Interactions between Indole-3-acetic Acid Producing
Acinetobacter sp. SW5 and Growth of Tomato Plant

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Many rhizobacteria can promote plant growth through various direct or indirect mechanisms, and their production of phytohormones such as indole-3-acetic acid (IAA) may have pronounced effects on growth and development of plants. Rhizobacterial strain isolated from rhizosphere of foxtail (Setaria viridis), Acinetobacter sp. SW5 produced 118.1 mg/L of IAA and 4.5 mg/L of gibberellin (GA3) in brain heart broth medium at 2 and 1 day of incubation, respectively. In a pot test the lengths of stem and root and fresh weight of the germinated tomato seedlings treated with Acinetobacter sp. SW5 significantly increased by 26.3, 33.3, and 105.3%, respectively compared to those of the uninoculated control in 12 weeks of cultivation. When the root exudate secreted from tomato seedlings was analyzed by HPLC, 3.75 ng mg tomato root⁻¹ of tryptophan which is an IAA precursor was detected. Acinetobacter sp. SW5 could produce 4.06 µM of IAA from root exudate from 8 tomato seedlings. Together with the capability of growth of Acinetobacter sp. SW5 in the tomato root exudates, this IAA secreted by bacteria might contribute to enhance the growth of tomato plants.

Keywords: Acinetobacter sp. SW5, indole acetic acid, plant growth promotion, tomato root exudates, tryptophan

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It has been well known that some rhizobacteria could enhance plant growth through various direct or indirect mechanisms, such as production of several phytohormones, nitrogen fixation, solubilization of insoluble phosphate, production of antipathogenic compounds, and they are called as plant growth promoting rhizobacteria (PGPR). The plant growth promotion by PGPR does not occur by any one of these mechanisms alone, and multiple mechanisms must be involved in it (Vessey, 2003; Babalola, 2010). In soils, some plant factors such as species and age also influence upon microbial community, and plant root exudates may strongly affect the biological processes in the rhizosphere including plant growth promotion by the inoculated PGPR. Furthermore, the introduction of PGPR into plant rhizosphere may affect the indigenous soil bacterial community and the plant factors can make this effect more complicated (Castro-Sowinski et al., 2007).

Nevertheless, the phytohormone production may be one of the important direct mechanisms of growth promotion and development of plants by PGPR. Although plants, of course, can synthesize phytohormones, they can also uptake and utilize exogenous sources such as microbially produced phytohormones (Lambrecht et al., 2000). Auxin which is a typical phytohormone and includes indole-3-acetic acid (IAA), indole-3-butyric acid, and other indolic compounds, can be secreted by many PGPR. The effects and action mechanisms of IAA in plants and biosynthetic pathways of IAA in plants and bacteria have been well known (Patten and Glick, 2002; Leveau and Lindow, 2005; Spaepen and Vanderleyden, 2011). However, in contrast to endogenous IAA produced in plants, production of IAA by PGPR in plant rhizosphere, action mechanisms and signal transduction pathway of exogenous IAA have not been thoroughly investigated. It have been reported that the high concentration of IAA may be deleterious to plant growth and...
stimulate lateral and adventitious roots (Leveau and Lindow, 2005; Spaepen and Vanderleyden, 2011), however, the interactions and effects between IAA-producing rhizobacteria and plants may be changed by some factors such as amount of IAA available, sensitivity of plant, interactions among phytohormones and other unknown factors (Patten and Glick, 2002; Spaepen et al., 2007). Recently it was reported that various compounds including tryptophan secreted in plant root exudates may affect the plant-microbe interactions in the rhizosphere (Kamilova et al., 2006; Kravchenko et al., 2011), and there have been many reports on IAA synthetic pathways and effects of secreted IAA from rhizobacteria on plant growth (Ahemad and Kibret, 2014). However, the rhizobacterial growth and IAA production in the secreted root exudates and subsequent its growth promotion of plant have not been reported yet. In this study, the growth promotion of tomato plant by a newly isolated rhizobacterial strain Acinetobacter sp. SW5 was examined and bacterial growth and IAA production from tomato root exudates was investigated.

