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Isolation and Characterization of the High Silicate and Phosphate Solubilizing Novel Strain *Enterobacter ludwigii* GAK2 that Promotes Growth in Rice Plants

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Abstract: Silicon (Si) and phosphorus (P) are beneficial nutrient elements for plant growth. These elements are widely used in chemical fertilizers despite their abundance in the earth's crust. Excessive use of chemical fertilizers is a threat to sustainable agriculture. Here, we screened different Si and P solubilizing bacterial strains from the diverse rice fields of Daegu, Korea. The strain with high Si and P solubilizing ability was selected and identified as *Enterobacter ludwigii* GAK2 through 16S rRNA gene sequence analysis. The isolate GAK2 produced organic acids (citric acid, acetic acid, and lactic acid), indole-3-acetic acid, and gibberellic acid (GA_1, GA_3) in Luria-Bertani media. In addition, GAK2 inoculation promoted seed germination in a gibberellin deficient rice mutant *Waito-C* and rice cultivar 'Hwayoungbyeo'. Overall, the isolate GAK2 increased root length, shoot length, fresh biomass, and chlorophyll content of rice plants. These findings reveal that *E. ludwigii* GAK2 is a potential silicon and phosphate bio-fertilizer.

Keywords: *Enterobacter ludwigii* GAK2; silicon; phosphorus; solubilization; plant growth

1. Introduction

Plants absorb various mineral elements as nutrients for their ideal growth [1]. Among them, phosphorus (P) is considered one of the major elements required for plant survival [2]. Similarly, the beneficial effect of silicon (Si) for healthy crop production has been reported [3]. Although Si is considered a non-essential element, a recent study has proved its beneficial role in quality production especially in the case of hyper-Si accumulator plants such as rice [4]. Both Si and P elements are abundantly available in the earth's crust as silicate and phosphate, respectively. However, their demand in a plant is fulfilled by an external fertilizer application. Excessive use of fertilizers causes hazardous effects in the natural ecological cycle that include phytotoxicity, water eutrophication, and several disorders associated with human health [5–7]. To mitigate these problems, the current study focused to meet the requirement of P and Si in a plant through the application of phosphate and silicate solubilizing bacteria to a phosphate- and silicate-based soil.

Microorganisms are reported as the major agents that contribute to mineral weathering [8]. Studies of mineral dissolution with cultures of fungi and bacteria have shown dramatic increases in the dissolution rates of minerals [9]. Microorganisms like *Proteobacteria*, *Aminobacter*, *Burkholderia*, *Collimonas*, *Janthinobacterium*, *Dyella*, and *Frateuria* have been reported to solubilize the biotite, which contains considerable amounts of silicate minerals [8]. Likewise, the strains *Enterobacter asburiae, Bacillus sp.*, *Pseudomonas sp.*, and *Burkholderia* are involved in phosphate solubilization [10]. Microorganism mediated phosphate dissolution occurs through the production of various enzymes, such as phosphatase and phytase [11]. However, the acidolysis phenomenon has been reported for the dissolution of silicates [12]. There is evidence of the potential role of phosphate solubilizing bacteria (PSB) in the enhancement of plant growth through P uptake by plants. However, the mechanism of silicate solubilization by silicate solubilizing bacteria (SSB) and uptake of silicon by plant lack sufficient information.

Microbial application as biofertilizer is gaining momentum in agriculture. Several microbial species have been reported for their beneficial impacts on the plant [13]. These microorganisms regulate various physiological functions in plants, which include germination, vegetative growth, cell division, biosynthesis of secondary metabolites, and mitigation of stresses [14]. Since SSB and PSB are involved in the dissolution of naturally available insoluble silicate and phosphate in agricultural soils, we hypothesized that these microbes could be employed as agents for the development of silicon- and phosphate-based fertilizers. Here, we describe the isolation, identification, and characterization of a novel strain that could promote rice plant growth through solubilization of both silicate ($Mg_2O_8Si_3$) and phosphate $Ca_3(PO_4)_2$.

2. Materials and Methods

2.1. Screening of Bacteria

Paddy soil and forest soil samples were collected from Gyeongbuk, South Korea, to isolate rhizosphere microbes. One gram of the soil sample was suspended in 9 mL of physiological saline (0.85% NaCl), diluted 6-fold, and used for bacterial isolation. Luria-Bertani (LB; Difco, Franklin Lakes, NJ, USA) agar medium was used to isolate the strains. One hundred microliters of the diluted sample solution was plated on solid medium and cultured at 30 °C for 3 days to isolate strains from the independent colonies. The isolated microorganisms were re-streaked and incubated in LB medium for 3 days.

