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Plant Growth-Promoting Trait of Rhizobacteria Isolated from Soil Contaminated with Petroleum and Heavy Metals

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Three hundred and seventy-four rhizobacteria were isolated from the rhizosphere soil (RS) or rhizoplane (RP) of *Echinochloa crus-galli*, *Carex leiorhyncha*, *Commelina communis*, *Persicaria lapathifolia*, *Carex kobomugi*, and *Equisetum arvense*, grown in contaminated soil with petroleum and heavy metals. The isolates were screened for plant growth-promoting trait (PGPT), including indole acetic acid (IAA) productivity, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and siderophore(s) synthesis ability. IAA production was detected in 86 isolates (23.0%), ACC deaminase activity in 168 isolates (44.9%), and siderophore(s) synthesis in 213 isolates (57.0%). Among the rhizobacteria showing PGPT, 162 isolates had multiple traits showing more than two types of PGPT. The PGPT-possesing rhizobacteria were more abundant in the RP (82%) samples than the RS (75%). There was a negative correlation (-0.656, $p<0.05$) between the IAA producers and the ACC deaminase producers. Clustering analysis by principal component analysis showed that RP was the most important factor influencing the ecological distribution and physiological characterization of PGPT-possesing rhizobacteria.

Keywords: Rhizobacteria, plant growth-promoting trait, indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophore(s)

With the increase of industrialization, environmental problems such as soil and/or groundwater contamination have grown as global issues [10]. Soil contamination is accelerated, in part, by atmospheric deposits, landfill discharge, and the use of sterilizers or fertilizers, and can induce secondary problems like groundwater and/or surface water contamination. Phytoremediation as an *in situ* biological method utilizing plants is environmentally sound and cost-effective [10, 19]. The remediation efficiency of phytoremediation is predominantly affected by various limiting factors that include environmental factors (pH, salinity, texture and fertility of soil, climate, precipitation) and pollutant toxicity (type and concentration of pollutants) [10, 15]. As a new strategy to overcome these disadvantages, rhizoremediation, where rhizobacteria are employed as assistants for phytoremediation, has been suggested [3, 4, 8, 11, 18].

Root exudates contain various nutrients, such as low molecular compounds, amino acid, glucose, organic acids, and vitamins [6]. Therefore, the rhizosphere plays an important role in constructing a proper habitat for soil microorganisms [1, 11]. Rhizobacteria also influence plant growth through soil and climatic conditions [2, 17, 22, 28]. Some rhizobacteria, such as plant growth-promoting rhizobacteria (PGPR), have synergistic relationships with plants, and as such influence directly or indirectly plant growth. Examples of this synergism include antibiotic production for plant protection from pathogens; N_2 fixation to supply the plant with nitrogen [20]; accumulation of Fe in siderophore(s) from Fe-insufficient soil and dissemination to the plant [12, 24]; and synthesis of phytohormones such as auxin, cytokinin, and indole acetic acid (IAA) [1, 30]. Some plant growth-promoting rhizobacteria also produce a plant growth-regulating enzyme [1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene) deaminase] that can inhibit the synthesis of ethylene that is often related with the plant aging process [26, 29]. In addition, some PGPR can generate soluble minerals such as phosphorus for ready absorption by the plant [14, 21].

Most of previous researches on PGPR in contaminated soils had been focused on their assistant for host plants to overcome contaminated-induced stress responses, thus providing improved remediation efficiency in contaminated soils [16, 27]. The ecological and physiological characteristics

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of PGPR in contaminated soils are directly or indirectly affected by abiotic parameters (soil texture, contaminants, temperature, pH, salinity, and so on) as well as biotic parameters (plants and soil microorganisms). Therefore, the information about the effects of biotic and abiotic parameters on PGPR performance is available to design the remediation strategy.

In this study, to compare the plant growth-promoting trait (PGPT) of rhizobacteria in contaminated soil, rhizobacteria were isolated from rhizoplane and rhizosphere soils associated with six species of plants in long-term petroleum and heavy-metal-contaminated soils. The IAA productivity, ACC deaminase activity, and siderophore(s) synthesis ability of the isolated rhizobacteria were evaluated, and then major factors influencing the ecological distribution and physiological characterization of PGPT-possesing rhizobacteria are discussed.

