Colonizing Ability of *Pseudomonas fluorescens* 2112, Among Collections of 2,4-Diacetylphloroglucinol-Producing *Pseudomonas fluorescens* spp. in Pea Rhizosphere

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*Pseudomonas fluorescens* 2112, isolated in Korea as an indigenous antagonistic bacteria, can produce 2,4-diacetylphloroglucinol (2,4-DAPG) and the siderophore pyoverdinin²¹¹² for the control of phytophthora blight of red-pepper. *P. fluorescens* 2112 was classified into a new genotype C among the 17 genotypes of 2,4-DAPG producers, by *phlD* restriction fragment length polymorphism (RFLP). The colonizing ability of *P. fluorescens* 2112 in pea rhizosphere was equal to the well-known pea colonizers, *P. fluorescens* Q8r1 (genotype D) and MVP1-4 (genotype P), after 6 cycling cultivations for 18 weeks. Four tested 2,4-DAPG-producing *Pseudomonas* spp. could colonize with about a 96% dominance ratio against total bacteria in pea rhizosphere. The strain *P. fluorescens* 2112 was as good a colonizer as other *Pseudomonas* spp. genotypes in pea plant growth-promoting rhizobacteria.

**Keywords:** *Pseudomonas fluorescens*, 2,4-diacetylphloroglucinol (2,4-DAPG), colonization, plant growth-promoting rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) can increase crop productivity by plant growth promotion and/or biological control of plant disease. Rhizobacteria are the subset of rhizosphere bacteria that exhibit root colonization [3]. Root colonization is an important and active process that may involve multiplication in or on roots as part of a competitive indigenous microflora [4]. In a previous study, *Pseudomonas fluorescens* 2112 was selected and identified from a red-pepper field soil in South Korea as a powerful biocontrol agent for the control of phytophthora blight and as a potential PGPR strain for the growth of pepper [8]. The strain produces an antifungal agent designated 2,4-diacyetylphloroglucinol (2,4-DAPG) and a siderophore called pyoverdinin²¹¹² [9, 10]. To date, several dozen strains of 2,4-DAPG-producing *P. fluorescens* spp. have been investigated concerning their biocontrol capability, production of antifungal metabolites, root colonization ability, and population monitoring by researchers at the United States Department of Agriculture-Agriculture Research Station (USDA-ARS) and Washington State University [1, 5–7, 14, 16, 17, 19, 20]. Rhizosphere colonization has also been monitored in several crops to discern population dynamics [2, 11, 12, 18, 21–23]. McSpadden Gardener _et al._ [17] identified 17 genotypes of *phlD*, the main gene for 2,4-DAPG production, by restriction fragment length polymorphism (RFLP) using repetitive-polymerase chain reaction (PCR) and *phlD*-PCR from terminal dilution culture (TDC) wells of strains grown in King’s medium B supplemented with rifampicin, ampicillin, chloramphenicol, and cyclohexamide. As well, to monitor rhizosphere populations of PGPR *Pseudomonas* spp., a rapid polymerase chain reaction (PCR)-based assay characterizing rhizosphere populations of 2,4-DAPG bacteria was used [16]. Among the 17 genotypes of 2,4-DAPG-producing *Pseudomonas fluorescens* spp., genotype P (MVP1-4) and D (Q8r1) strains were confirmed as superior in colonizing the roots of pea plants [5].

In this study, we determined the genotype of the Korean 2,4-DAPG producer, *P. fluorescens* 2112, and compared the pea plant root colonization competitiveness of this strain with genotypes P and D. We also monitored the changes of population dynamics of the strain density in pea rhizosphere during successive cycling.
Soil and Plant
The soil used in this study was collected in a non-cropped native vegetation area of Quincy, Washington, USA, in 2002. The soil was taken from the upper 30 cm of the soil profile, air-dried, and passed through a 0.5 cm mesh screen prior to use. The pea (Pisum sativum L.) cultivars used in this study were Columbia and were harvested in 2003 in Washington State.

