Synergistic Effects of Seed Endophytic Bacterial Consortia from *Lactuca serriola* on Phosphorus Solubilization

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Abstract

Plants have evolved in association with plant-beneficial bacterial endophytes. Some of these bacteria live inside seed, known as seed endophytes, are vertically transmitted to the next generation and contribute to early seedling development. In this study, we aimed to assess the plant growth-promoting (PGP) potential of these seed endophytes. To better simulate natural conditions, we constructed bacterial consortia to evaluate their synergistic effects. From the seed endophytes of *Lactuca serriola*, designated as a harmful non-indigenous plant in South Korea, we selected seven bacterial strains. These strains and their consortia were screened for four key PGP traits. Among them, two strains that played a key role in consortium function were selected and co-inoculated with different isolates in *L. serriola* to assess their impact under phosphorus deficiency. The *Kosakonia cowanii* SD1 and *Pantoea dispersa* SD25 consortium, and *Xanthomonas* spp. SD2 and *Stenotrophomonas maltophilia* SD8 consortium enhanced soil phosphorus availability. Notably, these two consortia displayed contrasting results in *in vitro* assays, suggesting that these combinations utilize different mechanisms to enhance soil phosphorus availability. These findings provide valuable insights into the role of seed endophytes in the adaptability and ecological success of non-indigenous plants, contributing to a better understanding of invasive plant management.

Introduction

Seed endophytes

Endophytes are non-pathogenic microbes that live within plant tissues without causing disease. They are known for plant growth-promoting (PGP) traits, including hormone modulation, nutrient acquisition, siderophore production, and stress mitigation [1]. Among them, seed endophytes resides in seeds and are often transmitted across generations [2]. Their early association enables them to support germination and initial plant development.

***Lactuca serriola* (Prickly lettuce)**

Lactuca serriola is an invasive plant originally from Europe, first reported in South Korea in 1978 [3]. Its rapid spread is driven by high seed production, vegetative propagation, and drought resistance [4]. It was designated as a harmful nonindigenous plant by the Korean Ministry of Environment in 2012.

Phosphate limitation

Phosphorus (P) is an essential macronutrient for plant growth, but is often limited in soil due to low bioavailability. Under phosphate deficiency, plants increasingly depend on microbial partners to mobilize insoluble phosphates [5]. Seed endophytes may play a key role in enhancing phosphorus acquisition under such nutrient stress conditions.

Objectives

- To characterize the plant growth-promoting traits of seed endophytic bacteria isolated from *L. serriola*.
- To evaluate the synergistic effects of bacterial consortia on phosphate solubilization.
- To assess the functional performance of consortia under phosphate-deficient conditions in *L. serriola*.

Methods

Figure 1. Wild *Lactuca serriola* and its seeds. In this study, seed sources was the Seongdong-ri (SD), Muan-gun, Korea population.

Seed endophytic bacteria

- Seven isolates from SD wild population (SDb)

SD1 : *Kosakonia cowanii*
SD2 : *Xanthomonas* spp.
SD7 : *Cronobacter dublinensis* subsp. *lausannensis*
SD8 : *Stenotrophomonas maltophilia*
SD10: *Pseudomonas* spp.
SD13: *Paenibacillus hunanensis*
SD25: *Pantoea dispersa*

Construction of bacterial consortia

- Single treatment
  - Individual 7 single isolates
- Dropout treatment
  - All except one (All – SDx)
- All treatment
  - Full 7-strain consortium

Quantification of PGP traits

- Four PGP traits were measured

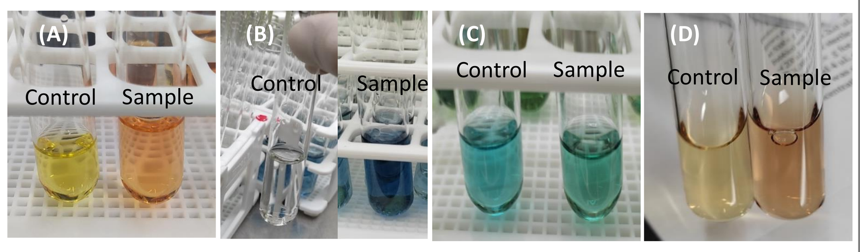

Figure 2. Colorimetric analysis of PGP test. (A) Indole-3-acetic acid (IAA) production. (B) Phosphate solubilization property (PPS). (C) Siderophore production (SPP). (D) 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity.

Dual-strain assay

- Selected strains (SD1 and SD2) were combined with other isolates to evaluate their synergistic effects on PGP traits and plant responses under phosphate-deficient conditions
  - Phosphate-deficient conditions simulated by adding tricalcium phosphate (TCP) to Hoagland solution.
  - Plant performance and soil properties were measured.

