

## RESEARCH ARTICLE

# Variation of the seed endophytic bacteria among plant populations and their plant growth-promoting activities in a wild mustard plant species, *Capsella bursa-pastoris*

Byungwook Choi | Seorin Jeong | Eunsuk Kim 

School of Earth Sciences and  
Environmental Engineering, Gwangju  
Institute of Science and Technology,  
Gwangju, South Korea

**Correspondence**

Eunsuk Kim, School of Earth Sciences  
and Environmental Engineering, Gwangju  
Institute of Science and Technology,  
Gwangju, South Korea.  
Email: eunsukkim@gist.ac.kr

**Funding information**

National Research Foundation of  
Korea, Grant/Award Number: NRF-  
2019R1F1A1058025; National Research  
Foundation of Korea, Grant/Award  
Number: NRF-2021R111A2047635

**Abstract**

Recent studies have revealed that some bacteria can inhabit plant seeds, and they are likely founders of the bacterial community in the rhizosphere of or inside plants at the early developmental stage. Given that the seedling establishment is a critical fitness component of weedy plant species, the effects of seed endophytic bacteria (SEB) on the seedling performance are of particular interest in weed ecology. Here, we characterized the SEB in natural populations of *Capsella bursa-pastoris*, a model species of weed ecology. The composition of endophytic bacterial community was evaluated using deep sequencing of a 16S rDNA gene fragment. Additionally, we isolated bacterial strains from seeds and examined their plant growth-promoting traits. Actinobacteria, Firmicutes, Alpha-, and Gammaproteobacteria were major bacterial phyla inside seeds. *C. bursa-pastoris* natural populations exhibited variable seed microbiome such that the proportion of Actinobacteria and Alphaproteobacteria differed among populations, and 60 out of 82 OTUs occurred only in a single population. Thirteen cultivable bacterial species in six genera (*Bacillus*, *Rhodococcus*, *Streptomyces*, *Staphylococcus*, *Paenibacillus*, *Pseudomonas*) were isolated, and none of them except *Staphylococcus haemolyticus* were previously reported as seed endophytes. Eight isolates exhibited plant growth-promoting traits like phosphate solubilization activity, indole-3-acetic acid, or siderophore production. Despite the differences in the bacterial communities among plant populations, at least one isolated strain from each population stimulated shoot growth of either *C. bursa-pastoris* or its close relative *A. thaliana* when grown with plants in the same media. These results suggest that a weedy plant species, *C. bursa-pastoris*, contains bacterial endophytes inside their seeds, stimulating seedling growth and thereby potentially affecting seedling establishment.

**KEYWORDS**

bacterial community, cultivable bacteria, natural variation, plant growth-promoting traits, seedling growth, weed ecology

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Ecology and Evolution* published by John Wiley & Sons Ltd.

## TAXONOMY CLASSIFICATION

Evolutionary ecology; Microbial ecology; Population ecology; Seed ecology

## 1 | INTRODUCTION

Successful establishment of seedlings in novel environments is a major fitness component of weedy plant species and a critical factor determining species' geographic range (Crawley, 1997). Soil microbiota, especially microorganisms in plants' rhizosphere, is proposed to form a mutualistic interaction with plants and consequently facilitate weed establishment (Coats & Rumpho, 2014; Trognitz et al., 2016). Notably, plant seeds harbor diverse seed endophytic bacteria (SEB) (Truyens et al., 2015), so seeds and SEB of weed plants would disperse simultaneously. Since SEB would constitute microbiota inside or in the rhizosphere of seedlings (Kaga et al., 2009; Puente et al., 2009), SEB, in addition to soil microorganisms, likely influence seedling establishment in novel environments.

Despite their plausible ecological significance (Elmore et al., 2019; Jeong et al., 2021; White et al., 2018), relatively little information is available on the characteristics of SEB in weed plant species. Most information on the SEB has come from studies using agriculturally important crop plants (Card et al., 2015; Pal et al., 2019; Shahzad et al., 2018). It should be noted that crop and wild plant species tend to have distinctive characteristics. Many crop plant species have a limited number of genotypes due to strong artificial selection, and their seeds are intensively managed to have consistent quality with pathogen-free status (Pérez-Jaramillo et al., 2016; White et al., 2019). Consequently, SEB of crop species are suggested to have lower diversity than wild plant species. Studies on the indigenous SEB of wild plant species are required to evaluate the ecological significance of SEB (Wassermann et al., 2019).

This study examined the bacterial community of seeds in one of the most common weedy species, *Capsella bursa-pastoris* (Brassicaceae) (Neuffer, 2011). *C. bursa-pastoris* is an annual weedy plant species worldwide, except for regions near the equator (Neuffer, 2011). As a model system of weed ecology, the adaptive divergence of *C. bursa-pastoris* along environmental gradients like altitude and latitude has been well documented (Huang et al., 2012; Neuffer, 2011).

Studies testing seed endophytes have often used DNA fingerprinting methods such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) or next-generation sequencing (NGS) (Beckers et al., 2016; Liu et al., 2019; Xu et al., 2014). While such techniques have the advantage of detecting uncultivable bacteria, they provide scant information on the functional role of microorganisms in plant performance. In contrast, culture-dependent methods can characterize the biological functions of isolated bacteria, although cultivable bacteria are only a subset of the whole microbiome. In particular, diverse assays can be conducted to evaluate whether SEB are able to produce so-called plant growth-promoting (PGP) molecules like indole-3-acetic acids (IAA), siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminases,

and phosphate-solubilizing molecules (Santoyo et al., 2016; Shahzad et al., 2018). Since culture-independent and -dependent methods provide complementary information on the SEB, this study used both methods.

Previous studies using crop plants suggested that the composition and function of SEB likely depend on cultivar genotypes and growing environments (Hardoim et al., 2012; Xu et al., 2014). Weedy plants occur in a broad geographic range with diverse environmental conditions. Distinctive genotypes tend to constitute natural populations in different environments (Linhart & Grant, 1996; Neuffer, 2011), implying that the composition and function of SEB in weedy species may be population specific. Thus, for a more general conclusion on the ecological role of SEB in weedy species, a comparative study at the population level was conducted.

Here, we collected seeds of *C. bursa-pastoris* populations along a latitudinal gradient in South Korea and characterized their SEB using both culture-independent and culture-dependent methods. NGS technique was used to compare SEB community among *C. bursa-pastoris* natural populations. In addition, SEB were isolated from seeds, and their PGP activities were assessed by examining the production of PGP molecules and the effect of SEB on seedling growth. Specifically, we addressed the following questions: (i) What kinds of endophytic bacteria inhabit inside seeds of *C. bursa-pastoris*? (ii) Do *C. bursa-pastoris* natural plant populations have differential seed bacterial communities? (iii) Do populations contain SEB-exhibiting PGP traits and promote seedling growth?

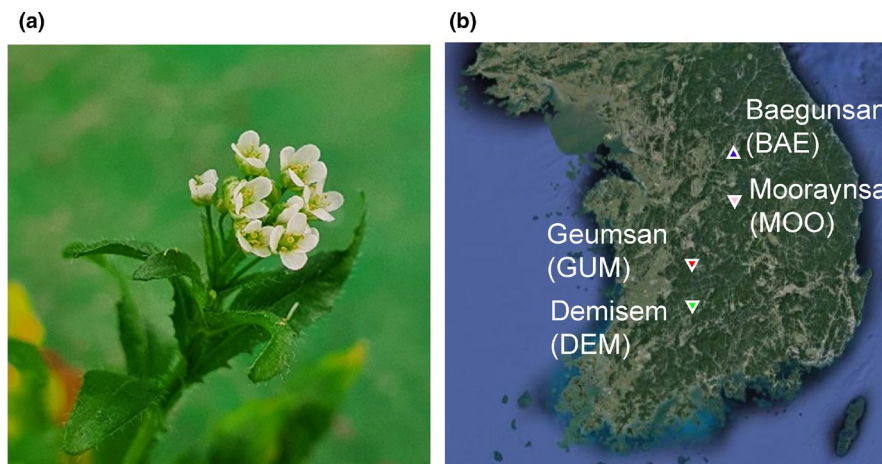
## 2 | MATERIALS AND METHODS

## 2.1 | Study populations and seed sources

We randomly chose four *C. bursa-pastoris* natural populations along a latitudinal gradient in South Korea in 2015 (Figure 1, Appendix S1). Seeds were collected from at least 20 maternal genotypes in each population. Germinants of field-collected seeds were grown for one generation in a growth chamber, and seeds from these plants were used to examine SEB. In detail, field-collected seeds were sown in pots (8 cm × 7.5 cm × 6 cm) containing commercial soil medium (Shinsung Mineral Co. LTD, Kyunggi-do, Korea). The soil medium consisted of cocopeat (51.5% v/v), peat moss (10.0% v/v), zeolite (10.0% v/v), perlite (15.0% v/v), and vermiculite (13.0% v/v) with final pH of 5.0–7.0. Pots were maintained in a growth chamber at 25°C and a 12-hour light/dark photoperiod with 100 μmol/s/m<sup>2</sup> photosynthetically active radiation (PAR). Humidity inside the chamber was not controlled.