Bacterial Strains and Growth Media
Four 2,4-DAPG-producing \( \textit{Pseudomonas} \) strains were used. \( \textit{Pseudomonas} \) STAD384 (genotype C), \( \textit{Pseudomonas} \) MVP 1-4 (genotype P), and \( \textit{Pseudomonas} \) Q8r1 (genotype D) were obtained from the collections of the USDA-ARS and Washington State University. Genotypes P and D were confirmed to be the best colonizing strains for pea rhizosphere [5]. Strain STAD384 was isolated in the wheat rhizosphere of plants obtained in 1997 from Oklahoma, USA; other than genotyping, less is known of this strain. Strains were cultured in a modified medium for \( \textit{Pseudomonas} \) spp., consisting of one-third strength King’s broth medium B (1/3 KMB) supplemented with ampicillin (40 \( \mu \)g/ml), chloramphenicol (13 \( \mu \)l/ml), and cyclohexamide (100 \( \mu \)g/ml, 1/3\( \times \) KMB) [16]. Inoculated DAPG-producing strains were isolated and cultured in 1/3 KMB\( ^{3+} \)B supplemented with rifampicin (100 \( \mu \)g/ml) (1/3\( \times \) KMB\( ^{3+} \)rif).

Soil Cycling of Pea Cultivation
\( \textit{Pseudomonas} \) spp. were inoculated into soil with the bacterial suspension in 1% methylcellulose solution [5] to establish an initial population density of approximately 10\( ^{4} \) CFU/g fresh weight of soil. Each pot (3 x 3 in\( ^{2} \)) was filled with 200 g of inoculated soil or non-inoculated soil. Five pea seeds were sown, and the seeds were covered with the same inoculated soil. Each pot received 33 ml or 2.5 mg/ml metalaxyl solution (active ingredient) after sowing for the control of \( \textit{Phytophthora} \) root rot. Pea plants were grown in a 22°C growth chamber with the 12-h photo-period after 5-day-old seedlings that had been emerging in a plastic bag in the same chamber. Plants were watered with 30 ml of tap water every day, and once a week, 30 ml of a fertilizer solution (Miracle-Grow) was applied. The bacteria were not inoculated into soil except at the beginning of the first cycle. Two plants were selected randomly from the five plants in each three pots after 3 weeks of cultivation to determine the rhizosphere-colonizing population density of the inoculated 2,4-DAPG-producing \( \textit{Pseudomonas} \) spp. The cycled soil was reused in the pots and sown with five pea seeds for the next 3 successive weekly cycles.

Population Density of Inoculated 2,4-DAPG Producer
The 2,4-DAPG-producing \( \textit{Pseudomonas} \) spp. of rhizosphere or pot soil were determined by the \( \text{phlD} \)-specific PCR-based TDC method [5]. Soil, rhizosphere soil, or cycled pea roots were harvested in a 50 ml capped tube and the bacteria were extracted with 15 ml of autoclaved distilled water (10 ml for soil). The tubes were vortexed and sonicated for 1 min. Extracted solutions of 100 \( \mu \)l were serially diluted in a 96-well microtiter plate prefilled with 200 \( \mu \)l of autoclaved distilled water per well to a dilution of 3\( ^{\text{nd}} \). Fifty microliter aliquots of the TDC dilution were inoculated to each well of a 96-well plate containing 200 \( \mu \)l of 1/3 KMB\( ^{3+} \) rif or 1/10 TSB. The inoculated plates were incubated at room temperature (24 ± 1°C), and their growth was measured using a Dynatech MR 500 microplate reader after 48 h (1/10 TSB) or 72 h (1/3 KMB\( ^{3+} \) rif).

An optical density at 600 nm of ≥0.07 was scored as positive. \( \textit{Pseudomonas} \) spp. of the TDC that scored positive growth was confirmed to contain \( \text{phlD} \) by PCR with the primers of B2BF and BPR4 [16]. Consequently, produced \( \text{phlD} \)s were determined by their genotypes using RFLP analysis with \( \text{MspI} \) and \( \text{HaeIII} \). The TDCs of \( \text{phlD} \) were used to calculate the population density of the \( \text{phlD} \) bacteria in the rhizosphere [16].

Population Density of Total Bacteria
The density of total culturable bacteria was determined by inoculating 50 \( \mu \)l aliquots from the one-third times serially diluted (3\( ^{\text{nd}} \)) rhizosphere extracts into the microtiter plates containing one-tenth strength tryptic soy broth (1/10 TSB) supplemented with 100 \( \mu \)g/ml cyclohexamide. Bacterial growth after 48 h at an \( \text{OD}_{600} \) ≥ 0.07 was scored as positive.