Materials and Methods

Isolation and identification of rhizobacteria

For the screening of efficient PGPR many bacterial strains were isolated from the rhizosphere soils of foxtail (Setaria viridis var. viridis) grown on the barren lakeside area at Lake Paro, S. Korea. Among the isolates strain SW5 which was aerobic Gram-negative nonmotile plump rod-shaped bacterium showed the highest production capability of IAA and it was identified as Acinetobacter sp. SW5 based on the similarity (97.875%) of its full 16S rRNA gene sequence analyzed by Macrogen Inc. (Korea) and compared with that of A. guillouiae ATCC 11171 T (GenBank accession No. X81659) using the BLAST analysis (Basic Local Alignment Search Tool; http://www.ncbi.nlm.nih.gov/BLAST/).

Plant growth promoting capability of Acinetobacter sp. SW5

Production of phytohormones by Acinetobacter sp. SW5, such as IAA, gibberellin (gibberellic acid; GA3), and abscisic acid (ABA) were determined by high performance liquid chromatography (HPLC). After incubation of bacteria in 100 ml brain heart broth (BHB) medium at 28°C, 150 rpm for 3 days, bacterial cells were removed from the culture medium by centrifugation (3,000 × g, 30 min). The pH of culture supernatant was adjusted to 2.5 and 15 ml of ethyl acetate was added. Extraction of phytohormones from the mixture on a vertical extraction shaker at 300 stroke/min for 10 min was repeated 3 times, and total 45 ml of ethyl acetate was recovered. Ethyl acetate was dried in a rotary vacuum evaporator, dissolved in 5 ml methanol and filtered through 0.45 µm pore membrane filter. Extracted phytohormones were analyzed by HPLC (Model Breeze, Waters, USA). Analytical conditions were as follows; Luna 5µ C18(2) LC column 250 × 4.6 mm (Phenomenex, USA), flow rates of 1 ml/min of 35% methanol (in 1% acetic acid) for IAA, 30% methanol (0.1 M H2PO4) for GA3 and 55% methanol (0.1 M acetic acid) for ABA, wavelengths of UV detector 280, 208, and 265 nm for IAA, GA3, and ABA, respectively (Karadeniz et al., 2006). The amount of bacterial protein was measured by Bradford assay (Bradford, 1976), and the production of phytohormone was also expressed based on bacterial protein.

Pot test for growth promotion of tomato plant by Acinetobacter sp. SW5

For the pot test to examine the plant growth promotion by Acinetobacter sp. SW5, soil was collected from a lakeside area of Lake Paro, S. Korea (~15 cm depth), and sieved through a mesh with 2 mm diameter to remove large organic and inorganic materials. The soil texture was loamy sand (sand 87.5%, silt 7.5%, clay 5.0%), soil pH was 6.70, and organic content measured by the loss-on-ignition method (Ball, 1964) was 2.1%. Sieved soil was mixed with the compost being marketed in S. Korea at a ratio of 7:3 and 200 g of mixed soil was poured into the small plastic containers [7 (D)×10 (H) cm]. The seedlings of tomato (Lycopersicon esculentum cv. Yegwang) which was germinated for 3 days in a Petri dish were planted (3 seedlings/pot × 16 pots) and 10 g of mixed soil was covered. The bacterial cells cultured in BHB were harvested by centrifugation (3,000 × g, 30 min), washed twice with sterile distilled water, enumerated on a hemocytometer, and 107 cells/g soil of bacteria suspended in 20 ml of distilled water were sprayed into the inoculated pots every other week. The inoculated pots and uninoculated control pots (8 each) were placed in a plant growth chamber (28°C, 6,000 lux for 14 h and 23°C, dark conditions for 10 h) and 20 ml of distilled water was sprayed everyday. After 12 weeks of time period, all the plants grown were cautiously harvested without the root loss, and washed with distilled water to remove the remaining soil particles. The lengths of the root and shoot and dry weight of each tomato plant were measured.