We randomly chose seven to eight maternal genotypes from each of the four *C. bursa-pastoris* populations and sterilized seed

**FIGURE 1** A photograph of *Capsella bursa-pastoris* (a) and seed sources (b) in the Korean Peninsula. The GPS coordinates of source populations are given in Appendix S1



surfaces following Johnston-Monje and Raizada (2011) with modification. Briefly, seeds were soaked in 0.1% Triton X-100 for 1 min, 3% sodium hydrochloride for 1 min, 70% ethanol for 1 min, and then washed three times with sterile distilled water. Surface sterilization was confirmed by incubating the sterilized seeds on potato dextrose agar (PDA) (# 213400, Difco Laboratories, Franklin Lakes, NJ, USA), R2A (# 218263, Difco), and Luria–Bertani (LB) agar (#7279, Acumedia, Lansing, MI, USA) at 25°C for a week. Only seeds that did not produce any microbial colonies were used to isolate bacterial endophytes and examine endophytic communities.

In order to visually confirm the occurrence of SEB, we used a fluorescence in situ hybridization (FISH) technique following Hewitson et al. (2010). Surface-sterilized seeds were fixed with 4% paraformaldehyde. Seeds were embedded in the frozen section compound (FSC 22 Clear, Leica) at – 80°C for 24 h, sectioned with 5 µm thickness using freezing microtome (DE/HM525NX, Thermo-Fisher Scientific, Waltham, MA, USA), and attached on a clear slide glass. The whole slide glass was pre-incubated in the hybridization buffer (0.9 M NaCl, 50 mM sodium phosphate (pH 7.0), 5 mM EDTA, 0.1% SDS, 0.5 mg of poly(A) per ml, 10× Denhardt solution, 35% (v/v) of formamide) at 45°C for 30 min. After adding 50 ng of oligodeoxynucleotide probe (EUB338) with dye, the slide glass was incubated in the hybridization buffer for 2 h at 45°C. The slide was in the washing solution (0.9 M NaCl, 0.1% SDS, 20 mM Tris hydrochloride (pH 7.2)) for 30 min at 48°C and washed twice with sterile distilled water. Pictures were taken using a Fluorescence microscope (EPI-Fluorescence & DIC Microscope, Carl Zeiss, Oberkochen, Germany) and edited using the iSolation FL auto program.

## 2.2 | Amplicon sequencing and clustering into operational taxonomic units (OTUs)

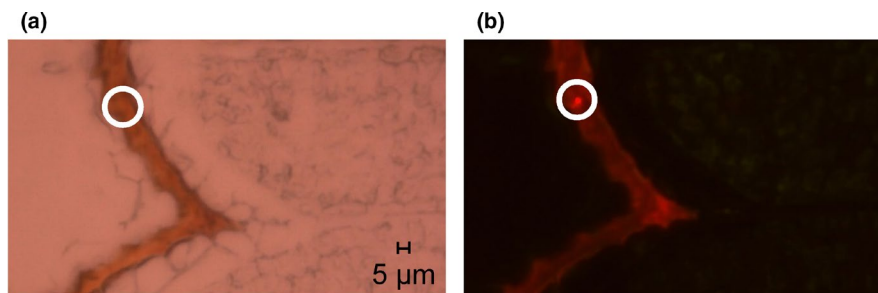
Equal amounts of seeds from seven individuals of each population were mixed to make three 500-mg samples. Each seed sample was homogenized using TissueLyser II (QIAGEN, Hilden, Germany). DNA was extracted from each sample using DNeasy Plant Mini Kit (QIAGEN), and bacterial 16S rDNA was amplified. We used two methods to reduce chloroplast and mitochondrial DNA in the

amplicon. First, we enriched bacterial cells in the homogenized seed mixture as described by Ikeda et al. (2009). Second, we amplified 16S rDNA using 799F (AACMGG-ATTAGATACCCCKG) and 1193R (ACGTCATCCCCACCTTCC). As shown by Beckers et al. (2016), the 799F – 1193R primer set amplifies mitochondrial and bacterial 16S rDNA but does not amplify chloroplast 16S rDNA. In addition, the size of amplified mitochondrial DNA (800 base pairs) was longer than that of bacterial DNA (approximately 450 bp). Amplified bacterial DNA was extracted from the gel and sequenced using the Illumina MiSeq platform by Macrogen Inc. (Seoul, Korea).

Sequence reads were processed with the Mothur v.1.43 pipeline following MiSeq SOP (Schloss et al., 2009). To trim sequence reads, we used *screen.seqs* with the options of zero maxambig, five maxhomop, and 430 maxlength. This procedure produced 86,980 high-quality reads from 1,307,899 raw reads. Contigs were clustered into OTUs with 97% sequence identity and aligned using the SILVA *nr\_132* database (Quast et al., 2012). Approximately 20% of the raw sequence reads were removed because they were chimeric or non-bacterial sequences including chloroplast, mitochondria, archaea, and eukaryotes. Singletons were removed by *remove.seqs* in Mothur (Allen et al., 2016). OTUs other than cyanobacteria were designated in the phyla or classes of Proteobacteria for further statistical analyses following Beckers et al. (2016).

## 2.3 | Bacterial isolation and identification

To isolate SEB (Figure 2), surface-sterilized seeds were ground using a sterile mortar and pestle. The material was spread on five different solid media: LB agar, LGI (50 g/L sucrose, 0.01 g/L ferric chloride hexahydrate, 0.8 g/L potassium phosphate tribasic, 0.2 g/L magnesium sulfate heptahydrate, 0.002 g/L sodium molybdate dihydrate, 7.5 g/L agar; pH 7.5) for diazotrophic bacteria, R2A for oligotrophic bacteria, King's B agar (#60786, Sigma-Aldrich, St. Louis, MO, USA), and commercial agar used for cooking for slow-growing bacteria based on previous studies (Gagne-Bourgue et al., 2013; Johnston-Monje & Raizada, 2011). The plates were incubated at 25°C for a month during which a bacterial colony larger than 2 mm was picked



**FIGURE 2** Images of fluorescent in situ hybridization (FISH) to detect seed endophytic bacteria inside seeds of DEM population. Seeds were sectioned using Cryomicrotome, and the EUB338 probes (Macrogen, Daejeon, Korea) were hybridized. The white circle in the right image indicates the position of endophytic bacteria. (a) is an image magnified  $400\times$  times by a light microscope. (b) is an image magnified  $400\times$  times with a fluorescence microscope

for subculture (Gagne-Bourgue et al., 2013). A total of 18 morphologically different colonies from the plates were selected, subcultured twice, and then preserved in 20% glycerol stock solutions at  $-80^{\circ}\text{C}$  until required.

For bacterial DNA extraction, a single colony was inoculated into liquid ISP2 medium in a round-bottom tube and incubated in a shaking incubator at 200 rpm at  $25^{\circ}\text{C}$ . DNA was extracted using Exgene Cell SV kits (Geneall, Seoul, Korea) following the manufacturer's instructions. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Coombs & Franco, 2003). Each PCR reaction contained a total of 50  $\mu\text{l}$  consisting of 5  $\mu\text{l}$  of  $10\times$  nTaq buffer, 5  $\mu\text{l}$  of 5  $\mu\text{M}$  dNTP, 1 U of nTaq polymerase (Enzynomics, Daejeon, Korea), 5  $\mu\text{l}$  of each primer set, and 200 ng of template DNA. The reaction conditions were previously described (Coombs & Franco, 2003). PCR products were purified using the EZ-pure PCR purification kit (Enzynomics) and sequenced (Macrogen Inc.). To identify bacterial isolates, we aligned nucleotide sequences using MEGA 6.0 (Tamura et al., 2013) and compared them with previously reported sequences of bacterial type strains using EZBcloud (Chunlab, Seoul, Korea). A bacterial isolate was assigned to a species with the highest 16S rDNA sequence similarity. The 16S rDNA sequences of the isolates have been deposited in NCBI GenBank, and their accession numbers are given in Appendix S2.