PCR Amplification and Genotyping by RFLP Analysis of \( \text{phlD} \)
In order to amplify the \( \text{phlD} \) gene of 2,4-DAPG-producing \( \textit{Pseudomonas} \) spp., 35 PCR cycles were performed using oligonucleotides in a MJ Research PTC-200 apparatus. The genotypes of \( \text{phlD} \) strains were determined by RFLP fingerprinting assay of PCR-amplified products after digestion with \( \text{HaeIII} \), \( \text{MspI} \), and \( \text{TaqI} \). For genotyping, a fresh colony on a LB plate was used as the PCR template. For the confirmation of dominant genotype strain in the rhizosphere, a cell suspension of TDC of harvested pea rhizosphere soil after 3 weeks of cultivation was used. PCR products were confirmed in 0.8% agarose gel in 0.5× Tris-borate-EDTA (TBE) buffer at 80 V. For RFLP analysis, 8 \( \mu \)l of PCR products was digested with each restriction enzyme at 37°C for 3 h. Digested fragments were analyzed on 2% agarose gels. Gel images were taken by a Kodak DC 120 digital imaging system.

Statistical Analysis
The rhizosphere population of 2,4-DAPG-producing \( \textit{Pseudomonas} \) spp. was converted to log colony forming units (CFU)/g weight of soil or fresh weight of root. The data were analyzed using STATISTIX 8.0 (Analytical Software).

RESULTS

\( \text{phlD} \) Gene and Genotype of \( \textit{P. fluorescens} \) 2112
\( \textit{P. fluorescens} \) 2112 displayed a \( \text{phlD} \) gene PCR product that was slightly larger (628 bp) than 600 bp, and the size was the same as in the \( \textit{P. fluorescens} \) Q8r1 and MVP1-4 strains acquired from an American 2,4-DAPG producer collection (Fig. 1). The \( \text{phlD} \) genotype of \( \textit{P. fluorescens} \) 2112 was confirmed to be genotype C among the 17 different \( \text{phlD} \) genotypes of 2,4-DAPG-producing fluorescent \( \textit{Pseudomonas} \) spp. by RFLP analysis with \( \text{HaeI} \), \( \text{MspI} \), and \( \text{TaqI} \) digestion; \( \text{TaqI} \)-digested fragments of \( \textit{P. fluorescens} \) 2112 were visualized as small bands of about 100 bp and as a larger band, identical to the pattern displayed by \( \textit{P. fluorescens} \) STAD 374 (genotype C).
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HaeIII digestion produced three bands; two bands exceeded 200 bp and one was above 100 bp (data not shown). *MspI* digestion revealed two bands, as found for STAD 384 and STAD 376 (genotype C). Therefore, a Korean collection of 2,4-DAPG producer, *P. fluorescens* 2112, was confirmed to be a new genotype C (Fig. 2). However, *phlD* of 2112 was slightly different in HaeIII- and TaqI-digested fragments in their band strength; the band of STAD 384 and STAD 376 were less dark than that of 2112 in agarose gels.

**Rifampicin**<sup>R</sup> **Mutation of *P. fluorescens* 2112**

For the population monitoring of *P. fluorescens* 2112 in the rhizosphere with KMB<sup>B</sup> rif, spontaneous mutation of the wild-type strain was induced by selecting resistant colonies on LB medium containing 200 µg/ml and 100 µg/ml rifampicin with six cycles of subculturing. Among the 48 rifampicin-resistant mutants, one strain, denoted P2112 M6, was chosen for further experiments involving growth curve determinations in M9 minimal medium and King’s B medium using a Safire Automatic OD Measuring Incubator. Although strain P2112 M6 was rif<sup>R</sup>, the strain could produce the 628 bp *phlD* PCR product, the same one in the wild-type strain. Thus, *P. fluorescens* 2112-M6 could be selected as a mutant of ampicillin<sup>R</sup>, chloramphenicol<sup>R</sup>, and rifampicin<sup>R</sup> for population monitoring.