Results

PGP trait responses in consortia treatments

K. cowanii SD1 and *Xanthomonas* spp. SD2 were key contributors, as their exclusion significantly reduced phosphate solubilization.

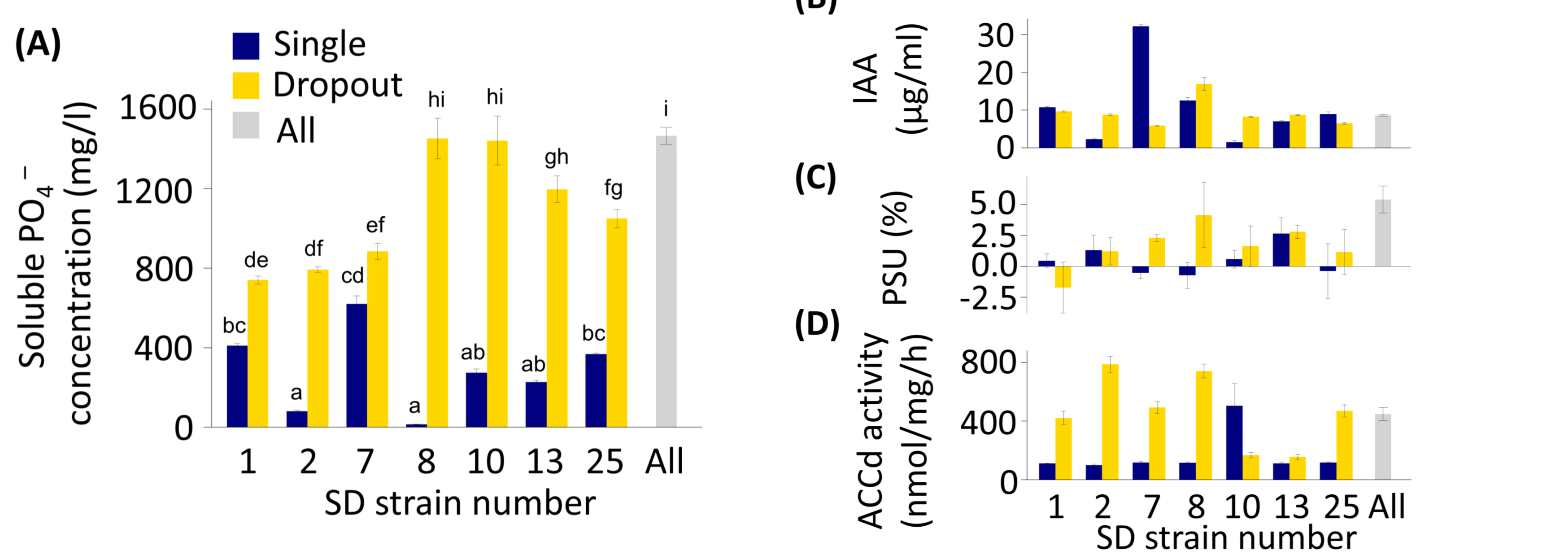

Figure 3. Quantitative PGP traits of SD source isolates. (A) Phosphate solubilizing property. (B) IAA production. (C) Siderophore production. (D) ACC deaminase activity. The numbers on the x-axis indicate the strain names of SD source bacterial isolates, with “All” representing the full consortium of all selected strains. Each bar represents the mean ± standard error. For phosphate solubilizing property, different letters indicate significant differences ($p < 0.05$) among bacterial strains and consortia compositions based on Tukey’s multiple comparison tests.

Results

Effects of dual-strain inoculation under phosphate deficiency

Consortia treatments outperformed individual isolates in enhancing phosphorus availability. Notable, *K. cowanii* SD1 + *P. dispersa* SD25 and *Xanthomonas* spp. SD2 + *S. maltophilia* SD8 combinations showed strong synergistic effects under phosphate-deficient conditions. *K. cowanii* SD1 and *P. dispersa* SD25 consortia enhanced phosphate solubilization both *in planta* and *in vitro* assays, suggesting direct microbial activity. In contrast, *Xanthomonas* spp. SD2 and *S. maltophilia* SD8 combination improved soil available phosphorus without corresponding *in vitro* effects, implying plant-mediated or rhizosphere-associated mechanism.