Materials and Methods

Sampling of Plants with Rhizosphere Soils

Six species of plants (*Echinochloa crus-galli*, K1; *Carex leiorhyncha*, K2; *Commelina communis*, H1; *Persicaria lapathifolia*, H2; *Carex kobomugi*, S1; *Equisetum arvense*, S2), grown in long-term petroleum-contaminated soil from a petroleum refinery facility located in Ulsan, South Korea, were carefully sampled. After removing the soil loosely adhering to the root by shaking the plant, the soil adhering firmly to the root of each plant was collected through brushing (rhizosphere soil sample). After brushing, the soil samples were dried under room temperature for 1 day. The root of each plant was washed with distilled water (DW) several times to remove the soil and the washed root collected by cutting and grinding manually with a ceramic mortar (Samwha Ceramic Co., Seoul, Korea) for 10 min (rhizoplane sample).

Colony Library of Rhizobacteria

One-gram fresh weight of the rhizosphere soil (RS) or ground rhizoplane (RP) was added to a 100-ml flask with 9.0 ml of sterilized DW. The flask was then shaken at 250 rpm for 30 min. The resulting suspension was decimally diluted (10^{-2}–10^{-6}) with sterilized DW and the diluted media were spread on LB-agar medium (Difco) and the plates incubated at 30°C for 3 days. After cultivation, representative colonies were selected based upon morphology and color properties, and transferred to new LB-agar media.

Evaluation of IAA productivity, ACC Deaminase Activity, and Siderophore(s) Synthesis Ability

All tests for IAA productivity, ACC deaminase activity, and siderophore(s) synthesis ability of the isolated rhizobacteria were carried out in triplicate.

To test IAA productivity, each isolate was inoculated in 5.0 ml of modified DF medium amended with 0.5 mg/ml of L-tryptophan [9], and incubated on a rotary shaker (180 rpm) at 30°C for 5 days. The composition of the medium was as follows: $(NH_4)_2SO_4$, 2.0 g; KH_2PO_4, 4.0 g; $Na_2HPO_4{\cdot}12H_2O$, 15.0 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; $FeSO_4{\cdot}7H_2O$, 1.0 mg; B (as H_3BO_3), 10.0 μg; Mn (as $MnSO_4{\cdot}H_2O$), 11.0 μg; Zn (as $ZnSO_4{\cdot}7H_2O$), 125.0 μg; Cu (as $CuSO_4{\cdot}5H_2O$), 78.0 μg; Mo (as $Na_2MoO_4{\cdot}2H_2O$), 17.0 μg; distilled water, 1.0 l. The resulting culture broth was mixed with Salkowski's reagent (150.0 ml concentrated H_2SO_4, 250.0 ml distilled water, 7.5 ml 0.5 M $FeCl_3{\cdot}6H_2O$) at a ratio of 1:2 (v/v), and allowed to stand at room temperature for 20 min. The developed pink color that indicates production of IAA was measured at 530 nm with a spectrophotometer (8453 UV–Visible Spectrophotometer; Agilent Technologies, U.S.A.). A two-sample t-test was carried out for comparison of the absorbance of each culture broth with a control (without inoculation) using SPSS 12.0K ($p<0.05$). The absorbance was converted into the concentration of IAA by a standard curve method with 3-indoleacetic acid (C_8H_6N-CH_2COOH; Showa Chemical Co. Ltd., Tokyo, Japan).

For testing ACC deaminase activity, the DF medium, including 3.0 mM of ACC instead of $(NH_4)_2SO_4$ as the sole nitrogen source, was prepared [7]. Each isolate was inoculated in the DF medium and incubated on a rotary shaker (180 rpm) at 30°C for 48 h. The optical density (OD) of the culture broth at 600 nm was measured every 4 h. A two-sample t-test was carried out for comparison of the OD at 48 h of each culture broth with a control (without inoculation) using SPSS 12.0K ($p<0.05$).

The ability of the isolates to produce siderophore(s) was determined using blue agar plates containing chrome azurol S (CAS) [25]. Each isolate was inoculated onto the plate and incubated at 30°C for 24 h. Orange halos around the isolate on the blue agar served as indicators of siderophore(s) excretion.

