Lysinibacillus xylanilyticus Strain GIC41 as a Potential Plant Biostimulant

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To identify Lysinibacillus strains with the potential to function as plant biostimulants, we screened 10 previously isolated Lysinibacillus strains from the rhizosphere and soil for their plant growth-promoting (PGP) effects. In vitro tests showed that all strains produced indole-3-acetic acid. In primary screening, the PGP effects of these strains were assessed on spinach seedlings grown on Jiffy-7 pellets; strains GIC31, GIC41, and GIC51 markedly promoted shoot growth. In secondary screening, the PGP efficacies of these three strains were examined using spinach seedlings grown in pots under controlled conditions. Only GIC41 exerted consistent and significant PGP effects; therefore, it was selected for subsequent experiments. The results of 6-week greenhouse experiments revealed that GIC41 markedly increased shoot dry weight by ca. 12–49% over that of the control. The impact of fertilization levels on the PGP efficacy of GIC41 was investigated using pot experiments. The application of a specific level of fertilizer was required for the induction of sufficient PGP effects by this strain. The phylogenetic analysis based on the 16S rDNA sequence identified GIC41 as L. xylanilyticus. Collectively, these results show the potential of strain GIC41 to function as a plant biostimulant.

Key words: Lysinibacillus, plant growth promotion, spinach, plant biostimulant

In agriculture, large amounts of chemical fertilizers are commonly used to increase crop production and meet the global food demands of the increasing population. Public concerns related to the impact of chemical fertilizers on the environment have also increased (Gupta et al., 2015). The long-term and indiscriminate use of chemical fertilizers degrades soil quality and has been linked to a number of environmental issues (Savci, 2012). Moreover, the production process of fertilizers involves the utilization of large amounts of energy (accounting for 1.2% of the world’s gross energy needs) and the emission of carbon dioxide (CO₂) and other greenhouse gases (Woods et al., 2015). The use of chemical fertilizers to increase crop productivity has been shown to promote plant growth (Sahu et al., 2019) and to form endospores that are highly resistant to a wide variety of abiotic environmental stresses, such as dryness, UV radiation, and high temperature, and the preparation of commercial formulations of plant biostimulants is easier and inexpensive. To enrich the portfolio of microbiome-based plant biostimulants, it is essential to search for these types of practical PGPB.

Lysinibacillus is a newly classified genus that was previously classified as Bacillus (Ahmed et al., 2007). Since Lysinibacillus species also have a demonstrated ability to form endospores, they are expected to serve as suitable agents for microbe-based products, including plant biostimulants (Ahsan and Shimizu, 2021). L. sphaericus, a well-known entomopathogen, has been formulated as mosquito insecticides that are commercially available worldwide (Berry, 2012). Recent studies demonstrated that the Lysinibacillus species are ubiquitous in nature, may be isolated from the plant rhizosphere, phyllosphere, and from inside plant tissues, and possess plant growth-promoting (PGP) traits, such as auxin production, phosphate solubilization, siderophore production, and nitrogen fixation (Vendan et al., 2010; Trivedi et al., 2011; Sharma and Saharan, 2015; Verma et al., 2016). Moreover, some Lysinibacillus strains have been shown to promote plant growth (Sahu et al., 2018; Shabanamol et al., 2018).
Our laboratory has a collection of various types of bacteria isolated from the rhizosphere, plant tissues, and soil, including strains of the genus *Lysinibacillus*. In the present study, we examined the PGP activities of our *Lysinibacillus* strains with the aim of selecting potential plant biostimulants.

**Materials and Methods**

**Bacterial strains**

The *Lysinibacillus* strains used in the present study were selected from our laboratory’s bacterial collection based on their partial 16S rRNA gene sequences. They are listed in Table 1. Strains GIC31, GIC41, GIC51, GIC81, and GIC119 were originally isolated from paddy field soil, whereas strain GUCS34 was isolated from tea garden soil. Strains T16 and T20 were derived from the rhizosphere soil of tomato (*Solanum lycopersicum* L.). The remaining two strains, C75 and W30, were isolated from the rhizosphere soil of Chinese chive (*Allium tuberosum* Rottler ex Spreng.) and Welsh onion (*Allium fistulosum* L.), respectively.