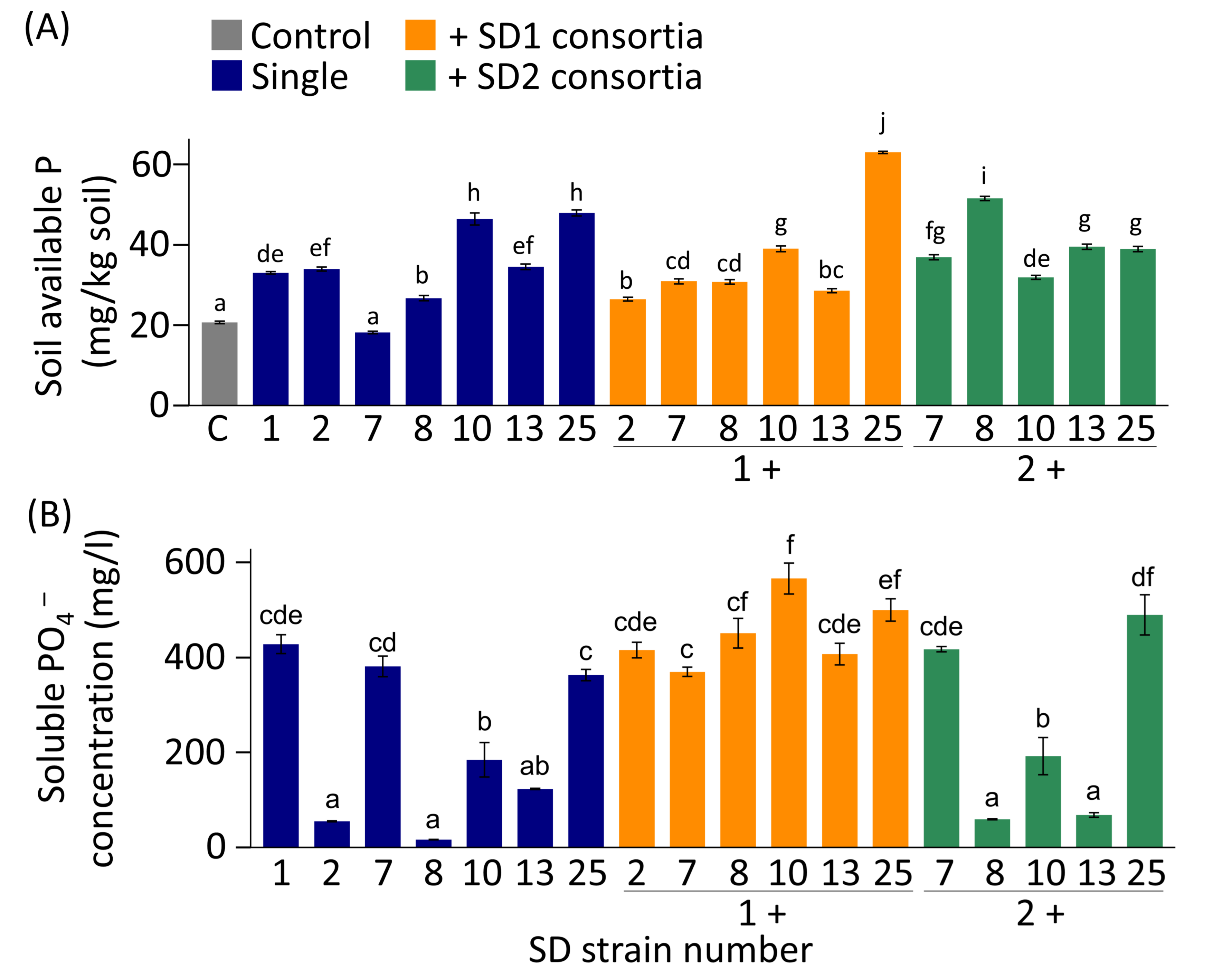

Figure 4. Effects of SD1-based and SD2-based dual-strain consortia and single SD source strains. (A) Soil available phosphorus measured from *in planta* experiments. (B) Quantitative phosphate solubilization property measured in *in vitro* phosphate solubilization assays. The numbers on the x-axis indicate the specific strains included in each treatment, and “C” represents the uninoculated control. Each bar represents the mean ± standard error. Different letters indicate significant differences ($p < 0.05$) among bacterial treatments based on Tukey’s multiple comparison tests.

These distinct patterns indicate that enhanced phosphorus availability may result from complex plant-microbe-microbe interactions that stimulate nutrient mobilization in the rhizosphere. These findings highlight the importance of complementary microbial interactions in consortium design and the potential application of seed endophyte-based inoculants for improving phosphorus availability under nutrient-deficient conditions.

Conclusion

- Seed endophytic consortia from *L. serriola* showed enhanced phosphate solubilization compared to individual isolates.
- Specific dual-strain combinations exhibited synergistic effects under phosphate-deficient conditions, which appear to operate through distinct microbial- and plant-mediated mechanisms.
- Microbial interaction and strain composition are critical factors in determining consortium performance.
- Seed endophyte-based consortia can serve as promising bio-inoculants for improving nutrient acquisition under low-phosphate environments.

Acknowledgement

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (No. RS-2024-00456189).

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