## 2.4 | Plant growth-promoting traits

We assessed four microbial traits that were suggested to promote plant growth directly (Glick, 2012). For assays, isolated bacterial strains were individually grown in test tubes containing 7 ml LB medium (#7178, Acumedia) at  $28^{\circ}\text{C}$  in a shaking incubator (210 rpm) for three days. Because all PGP assay procedures include incubation of bacterial strains at  $30^{\circ}\text{C}$  (see below), cells were cultured at  $28^{\circ}\text{C}$  before the assay to acclimate cells to  $30^{\circ}\text{C}$ . The bacterial cells were harvested by centrifugation and rinsed with sterilized deionized water (DW) twice. The cells were suspended in DW to the optical density (OD) of 1.2 at 600 nm, and triplicates of cell suspension were used for all assays.

The ability to solubilize inorganic phosphate was examined following Nautiyal (1999). Briefly, 20  $\mu\text{l}$  of prepared bacterial solution was inoculated in 8 ml of National Botanical Research Institute's Phosphate (NBRI-P) growth medium (10 g/L glucose, 5 g/L  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ , 0.25 g/L  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , 0.2 g/L KCl, 0.1 g/L  $(\text{NH}_4)_2\text{SO}_4$ ; pH 6.75) containing insoluble tricalcium phosphate (5 g/L  $\text{Ca}_3(\text{PO}_4)_2$ ), and incubated at  $30^{\circ}\text{C}$  in a shaking incubator (150 rpm) for two days. NBRI-P without bacterial inoculation was prepared as a negative control. Clear supernatant (100  $\mu\text{l}$ ) after centrifugation was transferred to a new clean glass tube with 4.2 ml of sterile DW, 500  $\mu\text{l}$  of 2.5% ammonium molybdate in 5 N sulfuric acid, and 200  $\mu\text{l}$  of  $\alpha$ -amino-naphthol solution. The mixture was incubated at room temperature for 30 min, and their absorbance at 660 nm was measured using Biospectrometer basic (Eppendorf, Hamburg, Germany). The phosphate level was estimated based on a standard curve that ranged from 0.1 to 2 mM of phosphate.

Siderophore production was quantitated following Schwyn and Neilands (1987) and Murakami et al. (2021). Prepared bacteria solution (20  $\mu\text{l}$ ) was inoculated in 8 ml of the  $10^{-2}$  diluted LB broth and incubated at  $30^{\circ}\text{C}$  in a shaking incubator (150 rpm) for four days. The supernatant (1 ml) was transferred to a glass tube with 700  $\mu\text{l}$  of Chrome Azurol S (CAS) solution (0.165 g/L CAS, 0.082 g/L  $\text{FeCl}_3$ , 0.397 g/L hexadecyltrimethylammonium bromide (HDTMA), in 100 mM piperazine buffer (pH 6.0) with 4 mM 5-sulfosalicylic acid). After incubation for one hour at room temperature, the absorbance was measured at 630 nm using the Biospectrometer basic. The percent siderophore unit (psu) was calculated as  $(\text{Ar}/\text{As})/\text{Ar} \times 100$  where Ar is the absorbance of CAS solution mixed with uninoculated media and As is the absorbance of CAS solution mixed with the supernatant of each sample culture.

We quantitated IAA following Patten and Glick (2002) with modification. We inoculated 20  $\mu\text{l}$  of a bacterial solution to 8 ml of LB broth containing 1000 mg/L of L-tryptophan (Sigma-Aldrich) and incubated the broth at  $30^{\circ}\text{C}$  in a shaking incubator (150 rpm) for two days. LB broth without bacterial inoculation was prepared as a negative control. After centrifugation, 1 ml of supernatant was recovered and mixed with 4 ml of Salkowski's reagent (11.5 M  $\text{H}_2\text{SO}_4$ , 9.2 mM  $\text{FeCl}_3$ , in sterilized DW). After incubation for 30 min at room temperature, the absorbance at 535 nm was measured. IAA concentration was estimated based on a standard curve that ranged from 1 to 50  $\mu\text{g}/\text{ml}$  IAA (Duchefa Biochemie, Haarlem, The Netherlands).

ACC deaminase activity was determined following Penrose and Glick (2003) with modification. We inoculated 20  $\mu$ l of bacterial solution into 7 ml of DF media (6 g/L  $\text{Na}_2\text{HPO}_4$ , 4 g/L  $\text{KH}_2\text{PO}_4$ , 2 g/L gluconic acid, 2 g/L citric acid, 0.2 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 ml of trace element mixtures containing 0.001 g/L  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g/L  $\text{H}_3\text{BO}_3$ , 0.011 g/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.125 g/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.078  $\text{L}^{-1}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.01 g/L  $\text{MoO}_3$ ) with 3 mM ACC (Sigma-Aldrich) and glucose. After the incubation at 30°C in a shaking incubator (150 rpm) for four days, bacterial cells were collected by centrifugation, rinsed with saline solution three times, and Tris-HCl (pH 7.5) one time. The pellet was suspended in 600  $\mu$ l of 0.1 M of Tris-HCl (pH 8.5) and mixed with 30  $\mu$ l of toluene for 30 s. Each bacterial mixture (200  $\mu$ l) was transferred to a new clean conical tube containing 20  $\mu$ l of 0.5 M ACC and incubated at 30°C for 15 min. After 1 ml of 0.56 M HCl was added, the mixture was centrifuged to transfer 1 ml of the supernatant to a new clean tube. A volume of 800  $\mu$ l of 0.56 M HCl and 300  $\mu$ l of 2, 4-dinitrophenylhydrazine (0.2% 2,4-dinitrophenylhydrazine in 2 M HCl) was added and incubated at 30°C for 30 min. After the addition of 2 ml of 2 N NaOH, the absorbance at 540 nm was measured using the BioSpectrometer basic. The concentration of  $\alpha$ -ketobutyrate was estimated based on a standard curve that ranged from 0 to 2 mM of  $\alpha$ -ketobutyrate (Sigma-Aldrich). The quantity of the whole protein was estimated using Lowry's methods (Lowry et al., 1951).

## 2.5 | In vitro growth assay

To confirm whether the isolated endophytic bacteria are able to promote plant growth, we grew plant seedlings and isolated bacteria together in a single plate. Seedlings germinated from surface-sterilized seeds were used following previous studies (Dovana et al., 2015; Maggini et al., 2020). First, we examined the effects of bacteria on the model plant species, *Arabidopsis thaliana* Col line, as other studies often tested bacterial PGP activity using model plant species. After surface sterilization, seeds of *A. thaliana* were sown on a square plate with 0.2 $\times$  Murashige and Skoog (MS) agar (#M022, Duchefa Biochemie, Haarlem, The Netherlands) containing 0.02% glycine, 0.5% inositol, and 0.5% sucrose. The plates were covered with aluminum foil and incubated at 25°C in the light for 16 h and 15°C in the dark for 8 h. Five days after incubation, 16 seedlings with a length of 1–2 cm were transferred to a new MS agar plate. The plates were incubated under the same conditions of the seed germination. Each bacterial isolate was streaked 7 cm below the root endpoint of seedlings in each plate, so that bacterial colonies did not contact plant seedlings (Figure 5e). Among 13 isolated bacteria, nine isolates were used for the experiment since four of them seldom grew in the MS agar. A plate that was not inoculated with any bacterial strain was a control. One-third of a Petri dish was not covered with aluminum foil for plants to receive light. Shoot and root lengths of seedlings were measured using a digital caliper at the time of streaking bacteria in each plate and after five days of incubation. The growth was calculated as the shoot or root length measured five days after bacterial inoculation minus those measured before inoculation.

We conducted the same procedures using *C. bursa-pastoris* seedlings. Seeds of ten maternal genotypes from each natural population were mixed and germinated in the same agar plates used for *A. thaliana*. Each plate had four seedlings from each plant population, resulting in a total of 16 seedlings. The plate inoculated with each bacterial isolate and a control plate without bacterial strain was replicated five times.

## 2.6 | Statistical analyses

All statistical analyses were performed using R version 3.6.1 (R Core Team, 2019). The *vegan* package was used for the analysis of the bacterial community. The abundance of the phyla/classes was Hellinger transformed to reduce the weighting of highly abundant phyla/classes and the overweighting of rare phyla/classes. Unclassified sequence reads at the phylum level were removed from the dataset. The Bray–Curtis dissimilarity matrix was calculated based on the Hellinger transformed abundance data. To examine the variable composition of the seed bacterial community among the four populations, we conducted a permutational analyses of variance (PERMANOVA) with 9999 permutations and analyses of similarity (ANOSIM) with 9999 permutations. Nonmetric multidimensional scaling (NMDS) was conducted using the Bray–Curtis dissimilarity distances. To identify bacterial phylum/class contributing to the differentiation of seed endophytic communities, we compared the composition of each phylum/class among source populations using one-way analyses of variance (ANOVA) with the population as a factor after arcsine transformation to satisfy normality. Pairwise comparisons between source populations were evaluated using Tukey's method in the *multcomp* package. As an alpha-diversity measurement, the Inverse Simpson index was calculated based on the number and abundance of OTUs for each population. Tukey's multiple comparison was conducted to compare the diversity indexes among natural populations.