Full-length 16S rRNA gene sequences were elucidated as suggested by Fu et al. (2020) for a more precise taxonomic classification. The 16S rRNA gene sequences of the strains were submitted to the GenBank database. These sequences were analyzed using a global alignment algorithm implemented in the EzBioCloud database (https://www.ezbiocloud.net/) (Yoon et al., 2017) and compared with the sequences of known *Lysinibacillus* species in the same database. A phylogenetic tree was constructed with the neighbor-joining method using MEGA version 7.24.2827 (Kumar et al., 2018).

**Inoculum preparation**

*Lysinibacillus* strains were cultured on nutrient broth (Nissui Pharmaceutical) at 30°C for 2 days with shaking at 200 rpm. Cells were harvested via centrifugation at 9,900×g for 10 min and washed twice with 10 mM MgCl₂·6H₂O. Washed cells were resuspended in 10 mM MgCl₂·6H₂O and adjusted to an optical density at 600 nm (OD₆₀₀) of 0.5 (ca. 10⁷ colony-forming units [CFU] mL⁻¹).

**Assessment of indole-3-acetic acid (IAA) production**

The abilities of bacterial strains to produce IAA were examined by the method suggested by Kumar et al. (2012) with some modifications. In brief, a 30-μL aliquot of the cell suspension of each strain was inoculated into 10 mL of tryptic soy broth (TSB; Becton, Dickinson and Company) supplemented with 0.5 mg of L-tryptophan and incubated with shaking at 200 rpm at 30°C for 2 days. After the incubation, the culture broth was centrifuged at 5,000×g for 2 days. DF medium without any nitrogen source was used as the control. Bacterial growth was assessed by measuring optical density at 530 nm.

**Assessment of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity**

The activity of ACC deaminase was evaluated according to the method described by Dell’Amico et al. (2005). In brief, bacterial strains were grown in TSB. After centrifugation, a 30-μL aliquot of the cell suspension (OD₆₀₀=0.5) of each strain was transferred to Dworkin and Faster (DF) medium containing 3.0 mM ACC as the sole nitrogen source and incubated with shaking at 200 rpm at 30°C for 2 days. DF medium without any nitrogen source was used as the control. Bacterial growth was assessed by measuring optical density at 600 nm.

**Assessment of nitrogen-fixing potential**

The nitrogen-fixing potential of bacterial strains was assessed by the method of Setten et al. (2013) with some modifications. Bacterial strains were grown for 24 h in L medium (Setten et al., 2013) containing 7.57 mM (NH₄)₂SO₄ at 30°C with shaking at 200 rpm. After the incubation, bacterial cells were harvested by centrifugation at 5,000×g for 10 min and washed twice with nitrogen-free L medium. Washed cells were resuspended in nitrogen-free L medium and adjusted to OD₆₀₀ of 0.5. A 50-μL aliquot of the cell suspension was then inoculated into 5 mL of nitrogen-free L medium in a 15-mL tube and incubated at 30°C with shaking at 200 rpm under aerobic (test tube covered with parafilm) and micro-aerobic (test tube with plastic screw caps) conditions. After 48 h of incubation, bacterial cell density (OD₆₀₀) was measured using a spectrophotometer.