Soil Characterization

The properties of the rhizosphere soil, such as pH, water content, organic content, concentration of heavy metals, total petroleum hydrocarbon (TPH), and anions were determined. After mixing the soil samples and distilled water in a 1:9 ratio (w/v), the pH of the soil suspension was determined with a pH meter (420A; Orion Research Inc., U.S.A.). For measurement of water content, the soil samples were dried at 105~110°C for 4 h and the water content was determined from the weight difference. Dried soil samples, at the same temperature as described above, were treated at 650°C for 30 min and the organic content was determined from the weight difference.

Hydrochloric acid (HCl, 30%, 1.8 ml) and 0.6 ml of 60% HNO_3 were added to 0.5 g of air-dried soil and boiled on a hot plate until the HCl and HNO_3 solution evaporated. Then, 10.0 ml of distilled water was added and filtered using filter paper (No. 6; Whatman International Ltd., Springfield Mill, U.K.). The Cd, Cu, Pb, Ni, and Zn concentrations in the filtrate were measured using an atomic absorption spectrophotometer (AAS Vario 6; Analytik Jena AG, Jena, Germany).

Hexane (5.0 ml) was added to a 5.0-g air-dried soil sample, shaken for 30 min, and allowed to stand for 30 min. The TPH concentration in the hexane layer was measured using gas chromatography (5890 Series II; Hewlett Packard, Santa Clara, CA, U.S.A.).

Distilled water (18.0 ml) was added to a 2.0-g air-dried soil sample, shaken for 30 min, and filtered (0.2 μm pore size filter; Whatman International Ltd., Springfield Mill, U.K.). The concentrations of Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-} in the filtrate were determined using ion chromatography (Waters Co., Milford, MA, U.S.A.) with a Waters 432 conductivity detector and Waters IC-Pak anion column (4.6 ID×150 mm).

RESULTS AND DISCUSSION

Physicochemical Property of Rhizosphere Soil

The values for pH, moisture content, and organic matter content in the rhizosphere soil samples were 6.5~8.3, 18~31%, and 6~20%, respectively (Table 1). The TPH concentration of each soil sample was arranged in the order of H2>H1> S1>S2>K1>K2 (Table 1). Total concentrations of heavy metals in the K1 and H1 samples were relatively higher, whereas those in K2 and H2 were relatively lower.

Comparison of IAA Productivity, ACC Deaminase Activity, and Siderophore(s) Synthesis Ability

Forty-seven colonies were selected from the RS of K1 and 24 colonies from the RP of K1; 23 colonies from the RS of K2 and 31 colonies from the RP of K2; 37 colonies from the RS of H1 and 38 colonies from the RP of H1; 31 colonies from the RS of H2 and 31 colonies from the RP of H2; 50 colonies from the RS of S1 and 16 colonies from the RP of S1; and 28 colonies from the RS of S2 and 18 colonies from the RP of *Equisetum arvense* (S2RP). The total number of selected colonies was 374.

Table 2 shows the results of the PGPT tests for the isolates from the RS and RP of the six species of plants. Percentages of bacteria not having PGPT were relatively higher in the KI sample (36.2% and 37.5% for RS and RP, respectively) and lower in the S1 sample (0% and 18.8% for RS and RP, respectively). Fifty-four of the total 216 rhizobacteria isolates from the RS samples lacked PGPT (25%), and 29 of the total 156 rhizobacteria isolates from the RP samples did not display PGPT (18.4%), indicating that the ratio of PGPT-prossessing rhizobacteria was higher in the RP than RS. Similar results indicated that the ratio of PGPR associated with perennial *Graminaceae* was higher in the RP than RS [7].

Percentages of rhizobacteria displaying binary activities of IAA production and ACC deaminase were the highest in the RS of the K1 and S2 samples (10.6% and 10.7%, respectively) and in the RP of the S2 sample (11.1%). Rhizobacteria percentages having IAA production and siderophore(s) synthesis activites were the highest in the RS of the K2 sample (34.8%) and in the RP of the H2 sample (22.6%). Rhizobacteria percentages having ACC deaminase and siderophore(s) synthesis activities were the highest in the RS and RP of the S1 sample (82.0% and 31.3%, respectively). Based on total rhizobacteria from the six species of plants, the ratio of rhizobacteria having ACC deaminase and siderophore(s) synthesis activities was relatively higher (26.4% and 20.9% for the RS and RP samples, respectively), whereas the ratio of rhizobacteria possessing IAA production and ACC deaminase activities was relatively lower (5.6% and 2.5% for the RS and RP samples, respectively).