We conducted separate ANOVA for *A. thaliana* and *C. bursa-pastoris* to evaluate the effect of bacterial inoculation on shoot and root growth. One-way ANOVA was performed for *A. thaliana*, and the model included bacterial isolate as a fixed factor. For *C. bursa-pastoris*, mixed model ANOVA was conducted with the source population, bacterial species, and their interaction as independent variables and the plate as a random factor. The effect of each isolated bacterium was evaluated by post hoc multiple comparisons between the plant growth with and without bacterial strain, and the statistical significance was adjusted based on Dunnett's method.

## 3 | RESULTS

### 3.1 | Endophytic bacterial communities of seeds in *C. bursa-pastoris* natural populations

Sequencing of amplicon libraries generated a total of 1,307,889 reads with a mean of 87,013 sequences per sample. Approximately

20% of the total sequences were of chloroplast or mitochondrial DNA, and approximately 80% of the remaining sequences were cyanobacteria or unclassified bacteria. The bacterial reads were assigned to 82 OTUs at a 97% cut-off in the Mothur pipeline. Rarefaction curves suggested that the dataset might be inadequate to capture the bacterial communities fully (Appendix S3), but the Goods' coverage estimates of all samples were over 99% (Appendix S3).

OTUs were assigned to eight phyla: Actinobacteria, Bacteroidetes, Chloroflexi, Deinococcus, Dependitiae, Firmicutes, Fusobacteria, and Proteobacteria. Although not evenly distributed, Actinobacteria, Firmicutes, Alphaproteobacteria, and Gammaproteobacteria occurred most commonly across four plant populations (Figure 3). Results of PERMANOVA ( $F = 2.67$ ,  $p < .05$ ) and ANOSIM ( $R = 0.35$ ,  $p < .05$ ) indicated that *C. bursa-pastoris* natural populations had a differential composition of seed endophytic phyla/classes. In particular, NMDS plot indicated that the bacterial community was likely divided into three groups at the phylum/class level, population BAE, population GUM, and population DEM and MOO. This was supported by the results of ANOSIM ( $R = 0.34$ ,  $p < .05$ ).

Additional ANOVA for each phylum showed that the composition of Actinobacteria ( $F = 3.72$ ,  $p = .06$ ) and Alphaproteobacteria ( $F = 3.48$ ,  $p = .07$ ) differed among plant populations (Appendix S4). In particular, the population GUM had a lower proportion of Actinobacteria ( $t = 3.34$ ,  $p = .04$ ) than the population MOO, and a higher proportion of Alphaproteobacteria ( $t = 3.02$ ,  $p = .06$ ) than the population BAE.

The number of OTUs from each sample varied from 21 to 45 (Figure 4b, Appendix S4). The Inverse Simpson index differed among endophytic communities of plant populations ( $F = 4.58$ ,  $p < .05$ ). Tukey's multiple comparisons showed that the endophytic community of the population GUM exhibited a higher Inverse Simpson index than that of population BAE ( $t = 3.015$ ,  $p = .07$ ) and population MOO ( $t = 3.072$ ,  $p = .06$ ). Although the population MOO had the largest number of OTUs, its inverse Simpson index was the lowest (Appendix S4). Sixty of 82 OTUs occurred only in a single plant population (Figure 4b), suggesting that both the species composition

as well as the proportion of seed endophytic communities differed among natural plant populations.

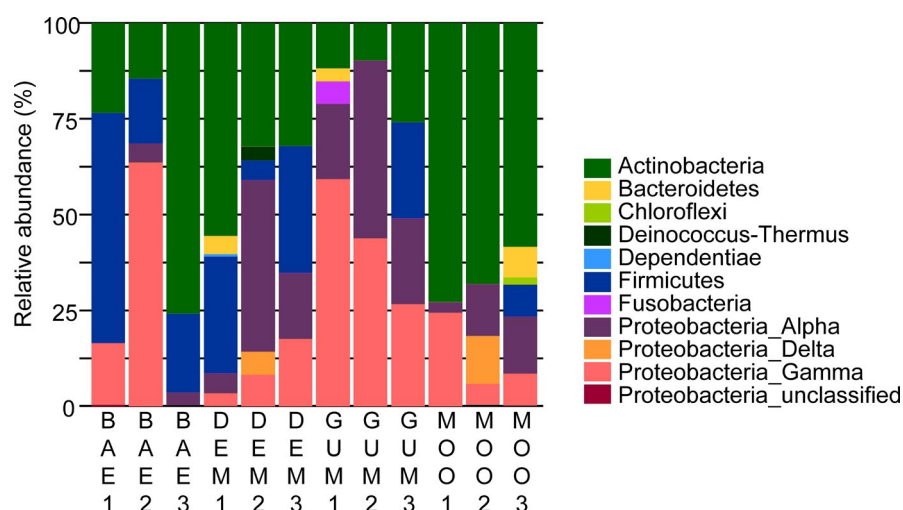
### 3.2 | Isolated endophytic bacteria and their PGP traits

A total of 13 cultivable bacterial species in six genera were isolated from four *C. bursa-pastoris* natural populations based on the similarities of partial 16S rDNA sequences (1203 – 1439 nucleotides) (Table 1). All isolates showed a high homology of 97% to 100% with previously known sequences. *Streptomyces* and *Bacillus* were the most dominant genera in the culture-dependent method. At the species level, four of five *Streptomyces* species and one of two *Bacillus* species occurred only in a single plant population.

Eleven out of fifteen isolates expressed variable PGP characteristics (Table 1). Compared to other bacterial strains, *Bacillus aryabhattai* B1 and *Streptomyces* (St.) *olivaceus* D2 produced a relatively high amount of PGP molecules in all testing assays. In contrast, *Streptomyces griseoplanus* D2, *Bacillus altitudinis* D6, *B. altitudinis* G4, and *Paenibacillus agarexedens* G5 exhibited low activities in all assays. *Streptomyces* strains except St. *griseoplanus* D3 produced more than 20 µg/ml IAA, 23% of siderophore units, and 278 mg/L soluble phosphate. *Rhodococcus corynebacterioides* B2, *Staphylococcus haemolyticus* D4, *Paenibacillus tritici* D5 exhibited ACC deaminase activity higher than 20 nmol α-KB/mg protein/h, but they showed low activity in other assays. Endophytic communities from three plant populations had at least one bacterial strain that exhibited positive activity in four PGP trait assays.

### 3.3 | Growth responses of seedlings to endophytic bacteria

Isolated bacterial strains affected shoot ( $F = 14.07$ ,  $p < .001$ ) and root growth ( $F = 3.18$ ,  $p < .01$ ) of *A. thaliana* (Figure 5a,b). *A. thaliana* grown with *Rhodococcus corynebacterioides* B2 ( $Z = 3.04$ ,  $p < .05$ ),



**FIGURE 3** Relative sequence abundance of bacterial phyla from seeds of four *Capsella bursa-pastoris* natural populations. Proteobacteria are shown at the class level following Beckers et al. (2016), and replicates are presented separately. Abbreviations of plant populations are given in Figure 1

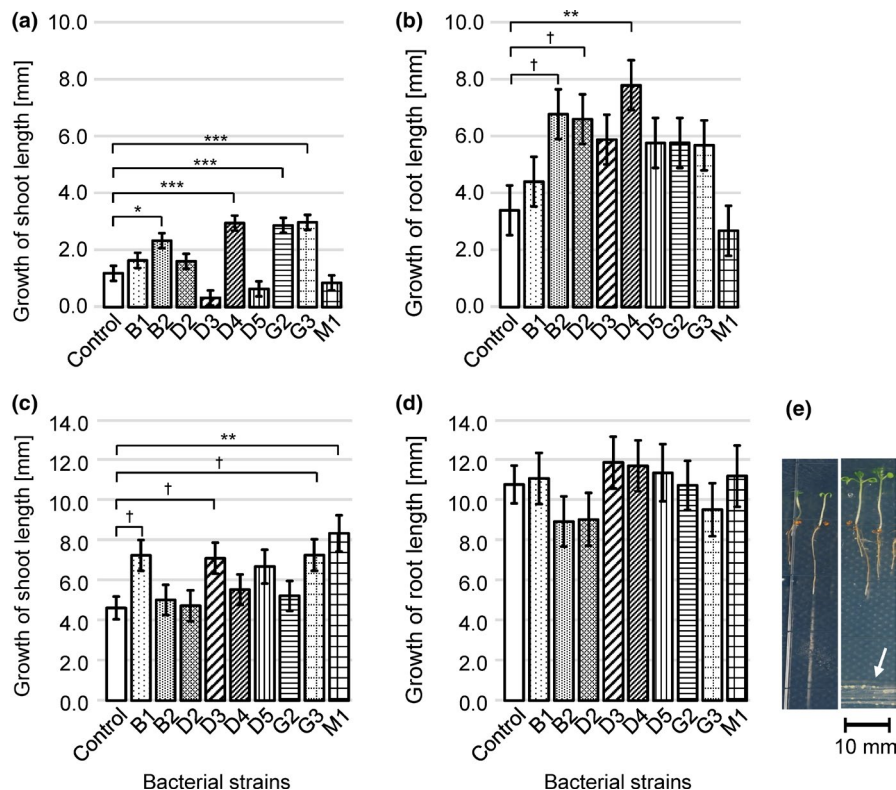


TABLE 1 Endophytic bacteria isolated from natural populations of *C. bursa-pastoris* and results of plant growth-promoting (PGP) assays