Interactions between tomato root exudate and Acinetobacter sp. SW5

For measurement of tryptophan in tomato root exudates, 8 tomato seedlings germinated for 3 days were cultivated on the distilled water-soaked filter papers (Whatman No. 2) in a sterile Petri dish placed in a plant growth chamber (28°C, 6,000 lux...
for 14 h and 23°C, dark conditions for 10 h) for 3 days. Tomato root exudates were extracted with 25 ml distilled water from the filter paper, concentrated at 45°C in a rotary vacuum evaporator (final volume 0.5 ml), and tryptophan was analyzed by HPLC (Kravchenko et al., 2011).

The root exudate secreted from 8 tomato seedlings was extracted with 40 ml distilled water as described above, and Acinetobacter sp. SW5 (10⁷ cells) was inoculated into it and incubated (30°C, 150 rpm, 24 h). Bacterial productions of IAA from tomato root exudates and tryptophan as a control were determined as described by Karadeniz et al. (2006). The growth of Acinetobacter sp. SW5 in the tomato root exudate extracted with 20 ml distilled water was also examined after inoculation of bacterial suspension in 2 ml sterile distilled water (OD₆₀₀: 1) and 4 days of incubation (30°C, 150 rpm).

All experiments were performed at least in triplicate. Statistical analysis was performed on all experimental data by a simple t-test, and means were compared using the SYSTAT software (Ver. 10, SPSS Inc.). The significance level was P<0.05.

Results and Discussion

Plant growth promoting capability of Acinetobacter sp. SW5

Acinetobacter spp. are widely distributed in soil and water, and some of them have been reported to enhance the growth of many plants (Indiragandhi et al., 2008; Gulati et al., 2009; Shi et al., 2011). In this study Acinetobacter sp. SW5 showed the highest production of IAA among many isolates, 1.37 ± 0.07 mg/mg protein at 2 days of incubation in BHB medium without the addition of IAA precursor tryptophan, and its concentration in the culture was 118.1 µg/L. This is much higher than 15 µg/ml of IAA at 2 days incubation by A. rhizosphaerae BIHB723 in 0.1% tryptophan added nutrient broth (Gulati et al., 2009), and 7.9-8.2 µg/ml of IAA by other strains of Acinetobacter spp. (Indiragandhi et al., 2008). Acinetobacter sp. SW5 also produced 4.5 mg/L of GA₃ (71.1 ± 0.9 µg/mg protein) after 24 h of incubation, which was also higher than that by another PGPR A. calcoaceticus SE370 in nutrient broth (Kang et al., 2009). However, ABA was not detected in the culture of Acinetobacter sp. SW5 after 2 days of incubation.

In a pot test to examine the plant growth promotion, Acinetobacter sp. SW5 significantly increased the lengths of shoot and root and fresh weight of tomato plants (n: 21) grown for 12 weeks by 26.3, 33.3, and 105.3%, respectively compared to those of the uninoculated control (Fig. 1). It has been reported in several studies that IAA production plays a critical role in the plant growth promotion by Acinetobacter spp. (Huddedar et al., 2002; Chaiharn et al., 2008; Rokhbakhsh-Zamin et al., 2011). Although many species of Acinetobacter are reported as PGPR (Indiragandhi et al., 2008; Gulati et al., 2009; Kang et al., 2009; Shi et al., 2011), this SW5 strain showed a high plant growth promoting capability. SW5 strain has a 97.875% of sequence homology of 16S rRNA gene compared with the type strain A. guillouiae ATCC 11171ᵀ reported recently by Nemec et al. (2010), and some general characteristics of SW5 strain were coincided with those of other Acinetobacter spp. (Baumann et al., 1968). Further analysis is necessary to classify this strain to species level. Other plant growth promoting mechanisms such as solubilization of insoluble phosphates, nitrogen fixation, production of other phytohormones and siderophore should be examined for the precise investigation of plant growth mechanisms in this SW5 strain.