2.2. Evaluation of the Ability to Solubilize Insoluble Silicate and Phosphate

The microorganisms isolated as described above were punched into petri plates with glucose agar medium (10 g L^{-1} glucose) containing 0.25% insoluble silicate (IS) ($Mg_2O_8Si_3$) to detect silicate solubilization and National Botanical Research Institute's Phosphate (NBRIP) medium with 0.25% insoluble phosphate (IP) [$Ca_3(PO_4)_2$] using a wood pick and cultured at 30 °C for 24–168 h [12,14]. The clear zone formed was identified and the diameter was measured. Among 100 screened isolates, 24 isolates with the maximum clear zone diameters were selected for further analysis.

2.3. Preliminary Bioanalysis Test

2.3.1. Detection of Indole-3-Acetic Acid (IAA)

For the detection of IAA, preliminary tests were performed in the 24 selected isolates using the Salkowski reagent [15]. In these tests, the bacterial broth culture was centrifuged at 8160× *g* (Centrifuge, Eppendorf 5415C, Hamburg, Germany) for 10 min and the supernatant was collected, followed by the addition of 0.5 mL of Salkowski reagent (50 mL 35% $HClO_4$, 1 mL 0.5 M $FeCl_3$) and then kept in the dark for 30 min. The change in the intensity of the pink color for each strain was noted on the basis of qualitative visual assessment.

2.3.2. Production of Ammonia

For the detection of ammonia, 100 µL of freshly grown culture of GAK2 in LB medium for 72 h was kept in tubes and diluted 100 times in double distilled water. LB broth was considered as control. Nessler reagent (0.5 mL) was added in the tube. The value was recorded by Ammonia medium range photometer (Range: 0.00 to 9.99 mg L^{-1}, MI405, Milwaukee, Szeged, Hungary).

2.3.3. Growth Promotion Test on Rice

The growth promotion test was performed on the gibberellin (GA) deficient rice dwarf mutant '*Waito*-C'. Seeds were placed on a petri plate (9 cm diameter) supplied with a filter paper and the bacterial cultures were inoculated and incubated at 28 °C in the growth chamber. To assess the growth promoting ability, root length, shoot length, and fresh plant biomass were measured.

2.4. Selection, Identification, and Phylogenetic Analysis of Microorganisms

The isolate with the largest clear zone diameter, maximum change in color intensity in the detection of IAA, and with the highest germination promoting ability, was selected for further analysis. After incubation at 30 °C for 3 days on LB solid medium, the selected strain was identified via polymerase chain reaction by amplification of 16S rRNA using the primer pair 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r (5′-TAC CTT GTT ACG ACT T-3′). The determined nucleotide sequence was subjected to homology analysis by searching through BLAST (http://www.ncbi.nlm.nih.gov/BLAST) Mega 6 version. The strain was registered on the National Center of Biotechnology Information with accession number KP676113.

2.5. Biochemical Analysis

2.5.1. Microorganism Culture

A 1% culture of the isolate was inoculated to 5 mL of LB medium in a test tube. A 1% culture of the isolate was inoculated to 250 mL of LB medium in a flask. The culture was incubated at 150 rpm, at 28 °C for two days. The optical density >1 of cell growth was measured at 660 nm using an ultraviolet-visible spectrophotometer (PG instrument T60U, Leicestershire, UK) and used for further experiment.

2.5.2. Quantification of Organic Acids

The organic acid was quantified by using the method described by Kang et al. [16]. In brief, the bacterial culture in the LB medium was centrifuged (Centrifuge, Eppendorf 5415C, Hamburg, Germany) 2040× g for 20 min. The culture supernatant was adsorbed using Sep-Pak (C18 cartridge, Waters, Milford, MA, USA), filtered through a 0.45-µm cellulose acetate membrane filter, and analyzed by high-performance liquid chromatography (HPLC; Waters 600, Milford, MA, USA) using a PL Hi-Plex H column (7.7 × 300 mm, Waters Co., Milford, MA, USA), detector refractive index (RI, Waters 410, Milford, MA, USA), and 5 mM H_2SO_4 as the solvent in distilled water. The flow rate was set to 0.6 mL min^{-1} with oven temperature at 65 °C, and the injection volume was 20 µL.