Ratios of rhizobacteria showing triple activities of IAA production, ACC deaminase, and siderophore(s) synthesis were the highest in the RS and RP of the H1 sample (13.5% and 10.5%, respectively). On the basis of total rhizobacteria, the ratios of rhizobacteria showing triple activities were 4.2% and 3.2% in the RS and RP samples, respectively.

Cattelan *et al.* [5] isolated rhizobacteria from soil associated with a leguminous plant and evaluated their PGPA:PGPR ratio to show activities of the siderophore(s) synthesis and IAA production to be 0.9% (1/166), and the PGPR ratio showing IAA production and ACC deaminase

Table 1. Physicochemical properties of the rhizosphere soil samples.

Items		Rhizosphere soil					
		K1	K2	H1	H2	S1	S2
pH		7.7	8.3	6.5	7.4	7.6	6.6
Moisture content (%)		31	31	29	23	18	18
Organic matter content (%)		19	6	20	11	10	8
TPH concentration (mg/kg dry soil)		161.5	88.0	437.8	1,378.7	340.5	268.4
Metal concentration (mg/kg dry soil)	Cu	85.6	12.3	95.9	10.9	65.0	43.9
	Cr	14.1	1.0	14.4	11.6	ND	ND
	Pb	81.3	31.7	87.0	20.2	84.8	46.7
	Ni	95.2	43.2	102.5	59.6	119.1	95.1
	Cd	1.4	1.2	0.7	0.4	1.4	2.4
	Zn	507.6	118.3	499.3	73.1	174.9	185.5
Anion content (mg/kg dry soil)	Cl^-	273	1219	157	157	10	10
	NO_3^-	85	84	78	76	5	4
	PO_4^{3-}	< 1	< 1	< 1	< 1	< 1	< 1
	SO_4^{2-}	597	230	118	107	4	< 3

ND, not determined.

K1, *Echinochloa crus-galli*; K2, *Carex leiorhyncha*; H1, *Commelina communis*; H2, *Persicaria lapathifolia*; S1, *Carex kobomugi*; S2, *Equisetum arvense*.

Table 2. Characterization of isolates from the rhizosphere soil (RS) and rhizoplane (RP) associated with plants.

Plant		No. of bacteria w/o PGPA	No. of bacteria with single activity			No. of bacteria with binary activities			No. of bacteria with triple activities
			IAA	ACC_d	Sid	IAA+ACC_d	IAA+Sid	ACC_d+Sid	IAA+ACC_d+Sid
K1 (%)	RS	17/47 (36.2)	4/47 (8.5)	10/47 (21.3)	4/47 (8.5)	5/47 (10.6)	2/47 (4.3)	5/47 (10.6)	0/47 (0.0)
	RP	9/24 (37.5)	1/24 (4.2)	3/24 (12.5)	5/24 (20.8)	1/24 (4.2)	2/24 (8.3)	3/24 (12.5)	0/24 (0.0)
K2 (%)	RS	6/23 (26.1)	0/23 (0.0)	3/23 (13.0)	4/23 (17.4)	1/23 (4.3)	8/23 (34.8)	1/23 (4.3)	0/23 (0.0)
	RP	11/31 (35.5)	2/31 (6.5)	7/31 (22.6)	1/31 (3.2)	0/31 (0.0)	6/31 (19.4)	3/31 (9.7)	1/31 (3.2)
H1 (%)	RS	13/37 (35.1)	1/37 (2.7)	6/37 (16.2)	4/37 (10.8)	1/37 (2.7)	4/37 (10.8)	3/37 (8.1)	5/37 (13.5)
	RP	3/38 (7.9)	1/38 (2.6)	1/38 (2.6)	16/38 (42.1)	1/38 (2.6)	4/38 (10.5)	8/38 (21.1)	4/38 (10.5)
H2 (%)	RS	9/31 (29.0)	1/31 (3.2)	1/31 (3.2)	10/31 (32.3)	1/31 (3.2)	3/31 (9.7)	4/31 (12.9)	2/31 (6.5)
	RP	1/31 (3.2)	0/31 (0.0)	1/31 (3.2)	11/31 (35.5)	0/31 (0.0)	7/31 (22.6)	11/31 (35.5)	0/31 (0.0)
S1 (%)	RS	0/50 (0.0)	0/50 (0.0)	5/50 (10.0)	2/50 (4.0)	1/50 (2.0)	0/50 (0.0)	41/50 (82.0)	1/50 (2.0)
	RP	3/16 (18.8)	0/16 (0.0)	0/16 (0.0)	5/16 (31.3)	0/16 (0.0)	3/16 (18.8)	5/16 (31.3)	0/16 (0.0)
S2 (%)	RS	9/28 (32.1)	4/28 (14.3)	7/28 (25.0)	1/28 (3.6)	3/28 (10.7)	0/28 (0.0)	3/28 (10.7)	1/28 (3.6)
	RP	2/18 (11.1)	0/18 (0.0)	4/18 (22.2)	4/18 (22.2)	2/18 (11.1)	3/18 (16.7)	3/18 (16.7)	0/18 (0.0)
Sum (%)	RS	54/216 (25.0)	10/216 (4.6)	32/216 (14.8)	25/216 (11.6)	12/216 (5.6)	17/216 (7.9)	57/216 (26.4)	9/216 (4.2)
	RP	29/158 (18.4)	4/158 (2.5)	16/158 (10.1)	42/158 (26.6)	4/158 (2.5)	25/158 (15.8)	33/158 (20.9)	5/158 (3.2)