| Source population | Strains | The closest type strain (accession number)                   | Plant growth-promoting activity |                                                         |                              |                                                      |                                                   |
|-------------------|---------|--------------------------------------------------------------|---------------------------------|---------------------------------------------------------|------------------------------|------------------------------------------------------|---------------------------------------------------|
|                   |         |                                                              | Similarity (%)                  | ACC deaminase activity (nmol $\alpha$ -KB/mg protein/h) | IAA production ( $\mu$ g/ml) | Siderophore production (psu, % of siderophore units) | Phosphate solubilization (mg/L of $\text{PO}_4$ ) |
| BAE               | B1      | <i>Bacillus aryabhatai</i> B8W2w (EF114313)                  | 1366/1374 (99.42)               | 30.86 $\pm$ 3.59                                        | 26.37 $\pm$ 0.34             | 20.66 $\pm$ 0.46                                     | 624.31 $\pm$ 26.91                                |
|                   | B2      | <i>Rhodococcus corynebacterioides</i> DSM 20151 (AF430066)   | 1322/1329 (99.47)               | 22.09 $\pm$ 1.18                                        | -                            | 3.57 $\pm$ 0.19                                      | 22.39 $\pm$ 1.03                                  |
| DEM               | D1      | <i>Streptomyces qinglanensis</i> 172205 (HQ660227)           | 1338/1339 (99.93)               | 2.09 $\pm$ 0.29                                         | 22.96 $\pm$ 0.68             | 26.06 $\pm$ 0.16                                     | 465.08 $\pm$ 60.35                                |
|                   | D2      | <i>Streptomyces olivaceus</i> NRRL B-3009 (JOFH01000101)     | 1337/1338 (99.92)               | 19.49 $\pm$ 1.08                                        | 22.17 $\pm$ 0.74             | 27.93 $\pm$ 0.10                                     | 499.77 $\pm$ 18.74                                |
|                   | D3      | <i>Streptomyces griseoplanus</i> NRRL B-3064 (AB184138)      | 1307/1322 (98.87)               | 1.88 $\pm$ 0.31                                         | -                            | 4.15 $\pm$ 0.29                                      | 18.92 $\pm$ 2.71                                  |
|                   | D4      | <i>Staphylococcus haemolyticus</i> MTCC 29970 (LILF01000056) | 1363/1363 (100)                 | 20.10 $\pm$ 0.26                                        | 4.36 $\pm$ 0.42              | 1.87 $\pm$ 0.33                                      | 12.30 $\pm$ 0.89                                  |
|                   | D5      | <i>Paenibacillus tritici</i> RTAE36 (CP009285)               | 1373/1373 (99.12)               | 21.63 $\pm$ 0.53                                        | -                            | 1.52 $\pm$ 0.37                                      | 22.39 $\pm$ 1.36                                  |
|                   | D6      | <i>Bacillus altitudinis</i> 41KF2b (ASJC01000029)            | 1288/1289 (99.92)               | 2.00 $\pm$ 0.16                                         | -                            | 1.56 $\pm$ 0.16                                      | 14.50 $\pm$ 2.46                                  |
| GUM               | G1      | <i>Streptomyces qinglanensis</i> strain 172205 (HQ660227)    | 1332/1333 (99.92)               | 1.40 $\pm$ 0.20                                         | 20.02 $\pm$ 1.27             | 28.92 $\pm$ 0.62                                     | 524.05 $\pm$ 37.66                                |
|                   | G2      | <i>Rhodococcus cercidiphylli</i> YIM 65003 (EU325542)        | 1203/1220 (98.61)               | 24.07 $\pm$ 0.77                                        | -                            | 28.34 $\pm$ 0.31                                     | 501.03 $\pm$ 23.63                                |
|                   | G3      | <i>Streptomyces anulatus</i> NRRL B-2000 (DQ026637)          | 1329/1329 (100.00)              | 0.94 $\pm$ 0.09                                         | 21.65 $\pm$ 0.46             | 23.11 $\pm$ 1.00                                     | 388.15 $\pm$ 26.31                                |
|                   | G4      | <i>Bacillus altitudinis</i> 41KF2b (ASJC01000029)            | 1353/1354 (99.93)               | 1.16 $\pm$ 0.05                                         | -                            | 3.97 $\pm$ 0.35                                      | 8.83 $\pm$ 3.63                                   |
|                   | G5      | <i>Paenibacillus ogarexensis</i> DSM 1327 (KF479658)         | 1377/1439 (97.55)               | 1.24 $\pm$ 0.03                                         | -                            | 2.72 $\pm$ 0.36                                      | 11.67 $\pm$ 1.43                                  |
| MOO               | M1      | <i>Streptomyces albus</i> NBRC 13014 (NR118467)              | 1242/1242 (100.00)              | 0.88 $\pm$ 0.05                                         | -                            | 28.11 $\pm$ 0.35                                     | 278.43 $\pm$ 14.05                                |
|                   | M2      | <i>Pseudomonas geniculata</i> ATCC 19374 (AB021404)          | 1307/1309 (99.85)               | 1.13 $\pm$ 0.09                                         | 34.32 $\pm$ 0.80             | 3.03 $\pm$ 0.48                                      | 31.22 $\pm$ 9.86                                  |

Note: Type strains with the highest 16S rDNA sequence similarity (GenBank accession number) are given. -, not detected.

**FIGURE 5** Responses of plant seedlings to isolated bacterial endophytes in the *in vitro* plate assays. The shoot and root growth was calculated as the difference in shoot and root length during a 5-day incubation of seedlings and a bacterial strain in the same plate. Averages of shoot and root growth and standard errors are given. (a) and (b) are the responses of *Arabidopsis thaliana* and (c) and (d) are the responses of *Capsella bursa-pastoris*. (e) is photographs of *C. bursa-pastoris* grown with (right) and without (left) *B. aryabhattai* inoculation (arrow). Abbreviations of bacterial strains are given in Table 1. Dunnett's multiple comparison was conducted between the control and each inoculated bacterium, and the significance levels of the differences are given. † $p < .10$ ; \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$



*geniculate* M2, was isolated in the culture-dependent method while Gammaproteobacteria was one of the dominant phyla in the NGS analysis.

Despite this limitation, only one (*S. haemolyticus* D4) out of fifteen isolates was assigned to bacterial species that have been previously reported as seed endophytes (Najnin et al., 2014). The other strains except *P. agarixedens* G5 were known to inhabit the rhizosphere or plant tissues other than seeds (Beneduzi et al., 2008; Gopalakrishnan et al., 2015; Hong et al., 2015; Hu et al., 2012; Kim et al., 2012; Qin et al., 2017; Verma et al., 2016; Wang et al., 2018). Given that SEB would be a subset of the bacterial community of phyllosphere or rhizosphere (Bertani et al., 2016; Cope-Selby et al., 2017), our result suggests testing more plant species would expand the current database of SEB.

Eleven out of fifteen isolates exhibited positive activity in testing assays. In particular, half of isolates (*B. aryabhattai* B1, *St. qinglanensis* D1 and G1, *St. olivaceus* D2, *R. cercidiphylli* G2, and *St. albus* M1) exhibited the phosphate-solubilizing activity that is comparable to other phosphate-solubilizing bacteria (Khan et al., 2014). This study examined whether isolated strains could dissolve tricalcium phosphate to determine phosphate-solubilizing activity. A limitation of this method is that it cannot measure the ability to dissolve metal phosphate, another common form of insoluble phosphate in soil (Bashan et al., 2013). Despite this limitation, the tricalcium phosphate method has been widely adopted in many studies as a useful screening method to identify candidate bacterial strains facilitating plant use of insoluble phosphates (Mahdi et al., 2021; Varga et al., 2020).