2.5.3. Analysis of IAA Production in *E. ludwigii* GAK2 Culture

The IAA content of the isolate culture was quantified according to the methods described by Lee et al. [17]. In brief, the organic layer obtained from the culture filtrate was vacuum-evaporated and the extracts were subjected to a reverse-phase C18 column. After the samples were dried through elution with methanol gradient (30%, 50%, and 100%), the methyl esters obtained from the sample were redissolved in ethyl acetate, and analyzed by gas chromatography-mass spectrometry. The IAA level was quantified using a standard peak value.

2.5.4. Extraction and Quantification of Gibberellin in GAK2 Culture

A protocol described by Lee et al. [17] was followed to extract and quantify GA content in bacterial culture. In Brief, *E. ludwigii* GAK2 was incubated in LB medium and GAs were obtained from its pure culture. The cultural filtrate was partitioned through ethyl acetate followed by the addition of 60% MeOH. The pH was adjusted to 8 and [$^2H_2$] GAs internal standard was added. The extracts obtained were dissolved in 60% MeOH and passed through Davisil column containing C18 powder. The eluent obtained was concentrated, dried to celite, and partitioned through SiO_2 column to extract the GAs. The extracts were eluted with 95:5 (v/v) ethyl acetate:hexane saturated with formic acid followed by drying and partition with phosphate buffer (pH 8). Formic acid was neutralized with 2 N NaOH and polyvinylpolypyrrolidone was added and stirred for 1 h. Thereafter, the pH was adjusted to 2.5 and the extract was portioned with ethyl acetate and vacuum-dried. The residue was separated using 100% MeOH and the solution obtained was dried. The GA fraction was prepared by passing the extract through HPLC. Detection of GA was conducted by injecting the GA fraction into a gas chromatograph coupled to a mass spectrometer (6890N network GC system, and 5973 network mass selective detector; Agilent Technologies). The retention time was determined by using Kovats retention index value of standard GAs.

2.6. Evaluation of the Effect of E. ludwigii GAK2 on Plant Growth

2.6.1. Pot Experiment

The pot experiment was conducted in a growth chamber (KGC-175 VH, KOENCON, Hanam, Korea) with a temperature of 28 °C during light hours and 22 °C during dark, photoperiod of 12/24 h, and relative humidity of 68 ± 4% at Kyungpook National University, Daegu, South Korea. Rice seeds of the cultivar 'Hwayoungbyeo', which were available in the Crop Genetics Laboratory of Kyungpook National University, were used for sowing. The rice seeds were sterilized by soaking in distilled water containing 0.01% Tebuconazole for 24 h and then thoroughly washed with distilled water. The seeds were allowed to germinate in a beaker containing distilled water and incubated for 3 days. The water was changed every day. The seeds were then sown in a sterilized nursery paddy field soil and allowed to grow for 2 weeks in a growth chamber. The soil was sterilized by autoclaving twice at 121 °C for 15 min. Two hundred grams of sterilized soil was filled in the pot (10 × 10 cm) and 0.4 g of either insoluble silicate ($Mg_2O_8Si_3$) or insoluble phosphate [$Ca_3(PO_4)_2$] was amended per pot (Appendix A). Approximately 100 mL of water was added to each pot and left over 24 h. After 24 h, pH was measured, and the seedlings were transplanted. The bacterial broth was prepared according to its standard optimal growth conditions and was diluted 10 times in distilled water. Approximately 50 mL of the diluted solution was inoculated in each pot after one week from transplantation, and then after one week from the first inoculation. The experimental setup included the Control (distilled water only), IS ($Mg_2O_8Si_3$), IP [$Ca_3(PO_4)_2$], GAK2 (Bacteria only), GAK2 + IS (bacteria combined with $Mg_2O_8Si_3$), and GAK2 + IP (bacteria combined with [$Ca_3(PO_4)_2$]) as treatments. Each treatment was replicated 8 times and each pot consisted of two rice seedlings. After 7 days from the second inoculation, the plants were uprooted and root soils were separated and immediately frozen in liquid nitrogen, stored in a cryogenic refrigerator, lyophilized, and homogenized with a grinder for analysis.

2.6.2. Analysis of Si Content of the Plant

The method described by Kang et al. [12] was followed to quantify the Si content of the plant. In brief, 0.5 g of the lyophilized crushed powder was soaked in 0.5 M HCl and rinsed through double distilled water before oven drying. A mixture of nitric acid, sulfuric acid, and perchloric acid (10:1:4 $v/v/v$) was subjected through the sample. The digested sample obtained was then analyzed by using the Inductively Coupled Plasma Mass Spectrometry (Optima 7900DV Perkin-Elmer, Waltham, MA, USA).