IAA, indole acetic acid; ACC_d, 1-aminocyclopropane-1-carboxylic acid deaminase; Sid, siderophore(s); K1, *Echinochloa crus-galli*; K2, *Carex leiorhyncha*; H1, *Commelina communis*; H2, *Persicaria lapathifolia*; S1, *Carex kobomugi*; S2, *Equisetum arvense*.

activities to be 2.6% (3/166); there was no PGPR having triple activities. Among the rhizobacteria associated with perennial *Graminaceae*, the PGPR ratio having siderophore(s) synthesis and ACC deaminase activities was 13.1% (11/84), and the PGPR ratio having triple plant growth-promoting traits was 2% (2/84) [7].

Percentages of rhizobacteria having IAA production, ACC deaminase, and siderophore(s) synthesis from the six species of plant samples are shown in Fig. 1. Rhizobacteria ratios with IAA production activities were arranged in the order of K2 (33.3%)>S2 (28.3%)>H1 (28.0%)>H2 (22.6%) >K1 (21.1%)>S1 (7.6%). The ratio of IAA-producing PGPR among rhizobacteria associated with perennial *Graminaceae* was 32% (27/84) [7]. Among the 45 rhizobacteria associated with *Brassica campestris ssp. pekinensis*, 44% could produce IAA [23]. However, only four had IAA productivity among the 116 rhizobacteria isolated from soil associated with *Glycine* max (L.) Merr. [5].

The rhizobacteria ratios showing ACC deaminase activity were S1 (80.3%)>S2 (50%)>H1 (38.7%)>K1 (38.0%) >H2 (32.3%)>K2 (29.6%) (Fig. 1). In particular, 96% of the PGPT-possessing rhizobacteria isolated from the RS of the S1 sample had ACC deaminase activity, even though only 21.7% of the PGPT-possessing rhizobacteria from the RS of the K2 sample could display its activity. Among the rhizobacteria associated with a leguminous plant, PGPR possessing ACC deaminase activity was 6.0% (7/116) [5]. However, the ratio of PGPR with ACC deaminase activity was 5% (5/84) in the RS and RP samples of perennial *Graminaceae* [7].

The siderophores play an essential role in the growth of plants with their ability to supply iron. Comparing the