All bacterial isolates with phosphate-solubilizing activity produced 20.66–28.92% unit siderophores. *Pseudomonas* strains producing a similar quantity of siderophore were shown to promote the shoot and root growth in rice, wheat, or bottle gourd (Agrawal et al., 2017). Siderophores excreted from bacterial cells can sequester ferric ions to facilitate plant absorption (Glick, 2012). In addition, siderophores can act as chelating agents for  $\text{Ca}^{2+}$  or metal ions that make phosphate ions insoluble (Wang et al., 2018). Siderophore production of isolates could synergistically contribute to their capacity of phosphate solubilization.

Six isolates exhibited a positive activity in the ACC deaminase assay. However, the value of ACC deaminase activity was lower than that of other *Streptomyces* spp. with 57–354 nmol  $\alpha$ -KB/mg protein/h or *Pseudomonas* spp. with 1400–40870 nmol  $\alpha$ -KB/mg protein/h (Agrawal et al., 2017; El-Tarabily, 2008). In response to environmental stresses, the ethylene level in a plant increases to reduce plant growth (Pierik et al., 2006). ACC deaminase can block the ethylene production of plants by cleaving ACC, the immediate precursor of ethylene, into  $\alpha$ -ketobutyrate and ammonia (Glick, 2012; Penrose & Glick, 2003). Consequently, ACC deaminase can ameliorate an adverse effect of environmental stress on plant growth. Notably, winter annuals like *C. bursa-pastoris* often exhibit the stress avoidance strategy, such that they finish their life cycle before facing stressful environmental conditions like hot and dry summer (Crawley, 1997). Thus, the advantage of winter annual plants might be small by harboring bacteria with high ACC deaminase activity. In an evolutionary ecological perspective, it would be of interest to examine whether plant life histories correlate with the abundance of SEB with ACC deaminase activities.

### 4.3 | Bacterial strains stimulating the growth of *C. bursa-pastoris* or *A. thaliana*

Four isolated strains (*B. aryabhattai* B1, *St. griseoplanus* D3, *St. anulatus* G3, and *St. albus* M1) stimulated shoot growth of *C. bursa-pastoris* (Figure 5). In previous studies, strains of those species were isolated from the rhizosphere of plants and shown to promote the growth of crop plant species (Bhattacharyya et al., 2017; Boubekri et al., 2021; Subramaniam et al., 2020; Wang et al., 2018). This study showed that some strains of four species could migrate into seed.

It is well acknowledged that soil microbiome can facilitate weed establishment (Trognitz et al., 2016). However, the ecological significance of SEB in weedy species has gained attention just recently. For instance, SEB can contribute to the seed germination and growth in invasive *Phragmites australis* (White et al., 2018), drought resistance in *Lactuca serriola* (Jeong et al., 2021), and the competitive ability by antagonistic effects on the competitor species (Elmore et al., 2019). Although soil microbiome has been postulated as a major source of mutualistic microorganisms for weedy plant species (Trognitz et al., 2016), our results imply that SEB might be another source of mutualistic microorganisms.

Among four strains stimulating *C. bursa-pastoris* shoot growth, *B. aryabhattai* G1, *St. anulatus* G3, and *St. albus* M1 produced siderophore and soluble phosphate (Figure 4, Table 1), suggesting those PGP traits likely play an important role in promoting the seedling growth. All those species are known to have the phosphate-solubilizing ability (Bhattacharyya et al., 2017; Boubekri et al., 2021; Subramaniam et al., 2020). In contrast, one isolate (*St. griseoplanus* D3) exhibited low activities in all testing PGP substances. Given that the phosphate-solubilization activity of *St. griseoplanus* depends on growth media (Wang et al., 2018), *St. griseoplanus* D3 might increase its activity in the Murashige and Skoog (MS) agar used for plant growth test. Alternately, other PGP mechanisms might contribute to plant growth. For instance, the production of gibberellins or volatile compounds is also suggested to enhance plant growth (Glick, 2012), although we did not test those characteristics.

Even though they are phylogenetically close relatives (Beilstein et al., 2008), seedlings of *A. thaliana* and *C. bursa-pastoris* reacted differently to the same isolated strains. Given the complex interaction between host plant species and endophytic bacteria (Carvalho et al., 2016), the PGP activity of endophytic bacteria has been hypothesized to depend on the host plant species (Long et al., 2008). Interestingly, a few bacterial strains isolated from *C. bursa-pastoris* exhibited the ability to promote the seedling growth of *A. thaliana*, suggesting that some endophytic bacteria can promote the growth of nonhost plant species. Endophyte host specificity does not necessarily exclude the possibility that endophytic bacteria can stimulate the growth of nonhost plant species. Although some endophytic bacteria could have PGP activities on a broad range of plant species, the mechanism of PGP activity likely differs among plant species (Long et al., 2008; Ma et al., 2011).

This study conducted in vitro seedling tests and showed that seven out of fifteen bacterial isolates stimulated shoot growth of

either *C. bursa-pastoris* or *A. thaliana*. However, it should be noted that in vitro tests might have a limited power to evaluate the diverse effects of endophytic bacteria on plant growth. For instance, we did not directly infect bacteria into individual plants, but the effects of endophytic bacteria might depend on their location inside the plant body and the developmental stage of the host plants (Truyens et al., 2015). In addition, the in vitro seedling test cannot evaluate plant tolerance to biotic and abiotic environmental stresses. We are conducting additional in vivo studies to examine plant performance in natural environments using seeds directly inoculated by isolated bacteria. The results are expected to provide more clear evidence on the interaction between endophytic bacteria and plant performance.

## 5 | CONCLUSIONS

This study revealed that the bacterial communities inside seeds differed among *C. bursa-pastoris* natural populations, indicating the diversity of SEB should be evaluated at the population level. Isolated bacteria from seeds produced a relatively high amount of PGP substances, and four strains stimulated shoot growth of *C. bursa-pastoris*. This result implies that SEB in addition to soil microorganisms can be a source of mutualistic microorganisms facilitating seedling establishment that is a critical fitness component of weedy plant species.

## ACKNOWLEDGMENTS

The authors thank Eujin Kim for help in measuring the morphological traits of plants. The authors also thank the associate editor and two anonymous reviewers for their valuable comments on the manuscript. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2019R1F1A1058025, NRF-2021R1I1A2047635)

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTION

**Byungwook Choi:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Methodology (lead); Writing – original draft (lead). **Seorin Jeong:** Data curation (supporting); Formal analysis (supporting); Methodology (supporting). **Eunsuk Kim:** Conceptualization (equal); Data curation (supporting); Funding acquisition (lead); Methodology (supporting); Supervision (lead); Writing – original draft (supporting); Writing – review & editing (lead).

## DATA AVAILABILITY STATEMENT

All data and R code for the analyses for this article are available in Dryad (<https://datadryad.org/stash/share/ANRIA2-7vado2aluuZvLhwwnrZVINTdqCUCjPF5sLQ0>, <https://datadryad.org/stash/share/ANRIA2-7vado2aluuZvLhwwnrZVINTdqCUCjPF5sLQ0>).