**Population Dynamics of 2,4-DAPG Producers in Rhizosphere**

The basal population sizes of four inoculated *phlD<sup>+</sup>* strains were determined to be log 4.37 (P2112), log 4.37 (STAD384), log 4.21 (MVP1-4), and 4.68 (Q8r1) CFU/g soil, and *phlD<sup>+</sup>* strain of non-inoculated control soil was log 3.55 CFU/g soil (Fig. 3). As the pea cultivation cycle proceeded, *phlD*<sup>+</sup> strains in the rhizosphere increased to log 7.3 and log 8.8. In particular, the population of P2112 was increased markedly early in the first or the second cycle. However, after the second cycle, the population did not increase. In contrast, the Q8r1 and MVP1-4 population increase steadily declined at the fifth cycle. In the fourth cycle, Q8r1 had the
Population Dynamics of Total Culturable Bacteria in Rhizosphere
During the cycling, the population sizes of total culturable bacteria did not vary significantly after cycle 1 and increased about 10^2 times from log 6.2 to 6.6 (Fig. 4). In the fifth cycle, all the inoculated pot soils had about 10^9 CFU/g soil of total culturable bacteria.

Plant Growth-Promoting Effect of *Pseudomonas* spp.
To confirm the plant growth-promoting effect by the *Pseudomonas* spp., the upper-ground portion of the stem and leaves of pea plants were weighed after each cycle. After the first three cycles, whereas the P2112 inoculated pots displayed 25.9% increased plant weight, the plant weight increase of strains STAD 384, MVP1-4, and Q8r1 was <5% (Fig. 5). Subsequently, the overall growth-promoting rate was about 5–10% for all species. The results supported the designation of the four strains of *Pseudomonas* spp. as PGPR.

Attractive Effect of Pea Root to Total Bacteria and *Pseudomonas* spp.
In order to show that *Pseudomonas* spp. were more avidly attracted to pea plant roots than other total bacteria, we compared the ratio of their log CFU from rhizosphere soil with that of non-rhizosphere pot soil. The ratios of log

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**Fig. 3.** Population dynamics of *phlD*+ *Pseudomonas* spp. in the rhizosphere of pea plants for five successive cycles (3 weeks/cycle). Error bars indicate the mean ± SD (n = 6) from the six independent experiments. Comparisons among groups were done by one-way ANOVA (p<0.05).

**Fig. 4.** Population dynamics of total culturable bacteria in the rhizosphere of pea plants for five successive cycles (3 weeks/cycle). Error bars indicate the mean ± SD (n = 6) from the six independent experiments. Comparisons among groups were done by one-way ANOVA (p<0.05).

**Fig. 5.** PGPR effect of 2,4-DAPG producing *Pseudomonas* spp. on the cycling of pea plants (3 weeks/cycle). Error bars indicate the mean ± SD (n = 6) from the six independent experiments. Comparisons among groups were done by one-way ANOVA (p<0.05).
plating cycle 0, phlD+ *Pseudomonas* spp. were 66–75% (mean 69.2%) against total culturable bacteria. However, as the cycles progressed, the ratio of phlD+ *Pseudomonas* spp. increased to above 90%; in cycle 1, the average ratio of *Pseudomonas* spp. to total culturable bacteria was 91.9% in pea rhizosphere soil. The average ratios of *Pseudomonas* spp. were 96.7% in cycle 3 and 96.6% in cycle 4. As seen in Table 2, Q8r1 was again the dominant species, displaying population dominance in cycle 4 (Table 3). These results supported the suggestion that the phlD+ *Pseudomonas* spp. became the dominant population in the rhizosphere of pea plants with successive cultivation cycles.

### DISCUSSION

Many studies have attempted to monitor the population dynamics of PGPR, such as the production of antibiotics in the rhizosphere or field soil. Researchers at USDA-ARS and Washington State University conducted studies that monitored 2,4-DAPG-producing fluorescent *Pseudomonas* spp. for the control of wheat and pea fungal disease. These groups identified 17 distinct phlD+ genotypes from several dozen phlD+ 2,4-DAPG-producing *Pseudomonas fluorescens* spp. [5–7, 14, 16, 17]. Another study developed a phlD-specific PCR-based terminal dilution end-point assay for the population monitoring of 2,4-DAPG-producing *Pseudomonas* spp.

Table 1. Attraction effect of pea root toward total culturable bacteria.