**Table 1. Lysinibacillus species identified by a BLAST analysis of nearly full-length 16S rRNA gene sequences**

| Strain | Origin | % Similarity | GenBank Accession Number |
|--------|--------|--------------|--------------------------|
| GIC31  | Paddy field soil | 99.3% with *L. xylanilyticus* DSM 23493 | MZ520561 |
| GIC41  | Paddy field soil | 99.5% with *L. xylanilyticus* DSM 23493 | MZ543950 |
| GIC51  | Paddy field soil | 98.27% with *L. xylanilyticus* DSM 23493 | MZ543951 |
| GIC119 | Paddy field soil | 99.09% with *L. xylanilyticus* DSM 23493 | MZ543965 |
| C75    | Chinese chive rhizosphere soil | 99.58% with *L. xylanilyticus* DSM 23493 | MZ543967 |
| GUCS34 | Tea garden soil | 99.67% with *L. pakistanensis* JCM 18776 | MZ543966 |
| T16    | Tomato rhizosphere soil | 99.25% with *L. capsici* PB300 | MZ543969 |
| GIC81  | Paddy field soil | 100% with *L. capsici* PB300 | MZ543964 |
| W30    | Welsh onion rhizosphere soil | 99.92% with *L. fusciformis* NBRC 15717 | MZ543970 |
| T20    | Tomato rhizosphere soil | 99.67% with *L. fusciformis* NBRC 15717 | MZ543972 |
Primary screening for PGP strains

The effects of the Lysinibacillus inoculation on the growth of spinach was evaluated to identify potential PGP strains. Spinach seeds (Spinacia oleracea L. cv. Banchu-summer-sky) were surface sterilized with 70% (v/v) ethanol for 1 min, followed by a treatment with 2% sodium hypochlorite for 5 min and thorough rinsing with sterilized distilled water. Seeds were then placed on moist filter paper in a Petri dish and vernalized at 4°C for 1 day. Thereafter, seeds were sown on Jiffy-7 pellets (Jiffy Products International) covered with a small amount of sterilized vermiculite and grown in a controlled environmental chamber (Biotron LH-220S, Standard; Nippon Medical and Chemical Instruments) at 23°C under a 12-h light/12-h dark cycle (light intensity, 30,000 Lx). After 7 days of sowing, spinach seedlings were drenched with 3 mL of the bacterial cell suspension (OD\text{600}=0.5). Control plants were treated with an equal volume of sterile MgCl\textsubscript{2}·6H\textsubscript{2}O (10 mM) without bacteria. Control and bacteria-treated seedlings were both maintained in the same controlled environmental chamber (light intensity, 30,000 Lx). Plants were regularly irrigated throughout the growing period. After 20 days of the drench treatment, spinach plants were harvested from pellets, and their leaf areas and shoot dry weights were measured. Image analysis software (LIA for Win32, https://www.agr.nagoya-u.ac.jp/~shinkan/LIA32.html) was used to measure the total leaf area of spinach plants from scanned images of all harvested leaves. To measure the shoot dry weight, spinach shoots (cut from the root and shoot junctions) were stored in a ventilated constant temperature dryer (ADVANTEC® DRM620TB; Toyo Seisakusho) at 80°C for 2 days. In this experiment, five plants were used for each treatment, and the experiment was repeated three times. Differences in the leaf area and shoot dry weight between the control and bacterial treatments were analyzed by Dunnett’s test (P<0.05).

Secondary screening for PGP strains

Strains GIC31, GIC41, and GIC51, the three best performing strains in primary screening, were subjected to further assessments in a pot experiment for their effects on spinach growth. Spinach seeds (cv. Banchu-summer-sky) were surface sterilized and vernalized as described above. Seeds were then sown in 150-mL plastic pots containing a double-autoclaved mixture of river sand and vermiculite at a ratio of 1:1 (v/v) and grown in a controlled environmental chamber (light intensity, 30,000 Lx). After 7 days of sowing, seedlings were drenched with 10 mL of the cell suspension of each strain (OD\text{600}=0.5). Control plants were drenched with the same amount of sterile MgCl\textsubscript{2}·6H\textsubscript{2}O (10 mM) without bacteria. Control and bacteria-treated seedlings were both stored in a ventilated constant temperature dryer (ADVANTEC® DRM620TB; Toyo Seisakusho) at 80°C for 2 days. In this experiment, five plants were used for each treatment, and the experiment was repeated three times. Differences in the leaf area and shoot dry weight between the control and bacterial treatments were analyzed by Dunnett’s test (P<0.05).