## ORCID

Eunsuk Kim  <https://orcid.org/0000-0001-8059-7334>

## REFERENCES

- Agrawal, T., Kotasthane, A. S., Kosharia, A., Kushwah, R., Zaidi, N. W., & Singh, U. (2017). Crop specific plant growth promoting effects of ACCd enzyme and siderophore producing and cynogenic fluorescent *Pseudomonas*. *3 Biotech*, 7, 27.
- Allen, H. K., Bayles, D. O., Looft, T., Trachsel, J., Bass, B. E., Alt, D. P., Bearson, S. M. D., Nicholson, T., & Casey, T. A. (2016). Pipeline for amplifying and analyzing amplicons of the V1–V3 region of the 16S rRNA gene. *BMC Research Notes*, 9, 380.
- Bashan, Y., Kamnev, A. A., & de-Bashan, L. E. (2013). Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: A proposal for an alternative procedure. *Biology and Fertility of Soils*, 49, 465–479. <https://doi.org/10.1007/s00374-012-0737-7>
- Beckers, B., Op De Beeck, M., Thijs, S., Truyens, S., Weyens, N., Boerjan, W., & Vangronsveld, J. (2016). Performance of 16S rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Frontiers in Microbiology*, 7, 650. <https://doi.org/10.3389/fmicb.2016.00650>
- Beilstein, M. A., Al-Shehbaz, I. A., Mathews, S., & Kellogg, E. A. (2008). Brassicaceae phylogeny inferred from phytochrome A and ndhF sequence data: tribes and trichomes revisited. *American Journal of Botany*, 95(10), 1307–1327.
- Beneduzi, A., Peres, D., da Costa, P. B., Bodanese Zanettini, M. H., & Passaglia, L. M. (2008). Genetic and phenotypic diversity of plant-growth-promoting *bacilli* isolated from wheat fields in southern Brazil. *Research in Microbiology*, 159(4), 244–250. <https://doi.org/10.1016/j.resmic.2008.03.003>
- Bertani, I., Abbruscato, P., Piffanelli, P., Subramoni, S., & Venturi, V. (2016). Rice bacterial endophytes: Isolation of a collection, identification of beneficial strains and microbiome analysis. *Environmental Microbiology Reports*, 8(3), 388–398. <https://doi.org/10.1111/1758-2229.12403>
- Bhattacharyya, C., Bakshi, U., Mallick, I., Mukherji, S., Bera, B., & Ghosh, A. (2017). Genome-guided insights into the plant growth promotion capabilities of the physiologically versatile *Bacillus aryabhattai* strain AB211. *Frontiers in Microbiology*, 8, 411.
- Boubekri, K., Soumare, A., Mardad, I., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., & Kouisni, L. (2021). The screening of potassium- and phosphate-solubilizing Actinobacteria and the assessment of their ability to promote wheat growth parameters. *Microorganisms*, 9, 470. <https://doi.org/10.3390/microorganisms9030470>
- Card, S. D., Hume, D. E., Roodi, D., McGill, C. R., Millner, J. P., & Johnson, R. D. (2015). Beneficial endophytic microorganisms of *Brassica*—A review. *Biological Control*, 90, 102–112. <https://doi.org/10.1016/j.biocontrol.2015.06.001>
- Carvalho, T. L., Ballesteros, H. G., Thiebaut, F., Ferreira, P. C., & Hemerly, A. S. (2016). Nice to meet you: Genetic, epigenetic and metabolic controls of plant perception of beneficial associative and endophytic diazotrophic bacteria in non-leguminous plants. *Plant Molecular Biology*, 90(6), 561–574. <https://doi.org/10.1007/s11103-016-0435-1>
- Choi, B., Jeong, H., & Kim, E. (2019). Phenotypic plasticity of *Capsella bursa-pastoris* (Brassicaceae) and its effect on fitness in response to temperature and soil moisture. *Plant Species Biology*, 34(1), 5–10.
- Coats, V. C., & Rumpho, M. E. (2014). The rhizosphere microbiota of plant invaders: An overview of recent advances in the microbiomics of invasive plants. *Frontiers in Microbiology*, 5, 368. <https://doi.org/10.3389/fmicb.2014.00368>
- Coombs, J. T., & Franco, C. M. M. (2003). Isolation and identification of Actinobacteria from surface-sterilized Wheat Roots. *Applied and Environmental Microbiology*, 69(9), 5603–5608. <https://doi.org/10.1128/AEM.69.9.5603-5608.2003>
- Cope-Selby, N., Cookson, A., Squance, M., Donnison, I., Flavell, R., & Farrar, K. (2017). Endophytic bacteria in *Miscanthus* seed: Implications for germination, vertical inheritance of endophytes, plant evolution and breeding. *GCB Bioenergy*, 9(1), 57–77. <https://doi.org/10.1111/gcbb.12364>
- Crawley, M. J. (1997). *Plant ecology*, 2nd ed. Blackwell Science Ltd.
- Dovana, F., Mucciarelli, M., Mascarello, M., & Fusconi, A. (2015). *In vitro* morphogenesis of *Arabidopsis* to search for novel endophytic fungi modulating plant growth. *PLoS One*, 10(12), e0143353. <https://doi.org/10.1371/journal.pone.0143353>
- Elmore, M. T., White, J. F., Kingsley, K. L., Diehl, K. H., & Verma, S. K. (2019). *Pantoea* spp. associated with smooth crabgrass (*Digitaria ischaemum*) seed inhibit competitor plant species. *Microorganisms*, 7, 143.
- El-Tarabily, K. A. (2008). Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropan e-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant and Soil*, 308, 161–174. <https://doi.org/10.1007/s11104-008-9616-2>
- Fürnkranz, M., Lukesch, B., Muller, H., Huss, H., Grube, M., & Berg, G. (2012). Microbial diversity inside pumpkins: Microhabitat-specific communities display a high antagonistic potential against phytopathogens. *Microbial Ecology*, 63(2), 418–428. <https://doi.org/10.1007/s00248-011-9942-4>
- Gagne-Bourgue, F., Aliferis, K. A., Seguin, P., Rani, M., Samson, R., & Jabaji, S. (2013). Isolation and characterization of indigenous endophytic bacteria associated with leaves of switchgrass (*Panicum virgatum* L.) cultivars. *Journal of Applied Microbiology*, 114(3), 836–853.
- Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012, 963401. <https://doi.org/10.6064/2012/963401>
- Gopalakrishnan, S., Srinivas, V., Prakash, B., Sathya, A., & Vijayabharathi, R. (2015). Plant growth-promoting traits of *Pseudomonas geniculata* isolated from chickpea nodules. *3 Biotech*, 5(5), 653–661. <https://doi.org/10.1007/s13205-014-0263-4>
- Granér, G., Persson, P., Meijer, J., & Alström, S. (2003). A study on microbial diversity in different cultivars of *Brassica napus* in relation to its wilt pathogen, *Verticillium longisporum*. *FEMS Microbiology Letters*, 224(2), 269–276.
- Hardoim, P. R., Hardoim, C. C., van Overbeek, L. S., & van Elsas, J. D. (2012). Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS One*, 7(2), e30438. <https://doi.org/10.1371/journal.pone.0030438>
- Hewitson, T. D., Wigg, B., & Becker, G. J. (2010). Tissue preparation for histochemistry: fixation, embedding, and antigen retrieval for light microscopy. In T. Hewitson & I. Darby (Eds.), *Histology protocols* (pp. 3–18). Springer.
- Hong, C. E., Jo, S. H., Moon, J. Y., Lee, J.-S., Kwon, S.-Y., & Park, J. M. (2015). Isolation of novel leaf-inhabiting endophytic bacteria in *Arabidopsis thaliana* and their antagonistic effects on phytopathogens. *Plant Biotechnology Reports*, 9(6), 451–458.
- Hu, H., Lin, H.-P., Xie, Q., Li, L., Xie, X.-Q., & Hong, K. (2012). *Streptomyces qinglanensis* sp. nov., isolated from mangrove sediment. *International Journal of Systematic and Evolutionary Microbiology*, 62, 596–600.
- Huang, H.-R., Yan, P.-C., Lascoux, M., & Ge, X.-J. (2012). Flowering time and transcriptome variation in *Capsella bursa-pastoris* (Brassicaceae). *New Phytologist*, 194(3), 676–689.
- Ikeda, S., Kaneko, T., Okubo, T., Rallos, L. E., Eda, S., Mitsui, H., Sato, S., Nakamura, Y., Tabata, S., & Minamisawa, K. (2009). Development of a bacterial cell enrichment method and its application to the community analysis in soybean stems. *Microbial Ecology*, 58(4), 703–714. <https://doi.org/10.1007/s00248-009-9566-0>