<table>
<thead>
<tr>
<th></th>
<th>Rhizosphere</th>
<th>Pot soil</th>
<th>Ratio (%)</th>
<th></th>
<th>Rhizosphere</th>
<th>Pot soil</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.43 ± 0.45b</td>
<td>6.90 ± 0.18a</td>
<td>122.2 ± 3.0d</td>
<td></td>
<td>Control</td>
<td>8.43 ± 0.36b</td>
<td>7.37 ± 0.20a</td>
</tr>
<tr>
<td>P2112</td>
<td>8.65 ± 0.28a</td>
<td>6.90 ± 0.27b</td>
<td>125.4 ± 10.0e</td>
<td></td>
<td>P2112</td>
<td>9.43 ± 0.80a</td>
<td>7.85 ± 0.20a</td>
</tr>
<tr>
<td>STAD</td>
<td>8.17 ± 0.19b</td>
<td>6.90 ± 0.25b</td>
<td>118.4 ± 5.9e</td>
<td></td>
<td>STAD</td>
<td>8.96 ± 0.43b</td>
<td>7.85 ± 0.19b</td>
</tr>
<tr>
<td>MVP1</td>
<td>8.49 ± 0.62a</td>
<td>6.90 ± 0.28a</td>
<td>123.0 ± 6.4f</td>
<td></td>
<td>MVP1</td>
<td>9.08 ± 0.51b</td>
<td>7.85 ± 0.17b</td>
</tr>
<tr>
<td>Q8r1</td>
<td>8.03 ± 0.14b</td>
<td>7.37 ± 0.19a</td>
<td>109.0 ± 8.4b</td>
<td></td>
<td>Q8r1</td>
<td>8.88 ± 0.37b</td>
<td>7.37 ± 0.27b</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD (n = 6) from the six independent experiments. Different letters at values in rows show significant difference using one-way ANOVA, post hoc tests by Duncan (p<0.05).

Media: Tryptic soy broth (TSB) containing cyclohexamide (Cx).

Table 2. Attraction effect of pea root toward phlD+ *Pseudomonas* spp.

<table>
<thead>
<tr>
<th></th>
<th>Rhizosphere</th>
<th>Pot soil</th>
<th>Ratio (%)</th>
<th></th>
<th>Rhizosphere</th>
<th>Pot soil</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.23 ± 0.35c</td>
<td>4.99 ± 0.26c</td>
<td>144.8 ± 10.9c</td>
<td></td>
<td>Control</td>
<td>7.32 ± 0.37c</td>
<td>4.99 ± 0.26c</td>
</tr>
<tr>
<td>P2112</td>
<td>8.26 ± 0.46c</td>
<td>5.94 ± 0.12c</td>
<td>139.1 ± 10.0d</td>
<td></td>
<td>P2112</td>
<td>8.26 ± 0.33c</td>
<td>5.94 ± 0.09c</td>
</tr>
<tr>
<td>STAD</td>
<td>7.68 ± 0.71b</td>
<td>5.94 ± 0.29b</td>
<td>129.3 ± 10.6d</td>
<td></td>
<td>STAD</td>
<td>7.92 ± 0.63d</td>
<td>5.46 ± 0.31b</td>
</tr>
<tr>
<td>MVP1</td>
<td>7.92 ± 0.63d</td>
<td>5.46 ± 0.31b</td>
<td>145.1 ± 12.7d</td>
<td></td>
<td>MVP1</td>
<td>9.08 ± 0.51b</td>
<td>7.85 ± 0.17b</td>
</tr>
<tr>
<td>Q8r1</td>
<td>6.92 ± 0.76d</td>
<td>4.51 ± 0.32d</td>
<td>153.4 ± 9.9d</td>
<td></td>
<td>Q8r1</td>
<td>8.88 ± 0.37b</td>
<td>7.37 ± 0.27b</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD (n = 6) from the six independent experiments. Different letters at values in rows show significant difference using one-way ANOVA, post hoc tests by Duncan (p<0.05).

Media: King’s medium B (KMB) containing cyclohexamide (Cx), ampicillin (Amp), chloramphenicol (Cm), and rifampicin (Rif).
Pseudomonas spp. [16]. The assay was shown to be a more accurate monitoring method for rhizosphere population tracking than traditional dilution plating on selective media or colony hybridization followed by PCR [6].

One Korean-isolate strain of Pseudomonas spp. that produces 2,4-DAPG and pyoverdine was isolated from strawberry root samples in Korea, and this strain was grouped into the same genotype group as STAD 384 and STAD 376 isolated in Oklahoma. However, the density of the PCR bands produced in RFLP was slightly different between P2112 and STAD 384, possibly due to a very minor inconsistency in sequences of the 628 bp phdD gene. This result highlights the very interesting issue concerning the worldwide diversity of genotypes of 2,4-DAPG-producing Pseudomonas spp. Country-specific studies of the quantity and variety of genotypes are necessary in to know which genotype is dominant in each rhizosphere in a particular geographic region.