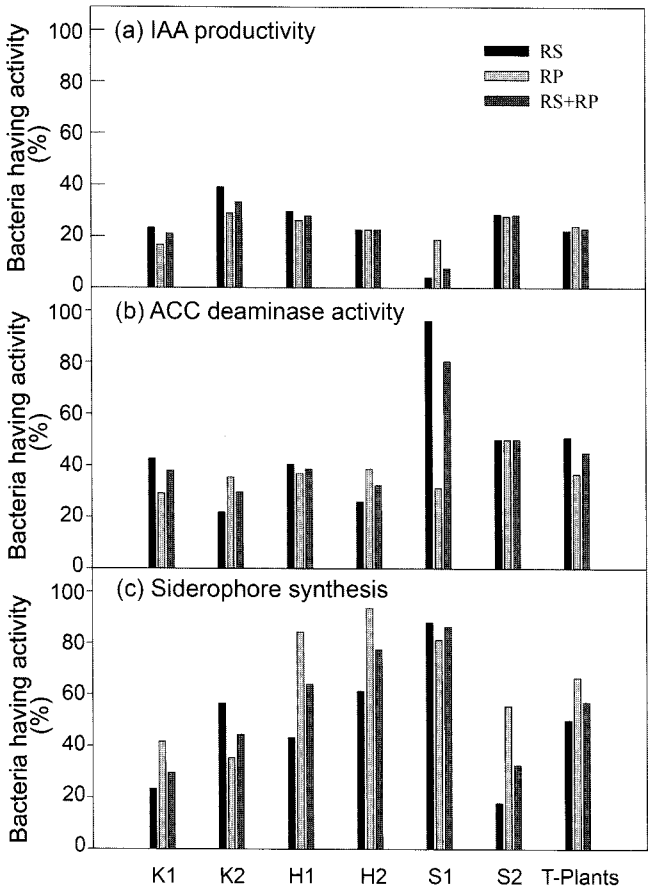

Fig. 1. Percentages of rhizobacteria having IAA productivity, ACC deaminase activity, or siderophore(s) synthesis ability in the rhizobacteria isolated from the RS and RP of six kinds of plants.
RS, rhizosphere soil; RP, rhizoplane.

rhizobacteria ratios showing IAA production or ACC deaminase activity, the ratios of siderophore(s)-synthesizing rhizobacteria were higher: S1 (86.4%)>H2 (77.4%)>H1 (64.0%)>K2 (44.4%)>S2 (32.6%)>K1 (29.6%) (Fig. 1). These ratios in the RS and RP of S1, the RS of H1, and the RS of the H2 samples were over 80%. Generally, soil bacteria were induced to synthesize siderophores in heavy-metal-contaminated soils because of Fe deficiency [11, 13]. Among the 374 rhizobacteria isolated in this study, 57% could synthesize siderophore(s). This result may be due to soil contamination with heavy metals, as well as TPH (Table 1). In uncontaminated soils, only 3.4 (4/116) or 13.3% (6/45) of the rhizobacteria could synthesize siderophore(s) [5, 23]. However, 23.8% (20/84) of rhizobacteria isolated from heavy-metal-contaminated soils could display siderophore(s) synthesis activity [7].

Correlation Analysis

The ecological and physiological properties of rhizobacteria in soils are directly or indirectly affected by abiotic parameters such as soil texture, contaminants, temperature, pH, salinity, and so on [16, 22, 27, 28]. When the relationship between the physicochemical properties of the soil samples (pH, moisture content, organic matter content, TPH/heavy metal/anion concentrations) and PGPT (IAA productivity, ACC deaminase activity, and siderophore(s) synthesis activity) was analyzed, no significant correlation existed (data not shown).

Table 3 shows the results of the correlation analysis among the PGPT-possessing rhizobacteria. There was a negative correlation (−0.866, $p<0.05$) between the IAA producers from the RS sample and the ACC deaminase producers from the RS samples. A negative correlation (−0.656, $p<0.05$) was also observed between total IAA producers and total ACC deaminase producers, without considering the RS or RP samples. This result well demonstrates that the ratio of rhizobacteria simultaneously possessing IAA productivity and ACC deaminase activity was relatively lower, compared with that having IAA production + siderophore(s) synthesis or ACC deaminase + siderophore(s) synthesis activities (Table 2). Nevertheless, there was no significant relationship among other PGPT-possessing rhizobacteria (Table 3).