- Jeong, S., Kim, T.-M., Choi, B., Kim, Y., & Kim, E. (2021). Invasive *Lactuca serriola* seeds contain endophytic bacteria that contribute to drought tolerance. *Scientific Reports*, 11, 1–12. <https://doi.org/10.1038/s41598-021-92706-x>
- Johnston-Monje, D., & Raizada, M. N. (2011). Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One*, 6(6), e20396. <https://doi.org/10.1371/journal.pone.0020396>
- Kaga, H., Mano, H., Tanaka, F., Watanabe, A., Kaneko, S., & Morisaki, H. (2009). Rice seeds as sources of endophytic bacteria. *Microbes and Environments*, 24(2), 154–162. <https://doi.org/10.1264/jsme2.ME09113>
- Khan, M. S., Zaidi, A., & Ahmad, E. (2014). Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. In M. S. Khan, A. Zaidi & J. Musarrat (Eds.), *Phosphate solubilizing microorganisms* (pp. 31–62). Springer.
- Kim, T. U., Cho, S. H., Han, J. H., Shin, Y. M., Lee, H. B., & Kim, S. B. (2012). Diversity and physiological properties of root endophytic actinobacteria in native herbaceous plants of Korea. *Journal of Microbiology*, 50(1), 50–57. <https://doi.org/10.1007/s12275-012-1417-x>
- Kukkurainen, S., Leino, A., Vähämäki, S., Kärkkäinen, H. R., Ahanen, K., Sorvari, S., Rugienius, R., & Toldi, O. (2005). Occurrence and location of endophytic bacteria in garden and wild strawberry. *HortScience*, 40, 348–352. <https://doi.org/10.21273/HORTSCI.40.2.348>
- Linhart, Y. B., & Grant, M. C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, 27(1), 237–277. <https://doi.org/10.1146/annurev.ecolsys.27.1.237>
- Liu, Y., Xu, P., Yang, F., Li, M., Yan, H., Li, N., Zhang, X., & Wang, W. (2019). Composition and diversity of endophytic bacterial community in seeds of super hybrid rice 'Shenliangyou 5814' (*Oryza sativa* L.) and its parental lines. *Plant Growth Regulation*, 87(2), 257–266. <https://doi.org/10.1007/s10725-018-0467-4>
- Long, H. H., Schmidt, D. D., & Baldwin, I. T. (2008). Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PLoS One*, 3(7), e2702. <https://doi.org/10.1371/journal.pone.0002702>
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2011). Inoculation of endophytic bacteria on host and non-host plants—Effects on plant growth and Ni uptake. *Journal of Hazardous Materials*, 195, 230–237. <https://doi.org/10.1016/j.jhazmat.2011.08.034>
- Maggini, V., Bandeira Reidel, R. V., De Leo, M., Mengoni, A., Gallo, E. R., Miceli, E., Biffi, S., Fani, R., Firenzuoli, F., Bogani, P., & Pistelli, L. (2020). Volatile profile of *Echinacea purpurea* plants after in vitro endophyte infection. *Natural Product Research*, 34, 2232–2237.
- Mahdi, I., Hafidi, M., Allaoui, A., & Biskri, L. (2021). Halotolerant endophytic bacterium *Serratia rubidaea* ED1 enhances phosphate solubilization and promotes seed germination. *Agriculture*, 11, 224. <https://doi.org/10.3390/agriculture11030224>
- Murakami, C., Tanaka, A. R., Sato, Y., Kimura, Y., & Morimoto, K. (2021). Easy detection of siderophore production in diluted growth media using an improved CAS reagent. *Journal of Microbiological Methods*, 189, 106310.
- Najnin, R. A., Shafrin, F., Polash, A. H., Zaman, A., Hossain, A., Taha, T., Ahmed, R., Tuli, J. F., Baua, R., Sajib, A. A., & Khan, H. (2014). A diverse community of jute (*Corchorus* spp.) endophytes reveals mutualistic host-microbe interactions. *Annals of Microbiology*, 65(3), 1615–1626.
- Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, 170, 265–270. <https://doi.org/10.1111/j.1574-6968.1999.tb13383.x>
- Neuffer, B. (2011). Native range variation in *Capsella bursa-pastoris* (Brassicaceae) along a 2500 km latitudinal transect. *Flora*, 206(2), 107–119. <https://doi.org/10.1016/j.flora.2010.03.001>
- Pal, G., Kumar, K., Verma, A., White, J. F., & Verma, S. K. (2019). Functional roles of seed-inhabiting endophytes of rice. In S. K. Verma & J. F. White (Eds.), *Seed endophytes* (pp. 213–236). Springer.
- Patten, C., & Glick, B. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, 78, 3795–3801.
- Penrose, D. M., & Glick, B. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum*, 118, 10–15. <https://doi.org/10.1034/j.1399-3054.2003.00086.x>
- Pérez-Jaramillo, J. E., Mendes, R., & Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology*, 90, 635–644. <https://doi.org/10.1007/s11103-015-0337-7>
- Pierik, R., Tholen, D., Poorter, H., Visser, E. J. W., & Voeseek, L. A. C. J. (2006). The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science*, 11, 176–183. <https://doi.org/10.1016/j.tplants.2006.02.006>
- Puente, M. E., Li, C. Y., & Bashan, Y. (2009). Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environmental and Experimental Botany*, 66(3), 402–408. <https://doi.org/10.1016/j.envexpbot.2009.04.007>
- Qin, S., Feng, W.-W., Wang, T.-T., Ding, P., Xing, K., & Jiang, J.-H. (2017). Plant growth-promoting effect and genomic analysis of the beneficial endophyte *Streptomyces* sp. KLBMP 5084 isolated from halophyte *Limonium sinense*. *Plant and Soil*, 416(1–2), 117–132.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590–D596. <https://doi.org/10.1093/nar/gks1219>
- R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation.
- Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda Mdel, C., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, 183, 92–99. <https://doi.org/10.1016/j.micres.2015.11.008>
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., & Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–7541. <https://doi.org/10.1128/AEM.01541-09>
- Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160, 47–56. [https://doi.org/10.1016/0003-2697\(87\)90612-9](https://doi.org/10.1016/0003-2697(87)90612-9)
- Shahzad, R., Khan, A. L., Bilal, S., Asaf, S., & Lee, I. J. (2018). What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. *Frontiers in Plant Science*, 9, 24. <https://doi.org/10.3389/fpls.2018.00024>
- Subramaniam, G., Thakur, V., Saxena, R. K., Vadlamudi, S., Purohit, S., Kumar, V., Rathore, A., Chitkineni, A., & Varshney, R. K. (2020). Complete genome sequence of sixteen plant growth promoting *Streptomyces* strains. *Scientific Reports*, 10, 10294. <https://doi.org/10.1038/s41598-020-67153-9>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. <https://doi.org/10.1093/molbev/mst197>

- Trognitz, F., Hackl, E., Widhalm, S., & Sessitsch, A. (2016). The role of plant-microbiome interactions in weed establishment and control. *FEMS Microbiology Ecology*, 92(10), fiw138. <https://doi.org/10.1093/femsec/fiw138>
- Truyens, S., Beckers, B., Thijs, S., Weyens, N., Cuypers, A., & Vangronsveld, J. (2016). The effects of the growth substrate on cultivable and total endophytic assemblages of *Arabidopsis thaliana*. *Plant and Soil*, 405(1), 325–336. <https://doi.org/10.1007/s11104-015-2761-5>
- Truyens, S., Weyens, N., Cuypers, A., & Vangronsveld, J. (2013). Changes in the population of seed bacteria of transgenerationally Cd-exposed *Arabidopsis thaliana*. *Plant Biology*, 15(6), 971–981.
- Truyens, S., Weyens, N., Cuypers, A., & Vangronsveld, J. (2015). Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports*, 7(1), 40–50. <https://doi.org/10.1111/1758-2229.12181>
- Varga, T., Hixson, K. K., Ahkami, A. H., Sher, A. W., Barnes, M. E., Chu, R. K., Battu, A. K., Nicora, C. D., Winkler, T. E., Reno, L. R., Fakra, S. C., Antipova, O., Parkinson, D. Y., Hall, J. R., & Doty, S. (2020). Endophyte-promoted phosphorus solubilization in *Populus*. *Frontiers in Plant Science*, 11, 1585–1600. <https://doi.org/10.3389/fpls.2020.567918>
- Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A. K., & Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of *Bacilli* associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *Journal of Basic Microbiology*, 56(1), 44–58.
- Wang, C., Cui, J., Yang, L., Zhao, C. A., Wang, T., Yan, L., & Liu, S. (2018). Phosphorus-release dynamics by phosphate solubilizing actinomycetes and its enhancement of growth and yields in maize. *International Journal of Agriculture and Biology*, 20, 437–444. <https://doi.org/10.17957/IJAB/15.0554>
- Wassermann, B., Adam, E., Cernava, T., & Berg, G. (2019). Understanding the indigenous seed microbiota to design bacterial seed treatments. In S. K. Verma & J. F. White (Eds.), *Seed Endophytes: Biology and Biotechnology* (pp. 83–99). Springer.
- White, J. F., Kingsley, K. I., Kowalski, K. P., Irizarry, I., Micci, A., Soares, M. A., & Bergen, M. S. (2018). Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (*Phragmites australis*). *Plant and Soil*, 422, 195–208. <https://doi.org/10.1007/s11104-016-3169-6>
- White, J. F., Kingsley, K. L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M. T., Verma, S. K., Gond, S. K., & Kowalski, K. P. (2019). Endophytic microbes and their potential applications in crop management. *Pest Management Science*, 75, 2558–2565.
- Xu, M., Sheng, J., Chen, L., Men, Y., Gan, L., Guo, S., & Shen, L. (2014). Bacterial community compositions of tomato (*Lycopersicon esculentum* Mill.) seeds and plant growth promoting activity of ACC deaminase producing *Bacillus subtilis* (HYT-12-1) on tomato seedlings. *World Journal of Microbiology and Biotechnology*, 30(3), 835–845.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Choi, B., Jeong, S., & Kim, E. (2022). Variation of the seed endophytic bacteria among plant populations and their plant growth-promoting activities in a wild mustard plant species, *Capsella bursa-pastoris*. *Ecology and Evolution*, 12, e8683. <https://doi.org/10.1002/ece3.8683>