Evaluation of PGP effects of strain GIC41 under glasshouse conditions

Strain GIC41 was selected as a potential PGP candidate because it performed the best in secondary screening. In this experiment, the PGP effects of this strain were evaluated under glasshouse conditions. Surface-sterilized and vernalized spinach seeds (cv. Banchu-summer-sky) were sown in plastic trays (Bee pot Y-49; Canelon Kaka) containing the commercial potting soil mix “Saika-ichiban” (IBIKO Corporation) and grown in a controlled environmental chamber at 23°C under a 12-h light/12-h dark cycle. After 7 days of sowing, seedlings were treated by drenching with the cell suspension of GIC41 (OD\text{600}=0.5) at 10 mL per plant. Control seedlings were treated with sterile MgCl\textsubscript{2}·6H\textsubscript{2}O (10 mM) instead of the GIC41 cell suspension. These seedlings were then transplanted into rectangular plastic containers (64×22×20 cm) containing the commercial potting soil mix (five seedlings per container) and grown on a bench in a glasshouse under natural sunlight for 6 weeks; during this period, plants were regularly irrigated without fertilization. Five containers were used as replicates for each treatment, and the experiment was repeated three times (designated as trials 1 to 3). Trials 1, 2, and 3 were conducted between March 26 and May 7, April 5 and May 17, and April 16 and May 28 in 2019, respectively.

At the end of the experiment in each trial, spinach plants were harvested and the shoot dry weight (after drying at 80°C for 2 days) was measured. The nitrogen and carbon contents of the shoots were measured using the dry combustion method with an NC analyzer (SUMIGRAPH NC TR22; Sumika Chemical Analy‐sis Service). To conduct this experiment, dried spinach shoots were ground in a blender to prepare homogenized samples. Thereafter, the nitrogen and carbon contents of the powder were assessed using the NC analyzer device. Since the growth of spinach plants in the control treatment markedly varied among the trials, shoot dry weight data obtained from the three trials were subjected to a fixed effects meta-analysis using the R package “meta” (ver. 2.6-1) (Schwarzer et al., 2015). Differences in nitrogen and carbon content between the control and GIC41 treatment were analyzed by the Student’s t-test (P<0.05).

Evaluation of PGP effects of GIC41 at different fertilization levels

In this experiment, the PGP effects of strain GIC41 were assessed at different fertilization levels. The experimental set-up was identical to that of the second screening experiment, except for the growth condition and fertilizer concentration. The experiment was conducted in a controlled environmental chamber (MLR-350; Sanyo Electric) at 23°C under a 12-h light/12-h dark cycle (light intensity, 20,000 Lx), and three different concentrations (0.05, 0.1, and 0.2%) of Hyponex solutions were provided to the spinach plants. After 20 days of drenching with the GIC41 cell suspension or sterile MgCl\textsubscript{2}·6H\textsubscript{2}O (10 mM), spinach plants were uprooted from the pots, and their shoot and root dry weights and leaf areas were measured. Five plants were used for each treatment, and the experiment was repeated three times. Differences in leaf areas and shoot and root dry weights between the control and bacterial treatment were analyzed by Tukey’s test (P<0.05).

Statistical analysis

All statistical analyses were performed with EZR version 1.41 (Saitama Medical Center, http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.6.1).

Nucleotide sequence accession numbers

The nucleotide sequences of the full-length 16S rRNA genes were deposited in the GenBank database under accession numbers MZ520561, MZ543950, MZ543951, MZ543965, MZ543967, MZ543966, MZ543969, MZ543964, MZ543970, and MZ543972 (Table 1).