Cluster Analysis

Cluster analysis was carried out by PCA with the ratios of rhizobacteria having IAA production, ACC deaminase, or siderophore(s) synthesis activity in the RS or RP of each plant sample (Fig. 2). The values for PC1 and PC2 were 68% and 32%, respectively. Higher values of PC1 and PC2 indicated a larger variation between the data. PGPT-possessing rhizobacteria in the RP samples could be grouped irrespective of PGPT types as well as plant species. ANOVA was carried out with the data from the PCA results (PC1 and PC2 values of each condition) to clarify the most important factor in grouping. The sampling position (RS or RP) significantly affected the patterns formed by the PC1 ($p<0.01$), although plant species as well as PGPT types affected slightly the patterns formed by the PC1 and PC2. Therefore, plant types were not important factors on distribution rates of the PGPT-possessing rhizobacteria; however, the distribution rates of PGPT-possessing rhizobacteria were affected by the RS and RP. Since the RP is the portion of a plant's root that lies at the surface of the soil, bacteria inhabiting on and/or in the RP are closely associated with a host plant. This close interrelationship might be an influence on the ecological distribution and physiological characterization of PGPT-possessing rhizobacteria.

In conclusion, the ratio of PGPT-possessing rhizobacteria in the RP was higher than that in the RS (75%), and a negative correlation between the IAA producers and the ACC deaminase producers (−0.656, $p<0.05$) was shown. There was no significant relationship between physicochemical

Table 3. Relationship among IAA production, ACC deaminase, and siderophore synthesis abilities of the rhizobacteria isolated from the rhizosphere soil and rhizoplane.

			IAA			ACC_d			Sid		
			RS	RP	RS+RP	RS	RP	RS+RP	RS	RP	RS+RP
IAA	RS	r	1								
		p	-								
	RP	r	0.746	1							
		p	0.089	-							
	RS+RP	r	-	-	1						
		p	-	-	-						
ACC_d	RS	r	−0.866*	−0.488	-	1					
		p	0.026	0.326	-	-					
	RP	r	0.357	0.683	-	−0.210	1				
		p	0.487	0.135	-	0.689	-				
	RS+RP	r	-	-	−0.656*	-	-	1			
		p	-	-	0.020	-	-	-			
Sid	RS	r	−0.554	−0.197	-	0.437	−0.439	-	1		
		p	0.255	0.708	-	0.387	0.384	-	-		
	RP	r	−0.516	−0.153	-	0.275	0.064	-	0.489	1	
		p	0.295	0.772	-	0.598	0.904	-	0.325	-	
	RS+RP	r	-	-	−0.409	-	-	0.204	-	-	1
		p	-	-	0.187	-	-	0.524	-	-	-

Correlation coefficient analysis was carried out for significance at the 5% level ($p<0.05$*).
IAA, indole acetic acid; ACC_d, 1-aminocyclopropane-1-carboxylic acid deaminase; Sid, siderophore(s);
RS, rhizosphere soil; RP, rhizoplane; *r*, Pearson correlation coefficient; *p*, probability.

properties of the soils and PGPT of the rhizobacteria. The RP was a key factor influencing ecological distribution and physiological characterization of PGPT-possessing rhizobacteria.

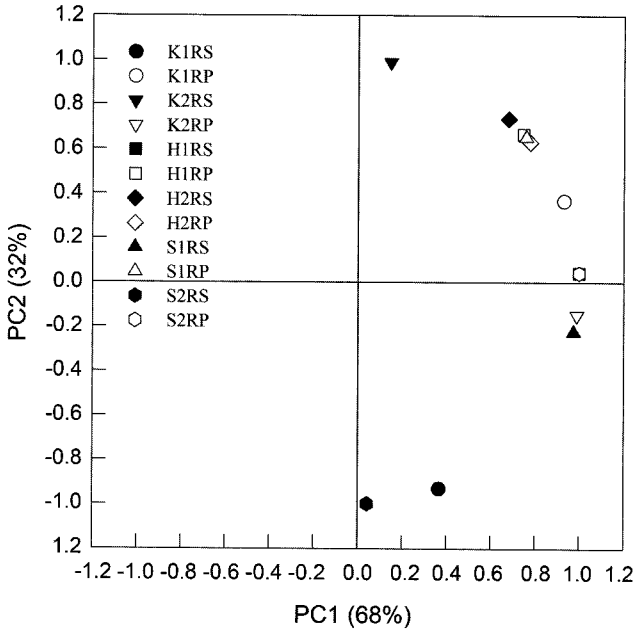

Fig. 2. Principal component analysis (PCA) of the percentages of PGPT-possessing rhizobacteria considering plant species, sampling position, and PGPT types.

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