2.7. Determination of Soil pH and Electrical Conductivity (EC)

The pH of the soil was determined both before and after the pot experiment. Before the experiment, 200 g soil per pot (10 × 10 cm) was filled in six pots. A randomly selected amount (0.2–2 g) of either magnesium trisilicate ($Mg_2O_8Si_3$) or calcium phosphate $Ca_3(PO_4)_2$ was mixed in each pot, and the pH of each pot was noted. Likewise, after the experiment, samples from each treatment were collected and pH and EC were recorded. The method described by Jackson [18] was used for the determination of EC of soil, while the procedure described by Kalra [19] was used for the determination of pH. The EC measurements were taken using the conductivity meter (YSI Model 32), calibrated at 0.014 dS m^{-1} with 0.01 M potassium chloride (KCl).

2.8. Statistical Procedures for Data Analysis

The present study was conducted in a completely randomized design. The data were statistically analyzed using SAS 9.4 software (SAS Institute, Cary, NC, USA). Significant differences among treatments were determined using the least significant difference (LSD) method or Student's t-test where applicable. Average values were expressed as mean ± SD, and $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Isolation Selection and Identification of Silicate Solubilizing Bacteria

The ability of the selected bacterial strains to convert the insoluble silicate and phosphate media to soluble forms was investigated. The strain that formed the highest clear zone diameter through metal solubilization was measured and selected for identification and characterization. Among all 100 isolates, 24 silicate solubilizing isolates were selected for further investigation. The strain GAK2 showed maximum silicate and phosphate solubilizing ability by forming a clear zone with diameter of >15 mm within 48 h on both glucose medium containing silicate and NBRIP medium containing phosphate (Table 1). A qualitative visual assessment of changes in the intensity of the pink color of GAK2 broth when passed through the Salkowski reagent test detected the strain ability to produce IAA (Figure 1A). The strain GAK2 also significantly enhanced growth attributes like root length, shoot length, and total biomass of the rice mutant '*Waito*-C' (Table 1). Moreover, the change of colorless media to yellow color detected the GAK2 ability to produce ammonia (Figure 1B). The ammonia content was found significantly higher on GAK2 culture broth (2.90 mg L^{-1}) as compared to control (0.09 mg L^{-1}). Therefore, this strain was selected for identification, characterization, and further analysis. The isolated strain showed 99% sequence homology to the *E. ludwigii* sequence determined through the BLAST search (Figure 2). The strain is registered in the National Center for Biotechnology Information database under accession number KP676113 (Supplementary Materials).

Table 1. Effect of *E. ludwigii* GAK2 on growth promoting attributes of gibberellin deficient rice mutant *Waito*-C.

	Root Length (cm)	Shoot Length (cm)	Total Fresh Weight (cm)
Control	3.63 ± 0.4 b	4.63 ± 0.50 b	0.59 ± 0.16 b
GAK2	5.86 ± 0.4 a	6.36 ± 0.56 a	1.01 ± 0.21 a

Each value represents mean ± SD (n = 8). Different letters in the column after mean values represent significant differences at $p < 0.05$ determined by student t-test.

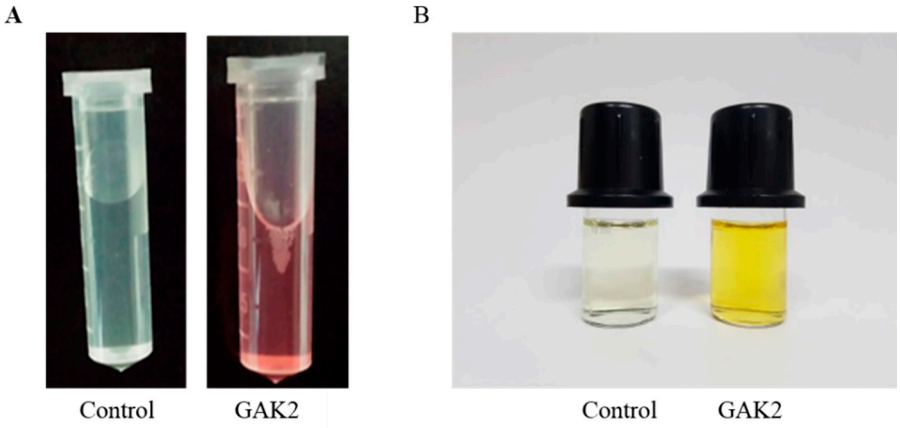

Figure 1. (**A**) Detection of indole-3-acetic acid; (**B**) Detection of ammonia production.