The phdD genotype D (Q8r1-96) and genotype P (MVP1-4) have been reported to be significantly greater than other genotypes of Pseudomonas spp. in the population density of pea plant rhizosphere [5]. Thus, we wanted to know if the Korean strain, P. fluorescens 2112, has the same competitiveness as Q8r1 and MVP1-4 in pea rhizosphere of Quincy soil. P. fluorescens 2112 was also very effective in colonization to the same degree as genotypes P (MVP1-4) and D (Q8r1) phdD. Additionally, in cycles 1 and 2, P. fluorescens 2112 was the highest populating strain in pea plant rhizosphere. These results support the view that a foreign P. fluorescens 2112 could also exhibit good colonization capacity in soil encountered in Washington state; P. fluorescens Q8r1 increased to log CFU 8.8 (cycle 4) to display the highest population density. In the case of Q8r1, it is conceivable that the strain harmonizes with the indigenous soil bacteria in Quincy soil. Thus, the rate of increase might be expected to be slower than the ectogeneous bacterial strain P. fluorescens 2112.

Concerning the population density of the total culturable bacteria in the total rhizosphere, all four strains in the inoculated soil increased to an average of log 8.7 CFU/g. However, the density of the PCR bands produced in RFLP was slightly different between P2112 and STAD 384, possibly due to a very minor inconsistency in sequences of the 628 bp phdD gene. This result highlights the very interesting issue concerning the worldwide diversity of genotypes of 2,4-DAPG-producing Pseudomonas spp. Country-specific studies of the quantity and variety of genotypes are necessary in to know which genotype is dominant in each plant rhizosphere in a particular geographic region.

Table 3. Dominant population ratio of phdD Pseudomonas spp. in rhizosphere in successive cycles.

<table>
<thead>
<tr>
<th>Cycle 0 (soil)</th>
<th>Pseudomonas (phdD)</th>
<th>Total bacteria*</th>
<th>phdD/total bacteria</th>
<th>Cycle 1</th>
<th>Pseudomonas (phdD)</th>
<th>Total bacteria</th>
<th>phdD/total bacteria</th>
<th>Cycle 2</th>
<th>Pseudomonas (phdD)</th>
<th>Total bacteria</th>
<th>phdD/total bacteria</th>
<th>Cycle 3</th>
<th>Pseudomonas (phdD)</th>
<th>Total bacteria</th>
<th>phdD/total bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.55 ± 0.28b</td>
<td>5.77 ± 0.00b</td>
<td>61.5 ± 4.8b</td>
<td>Control</td>
<td>5.66 ± 0.76b</td>
<td>8.36 ± 0.48ab</td>
<td>67.7 ± 9.4b</td>
<td>Control</td>
<td>7.23 ± 0.35c</td>
<td>8.43 ± 0.45ab</td>
<td>85.7 ± 4.4b</td>
<td>Control</td>
<td>7.15 ± 0.48b</td>
<td>8.43 ± 0.36b</td>
<td>91.5 ± 3.3b</td>
</tr>
<tr>
<td>P2112</td>
<td>4.37 ± 0.28a</td>
<td>6.61 ± 0.28a</td>
<td>66.1 ± 6.8a</td>
<td>P2112</td>
<td>8.01 ± 0.35a</td>
<td>8.78 ± 0.43a</td>
<td>91.2 ± 5.3b</td>
<td>P2112</td>
<td>8.15 ± 0.48a</td>
<td>8.43 ± 0.36b</td>
<td>91.5 ± 3.3b</td>
<td>P2112</td>
<td>8.32 ± 0.75b</td>
<td>9.43 ± 0.80a</td>
<td>96.8 ± 4.0b</td>
</tr>
<tr>
<td>STAD384</td>
<td>4.37 ± 0.28a</td>
<td>6.40 ± 0.48a</td>
<td>68.3 ± 9.1a</td>
<td>STAD384</td>
<td>7.31 ± 0.46a</td>
<td>8.10 ± 0.26a</td>
<td>90.2 ± 5.9a</td>
<td>STAD384</td>
<td>8.34 ± 0.43a</td>
<td>8.96 ± 0.43b</td>
<td>94.8 ± 6.9ab</td>
<td>STAD384</td>
<td>8.33 ± 0.58a</td>
<td>9.08 ± 0.51b</td>
<td>95.6 ± 8.3ab</td>
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<tr>
<td>MVP1-4</td>
<td>4.21 ± 0.00a</td>
<td>6.24 ± 0.00ab</td>
<td>67.5 ± 0.00a</td>
<td>MVP1-4</td>
<td>7.60 ± 0.71a</td>
<td>8.09 ± 0.48a</td>
<td>93.9 ± 8.4a</td>
<td>MVP1-4</td>
<td>8.10 ± 0.38a</td>
<td>8.88 ± 0.37ab</td>
<td>99.3 ± 7.0a</td>
<td>MVP1-4</td>
<td>8.10 ± 0.38a</td>
<td>8.88 ± 0.37a</td>
<td>99.3 ± 7.0a</td>
</tr>
<tr>
<td>Q8r1</td>
<td>4.68 ± 0.48a</td>
<td>6.24 ± 0.00ab</td>
<td>75.0 ± 7.6a</td>
<td>Q8r1</td>
<td>7.57 ± 0.63a</td>
<td>8.19 ± 0.35a</td>
<td>92.4 ± 8.8a</td>
<td>Q8r1</td>
<td>7.15 ± 0.48b</td>
<td>8.47 ± 0.23ab</td>
<td>96.2 ± 4.4a</td>
<td>Q8r1</td>
<td>7.15 ± 0.48b</td>
<td>8.47 ± 0.23ab</td>
<td>96.2 ± 4.4a</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD (n = 6) from the six independent experiments. Different letters at values in rows show significant difference using one-way ANOVA, post hoc tests by Duncan (p<0.05).