Interactions between tomato root exudate and Acinetobacter sp. SW5

Because the plant growth promotion and remaining in rhizosphere of Acinetobacter sp. SW5 were confirmed in the pot test, the involvement of IAA between this bacterium and tomato root was subsequently examined. When the tomato root exudates extracted from 3-day grown tomato seedlings was analyzed, the main IAA precursor, tryptophan was detected at 3.75 ± 0.95 ng/mg tomato root (Fig. 2). This concentration was much higher than 1.6 ± 0.3, and 0.87 ± 0.1 ng/mg tomato extracted from the root of L. esculentum cv. Karmello and L.

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Fig. 1. Stem length (A), root length (B), and fresh weight (C) of 12-week-grown tomato plants inoculated with 1×10⁷ cells/g soil of Acinetobacter sp. SW5. The asterisk (*) means statistically significant difference from control (P<0.05).
esculentum cv. Aromato, respectively (Kravchenko et al., 2004), but similar to 3.69 ± 0.48 ng/mg tomato extracted from *L. esculentum* cv. Carmello (Kamilova et al., 2006). Although the cultivar of tomato used in this study, Yegwang was different from those in other studies, the tryptophan concentration in the tomato root exudates seems to be quite high. IAA was not detected from the tomato root exudates (data not shown) and similar studies also showed the production of various organic acids sugars and other metabolites but IAA (Kamilova et al., 2006; Kravchenko et al., 2011; Ahemad and Kilbret, 2014).

When *Acinetobacter* sp. SW5 was inoculated into the root exudate extracted from 8 tomato seedlings grown for 3 days, 4.06 ± 0.13 μM (0.71 ± 0.02 μg/ml) of IAA was produced after 24 h of incubation, which might be formed mainly from IAA precursor tryptophan present in the root exudate. IAA was also produced by same bacteria from synthetic tryptophan added into distilled water, but its concentration was much lower than
that from the tomato root exudates even though the concentration of tryptophan added (1 μM) was more than 2 times higher than that in the tomato root exudates. This result suggested that Acinetobacter sp. SW5 could produce IAA using other precursors present in the tomato exudates. Besides IAA, Acinetobacter sp. SW5 produced some other metabolites from root exudates and tryptophan (Fig. 3). One major peak of 8.3 min retention time was identified as indole-3-acetamide (IAM) in HPLC analysis with standard chemicals, which is one of the common metabolic intermediate of indole-3-acetamide pathway of IAA biosynthesis (Spaepen et al., 2007). There are several pathways for IAA biosynthesis, and each IAA producing microbe uses one or more different pathways. Among them IAM pathway is the best characterized pathway in bacteria, and many bacteria were reported to use this pathway for IAA synthesis (Spaepen et al., 2007). To date, there has been just few report on IAA synthesis pathway in Acinetobacter sp. (Bhawsar et al., 2012), in which A. haemolyticus A19 utilized tryptamine pathway and indole-3-acetonitrile (IAN) pathway together, however, IAM was not detected. In our previous study Acinetobacter sp. SW5 utilizes several pathways of IAA synthesis including IAM pathway and tryptophan independent pathway (Kim and Song, 2012), and also the activity of nitrilase which catalyzes IAN to IAA was detected although IAN was not detected in this study. Some other minor peaks suggest the involvement of other IAA biosynthesis pathways in this bacterium which should be investigated in a future study.

When Acinetobacter sp. SW5 was inoculated into the tomato root exudates, the growth of bacteria increased by 19% compared to that in the distilled water (Fig. 4). In addition to tryptophan, the tomato root excreted many compounds which might be utilized for the growth of Acinetobacter sp. SW5 (Fig. 2), and this kind of bacterial growth may occur in the real plant rhizosphere. Kamilova et al. (2006) and Kravchenko et al. (2011) also detected various organic acids, carbohydrates and amino acids from tomato root exudate, which were utilized for the growth of some rhizobacteria. Lambrecht et al. (2000) suggested that the plant growth may be promoted by IAA produced by PGPR which growth can be helped by organic compounds secreted by plants, and our study supports this model. There were many other compounds secreted from tomato root (Fig. 3), and some of them may be involved directly or indirectly in plant growth promotion.
Interactions between Acinetobacter sp. SW5 and tomato growth