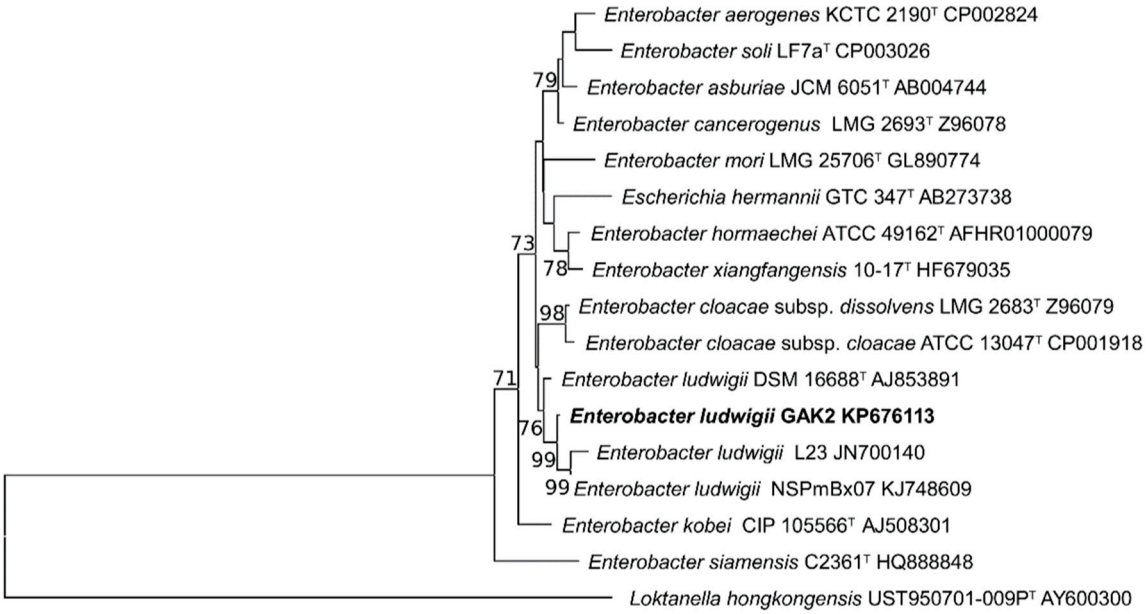

Figure 2. Phylogenetic tree constructed by the maximum parsimony method using 16S rRNA gene sequence analysis (100% bootstrap support).

3.2. Biochemical Analysis of E. ludwigii GAK2 Culture

The *E. ludwigii* GAK2 has an innate ability to produce organic acid, indole-3-acetic acid and gibberellin. Quantification results showed that the isolate produced organic acids such as citric acid (113.8 μg mL^{-1}), lactic acid (20.3 μg mL^{-1}), and acetic acid (109.5 μg mL^{-1}) in glucose medium. The GA_1 and GA_3 content of the culture filtrate were 0.017 ng mL^{-1}, and 0.08 ng mL^{-1} respectively. Moreover, the IAA concentration of GAK2 culture increased from 15.1 mg mL^{-1} to 104.6 mg mL^{-1} in a week (Figure 3).