One cycle was 3 weeks culture of pea plant.

*Total culturable bacteria.
These results support the view that the number of phID- Pseudomonas spp. could proliferate better (i.e., 10×) than other soil bacteria in plant roots.

Lugtenberg et al. [13] reported that 40% of Pseudomonas fluorescens spp. can stimulate plant growth, and the colonization of Pseudomonas spp. in rhizosphere was suggested to be an important mechanism for plant growth-promoting (PGP) effect. The weight per plant of the upper-ground stem and leaves of the pea plant in P2112-inoculated pots had the highest PGR effect, and the promoting rate was 121.4% compared with that of non-inoculated control pot; P. fluorescens STAD384-, MVP1-4, and Q8r1-inoculated pots displayed a plant weight increase of 103.6%, 106.3%, and 108.1%, respectively. Among the four strains, P. fluorescens 2112 had apparent PGPR ability, but the other three strains had weak PGPR effects. The growth-promoting effect of pea plants using fluorescent Pseudomonas increased, consistent with other reports on PGPR and their effects.

In general, many soil bacteria show positive chemotactic behavior toward plant-root exudates [13]. Presently, Pseudomonas spp. were more attracted to pea roots than total bacteria (Tables 1 and 2). The population ratio of Pseudomonas spp. in pea rhizosphere toward non-rhizosphere pot soil was more than 31.2%, compared with that of the total bacteria in the third cycle. Regards the average ratio of the second, third, and fourth cycles, Pseudomonas spp. were 21.5% more attracted to pea roots. These results mean that Pseudomonas spp. have more affinity for the rhizosphere than other soil bacteria. The average percentage of phID- Pseudomonas relative to total bacteria increased from 69.2% at non-plating cycle 0 to 91.9% at cycle 1, 92.2% at cycle 2, 96.7% at cycle 3, and 96.6% at cycle 4 (Table 3). These results indicate that the fluorescent Pseudomonas spp. can predominate in the pea rhizosphere in long-term cultivation.

The prior collective data showed that the strains from regions of the United States other than Washington state were of similar genotypes of 2,4-DAPG-producing Pseudomonas spp. among the biocontrol agents for each area. In this report, a foreign 2,4-DAPG producer was also shown to be capable of effectively colonizing roots of pea plants grown in soil recovered from Washington state; the present genotype competed well with genotypes D (Q8r1) and P (MVP1-4). This suggests that foreign-born biocontrol-PGPR could colonize the agriculture soil of other countries.

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References


