting effect on *Lemna minor*. We noticed that the green color of the frond of Lemna/P23 was darker than that of sterilized *Lemna minor* plants. When we compared their chlorophyll content (chlorophylls/wet weight), we confirmed that it was higher in the Lemna/P23 plant (103.1 mg/100 g) than in the sterilized plants (62.1 mg/100 g) (Fig. 1A, D). These findings suggest that an increase in photosynthetic activity associated with the increase in chlorophyll content is one of the reasons for the growth-promoting effect of strain P23 in *Lemna minor*. It may be of importance to note that the ratio of chlorophyll *a* to chlorophyll *b* was not significantly different between the uncolonized *Lemna* ( $a/b = 2.36$ ) and the colonized Lemna/P23 ( $a/b = 2.23$ ) plants.

Further, we examined the effects of strain P23 on several vegetable plants in a hydroponic culture. *Escherichia coli* DH5 $\alpha$  was also used as a reference for comparison. The vegetables evaluated in this study were *Brassica rapa* var. *chinensis* (pak choi; Sakata Seed, Japan), *Raphanus sativus* L. cv. *Raphanus sativus* (radish; Nihon Nousan Shubyoku, Japan), and *Lactuca sativa* L. cv. Great Lakes (lettuce; Atariya Noen, Japan). Sterilized seeds were germinated on solid Hoagland medium 2 (H2) (pH 6.0) containing 606.6 mg/L  $\text{KNO}_3$ , 944.6 mg/L  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 246.5 mg/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 230.1 mg/L  $\text{NH}_4\text{H}_2\text{PO}_4$ , 9 mg/L Fe-EDTA, 3.7 mg/L KCl, 1.54 mg/L  $\text{H}_3\text{BO}_3$ , 0.57 mg/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 mg/L  $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.12 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.08 mg/L  $\text{H}_2\text{MoO}_4$ , and 1.5% agar. Ten young seedlings with three or four leaves were then transferred to hydroponic culture boxes (15 cm  $\times$  15 cm  $\times$  10 cm; Hipack S-38, ENTEC, Japan) containing 1.5 L H2 medium at different dilutions ( $\times 1$  H2,  $\times 1/10$  H2,  $\times 1/100$  H2) with and without bacteria. After incubating the young seedlings of the vegetable plants with and without strains P23 and DH5 $\alpha$  for one week to allow colonization, ten seedlings were selected from each of the following conditions: seedlings only,

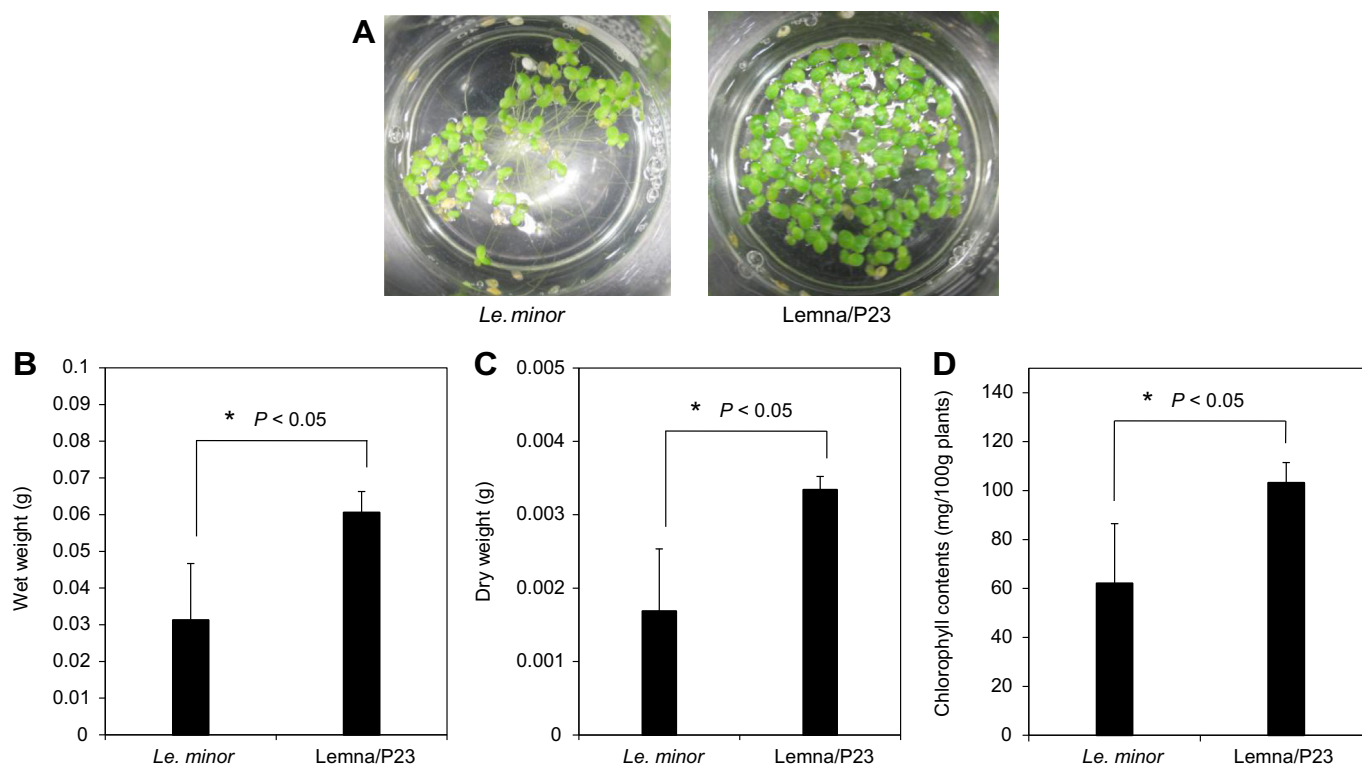


FIG. 1. Effects of PGPB *A. calcoaceticus* P23 on the growth and chlorophyll content of *Lemna minor*. (A) Bird's-eye view of the culture flasks on the seventh day after transferring the plants to bacteria-free fresh H1. (B) Wet weight of all plants present in a flask for each combination. (C) Dry weight of all plants in a flask. (D) Chlorophyll content of four randomly chosen fronds. Values are the average of three independent experiments and error bars indicate standard deviation (SD). Statistical significance was analyzed by Student's *t*-test, and the results were shown above the bars.

Download English Version:

<https://daneshyari.com/en/article/20532>

Download Persian Version:

<https://daneshyari.com/article/20532>

[Daneshyari.com](https://daneshyari.com)