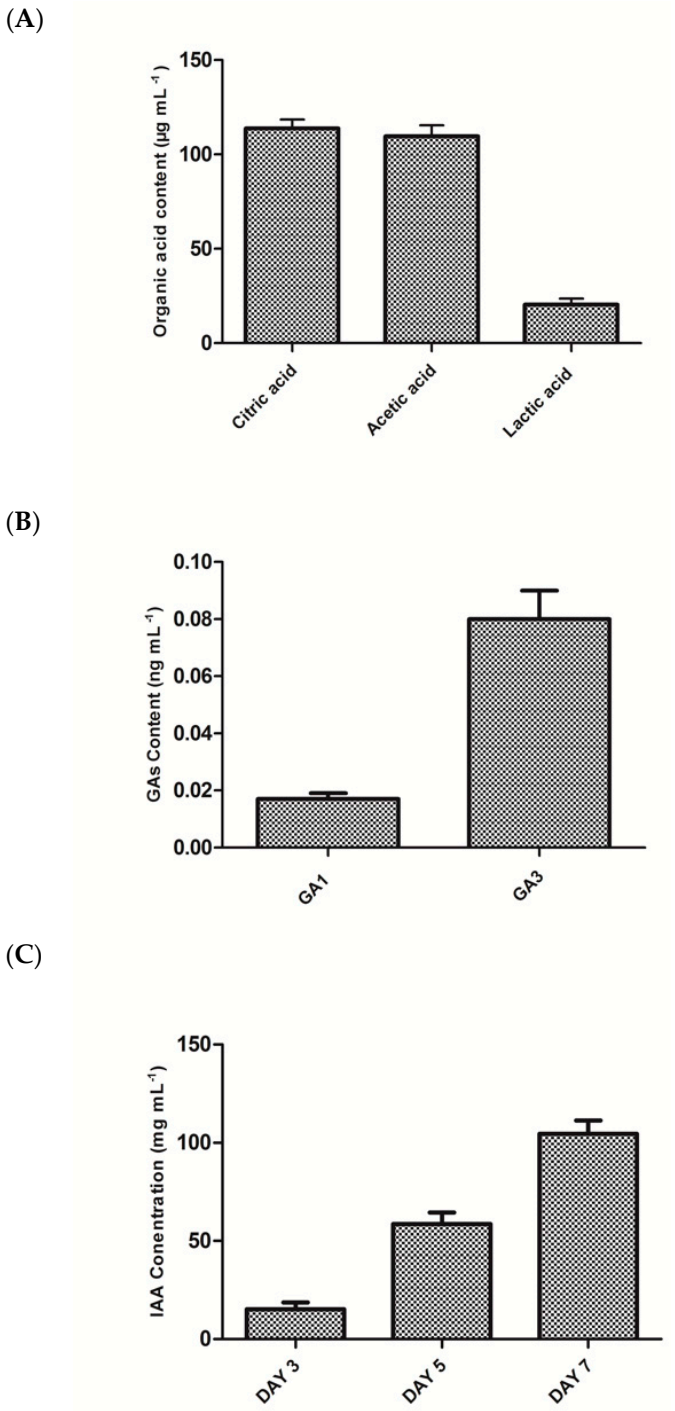

Figure 3. Quantification of (**A**) organic acid (**B**) gibberellin (GAs) and (**C**) indole-3-acetic acid (IAA) of the bacterial culture. Bars represent mean ± standard deviation (n = 3).

3.3. Plant Growth Promoting Characteristics

In our study, *E. ludwigii* inoculation had beneficial effects for plants for both IS and IP rich soils. GAK2 application along with IS significantly increased the plant growth promoting attributes including root length, shoot length, shoot weight, root weight, and chlorophyll content compared with the control treatment and sole application of IS. Similarly, GAK2 application along with IP significantly increased all the growth promoting attributes (except chlorophyll content), compared with the control (Table 2).

Table 2. Influence of *E. ludwigii* GAK2 on plant growth promoting attributes of rice plant.

Treatment	F.RW (g plant^{-1})	F.SW (g plant^{-1})	RL (cm)	SL (cm)	Chl (SPAD)
Control	0.47 ± 0.04 c	2.20 ± 0.28 c	8.85 ± 0.21 c	46.50 ± 0.7 b	44.05 ± 0.77 ab
IS	0.66 ± 0.18 bc	2.40 ± 0.28 bc	9.95 ± 0.35 bc	45.8 ± 0.21 b	41.65 ± 5.58 b
IP	0.84 ± 0.11 bc	2.25 ± 0.21 c	10 ± 0.28 abc	46.10 ± 2.9 b	44.65 ± 1.62 ab
GAK2	0.88 ± 0.34 bc	3.07 ± 0.38 ab	10.75 ± 0.35 ab	52.75 ± 1.06 a	42.72 ± 0.73 ab
GAK2 + IS	1.43 ± 0.09 a	3.25 ± 0.25 a	11 ± 0.35 a	53.25 ± 0.35 a	47.9 ± 1.55 a
GAK2 + IP	0.99 ± 0.13 b	3.21 ± 0.35 a	11.25 ± 1.06 a	52.76 ± 1.07 a	43.65 ± 0.63 ab

IS: Insoluble Silicate, IP: Insoluble Phosphate, F.RW: Fresh root weight, F.SW: Fresh shoot weight, RL: Root length, SL: Shoot length, Chl: Chlorophyll content, SPAD: Soil Plant Analysis Development Chlorophyll Meter. Each value represents mean ± SD (n = 8). Different letters in the column after mean values represent the least significant differences at $p < 0.05$.

3.4. Silicon Content in Plant

Our study showed that GAK2 inoculation significantly increased Si content in rice plant tissues on insoluble silicate ($Mg_2O_8Si_3$) based soil compared with the control treatment. Similarly, P content was higher on IP + GAK2 based soil compared with the control treatment (Table 3).

Table 3. Determination of pH, electrical conductivity, phosphorus content, and silicon content in rice.

Treatment	EC (ds m^{-1})	pH	P (mg Kg^{-1} D.W.)	Si (mg Kg^{-1} D.W.)
Control	0.72 ± 0.02 c	5.08 ± 0.14 de	4063 ± 4.33 c	156.28 ± 8.06 c
IS	0.83 ± 0.04 c	6.55 ± 0.07 a	4030 ± 189.54 c	128.08 ± 7.37 d
IP	1.05 ± 0.07 b	5.45 ± 0.07 c	4931 ± 57.34 b	115.68 ± 6.56 d
GAK2	1.07 ± 0.07 b	4.96 ± 0.05 e	4133 ± 211.26 c	194.06 ± 4.67 b
GAK2 + IS	1.27 ± 0.07 a	6.16 ± 0.07 b	3884.9 ± 67.08 c	232.41 ± 11.18 a
GAK2 + IP	1.11 ± 0.02 b	5.31 ± 0.15 cd	5549.7 ± 263.46 a	127.46 ± 8.62 d

IS: Insoluble Silicate, IP: Insoluble Phosphate, EC: Electrical Conductivity, ds: deciSiemens, D.W.: Dry Weight. Each value represents mean ± SD (n = 3). Different letters in the column after mean values represent the least significant differences at $p < 0.05$.

3.5. Measurement of Electrical Conductivity and pH

Before the experiment, preliminary test of pH revealed that increase in silicate ($Mg_2O_8Si_3$) concentration leads to an increasing trend of pH. Addition of 0.4 g of $Mg_2O_8Si_3$ to 200 g experimental soil per pot after 24 h showed pH values ranging from 5.6–6. The pH value of the experimental soil remained constant with amendment of $Ca_3(PO_4)_2$. After the experiment, the pH value of IS was significantly higher compared with the control, while electrical conductivity was significantly higher in the GAK2 + IS treatment compared with the control treatment (Table 3).

4. Discussion

Rice has been reported as a hyper-silicon accumulator plant [4]. However, it can only absorb Si as monosilicic acid H_4SiO_4 [20] and P as orthophosphate ($H_2Po_4^-$ and HPO_4^{2-}) [21]. Although the earth crust is rich in silicon and phosphorus, they are available in insoluble forms and plants are unable to uptake them. The present study aimed to meet the Si and P requirements of rice plant through employing SSB and PSB. We conducted the screening of microbes from a diverse agriculture zone, Daegu, South Korea, and identified a novel bacterial strain that could solubilize both silicate and phosphate. The strain represents 99% homology to *E. ludwigii* based on phylogenetic analysis. Genus *Enterobacter* is very diverse and feasible for its potential use in agriculture as a plant growth promoter [22,23]. The results of the present study showed that *E. ludwigii* GAK2 could be useful in regulating the levels of phytohormones and ammonia production as well as Si and P solubilization. Ammonia production is associated with nitrogen fixation in plants that enhance symbiotic association of plant and microorganisms [24]. Phytohormones mitigate various biotic and abiotic stresses [25], and the organic acid enhances the degree and rate of metal dissolution and promotes plant growth [26].

Therefore, we conducted a pot experiment by cultivating rice in silicate- or phosphate-based media with or without GAK2 application. The GAK2 treated rice plants along with IS or IP application significantly increased Si and P contents compared with the plants treated with IS or IP alone or with control plants. These plants had higher shoot length, root length, shoot fresh weight, root fresh weight, and chlorophyll content. Our results are in line with Kang et al. [14] where the silicate-solubilizing bacterial strain *Burkholderia eburnea* CS4-2 inoculation promoted the growth attributes of japonica rice. Likewise, Patra and Neue [27] reported that Si and P increased the rice grain yield, and that P concentration was influenced by Si concentration in rice. Thus, P and Si dissolution by GAK2 might have played a role in promoting the growth attributes of the plant.

Organic acids have been predicted to play multiple functions in the soil, including microbial chemotaxis, nutrient acquisition by roots, mineral weathering, and metal detoxification [28]. Our experiment revealed that *E. ludwigii* GAK2 could produce organic acids such as citric acid, lactic acid, and acetic acid. Therefore, the higher Si content of the bacteria treated plants can probably be due to the organic acid generated by the microorganisms that solubilized the insoluble metal and, hence, increased the absorption amount. This is consistent with the results of Vyas and Gulati [29], where bacteria like *Pseudomonas* produced the organic acid like oxalic, malic, lactic, 2-ketogluconic, formic, succinic, and citric acids and promoted the growth of maize. Our results are also consistent with Liu et al. [30], who showed that *Bacillus mucilaginosus* decomposed minerals and insoluble silicic acid through the release of silicon and potassium ions to form organic acids and polysaccharides. It is thought that organic acids establish organic ligands and enhance silicate dissolution through the formation of destabilizing-framework surface complexes and metal complexation [9,31].

IAA has been reported to play a key role in plant growth promotion [32,33]. It is well known that the presence of auxin enhances the influence of bacteria in the rhizosphere of a plant [34]. Our experiment revealed that *E. ludwigii* GAK2 could produce IAA in a pure culture. Since GAK2 could produce IAA on culture media, inoculation of the bacterial culture might have led to production of IAA in the rhizosphere of the plants, stimulating plant growth and development.

Likewise, the beneficial role of gibberellin in improving plant metabolism and its ubiquitous production by plants, fungi, and bacteria have been widely reported [35]. Up to now, 136 kinds of GAs have been isolated from plants, bacteria, and fungi. Among these, physiological activity is limited to GA_1, GA_3, and GA_4 [36]. Our investigation revealed that *E. ludwigii* GAK2 produced biologically active gibberellin (GA_1 and GA_3) on culture media. Several authors have reported that the inoculation of microbes producing GA on pure culture could improve growth and development of various crops, like *Serratia nematodiphila* PEJ1011 in pepper plant and *Capsicum annuum* L. [37], *Photorhabdus temperata* M1021 in rice [38], *Promicromonospora* sp. SE188 in tomato [39], and rhizobacteria in red peppers [40]. Thus, the results of the current study revealed that *E. ludwigii* GAK2 inoculation had a beneficial effect on plant growth and development.

Moreover, various factors such as pH, temperature, and time affect metal dissolution [41]. Various factors such as temperature, dissolved silicon concentration, and the activity of bacteria also affect the dissolution rate of SiO_2 [42]. A preliminary test of soil pH revealed that amendment with 0.4 g of $Mg_2O_8Si_3$ or $Ca_3(PO_4)_2$ per 200 g soil increased the pH value to the range of 5.6–6.0. Since our investigation proved that the microbial activity thrives better at the pH ranging from 5–7, the microbes might have actively participated in the Si and P dissolution. In the present study, we considered the soil pH to select the particular level 2 g of either $Mg_2O_8Si_3$ or $Ca_3(PO_4)_2$ per kg soil. Further studies are needed to test the potential of SSB and PSB for Si and P dissolution in field condition, for promoting plant growth and yield.

5. Conclusions

Silicon and phosphorus content and plant growth promoting attributes were considerably affected by *E. ludwigii* GAK2 inoculation in silicate and phosphate mediated soil. Phytohormones, ammonia, and organic acid production by GAK2 were effective in plant growth promotion and nutrient

dissolution. To the best of our knowledge, this is the first study showing the innate ability of the strain *E. ludwigii* GAK2 to solubilize both silicate and phosphate that produce organic acids, IAA, and GA. Therefore, *E. ludwigii* GAK2 might be employed as an efficient inoculant for plants as a silicon and phosphate biofertilizer to promote plant growth especially on agricultural land containing abundant amounts of insoluble silicate and phosphate.

Supplementary Materials: The accession number of *E. ludwigii* is available from https://www.ncbi.nlm.nih.gov/nuccore/830699107.

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Appendix A

The insoluble properties of magnesium trisilicate are available from http://www.chemicalbook.com/ChemicalProductProperty_EN_CB8205317.htm, while the solubility limit of tricalcium phosphate is available from the link https://pubchem.ncbi.nlm.nih.gov/compound/Calcium_phosphate#section=Melting-Point.

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