Results

Molecular characterization of Lysinibacillus strains

The 10 Lysinibacillus strains used in the present study were identified by a sequence analysis of the nearly full-length 16S rRNA genes. The results obtained revealed that these strains possessed high similarities to species of L. xylanilyticus, L. paskitanensis, L. capsici, and L.
Lysinibacillus strains were tested in vitro for their abilities to produce IAA, siderophores, and ACC deaminase, solubilize phosphate, and fix nitrogen. All strains were capable of producing IAA, but lacked the other traits (Table 2). Among the strains tested, IAA production was the highest in C75 at
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Table 2. *In vitro* plant growth-promoting traits of *Lysinibacillus* strains

| Strain | IAA production (μg mL⁻¹) | Phosphate solubilization | Siderophore production | ACC deaminase production | Nitrogen fixation |
|--------|--------------------------|--------------------------|------------------------|--------------------------|------------------|
| GIC31  | 1.2                      | —                        | —                      | —                        | —                |
| GIC41  | 1.5                      | —                        | —                      | —                        | —                |
| GIC51  | 1.0                      | —                        | —                      | —                        | —                |
| GIC119 | 1.0                      | —                        | —                      | —                        | —                |
| C75    | 2.0                      | —                        | —                      | —                        | —                |
| GUCS34 | 1.8                      | —                        | —                      | —                        | —                |
| T16    | 1.4                      | —                        | —                      | —                        | —                |
| GIC81  | 1.2                      | —                        | —                      | —                        | —                |
| W30    | 1.1                      | —                        | —                      | —                        | —                |
| T20    | 1.9                      | —                        | —                      | —                        | —                |

“—” indicates negative.

Fig. 2. Influence of the *Lysinibacillus* treatment on the leaf area (A) and shoot dry weight (B) of spinach plants grown on Jiffy-7 pellets. Spinach plants were sampled 20 days after treatment. Bars represent the mean±standard deviation of three independent experiments. Significant differences between the control and treatment groups are indicated by asterisks (Dunnett’s test, *P* < 0.05, **P** < 0.01).

Fig. 3. Influence of the *Lysinibacillus* treatment on the leaf area (A) and shoot dry weight (B) of spinach plants grown in pots containing a river sand-vermiculite mixture. Spinach plants were sampled 20 days after treatment. Bars represent the mean±standard deviation of three independent experiments. Significant differences between the treatments are indicated by different letters (Tukey’s multiple comparison of means, *P*<0.05).

2.0 μg mL⁻¹ of IAA, followed by T20 and GUCS34 at 1.9 and 1.8 μg mL⁻¹ of IAA, respectively.

Screening for effective PGP *Lysinibacillus* strains in spinach

Two-step screening was conducted to identify potential *Lysinibacillus* strains that enhance spinach growth. In primary screening, the PGP effects of *Lysinibacillus* strains were evaluated on spinach seedlings grown on Jiffy-7 pellets under controlled conditions. The majority of *Lysinibacillus* strains promoted spinach growth over that of control plants (Fig. 2). Significant enhancements in leaf areas were observed in plants treated with strains GIC31, GIC41, and GIC51. In addition, strains GIC31 and GIC41 significantly increased the dry weights of spinach plants.

In secondary screening, the PGP effects of the top three performers in primary screening (strains GIC31, GIC41, and GIC51) were assessed on spinach plants grown in pots containing a river sand-vermiculite mixture that were fertilized once a week under controlled conditions. Only strain GIC41 significantly increased both the leaf area and shoot dry weight (Fig. 3); therefore, this strain was selected for subsequent experiments.

Efficacy of GIC41 for spinach growth under glasshouse conditions

The PGP effects of strain GIC41 on spinach plants grown under glasshouse conditions were evaluated in three independent trials. The results obtained demonstrated that the drench treatment with strain GIC41 on the transplanting
day consistently promoted spinach growth across all trials, resulting in an approximate 12–49% increase in the shoot dry weight over that with the control treatment (Fig. 4A and B). A meta-analysis of data from three trials indicated that the mean difference (MD) between the GIC41 and control treatments was 0.95 (95% confidence interval: 0.58–1.31) (Fig. 4B), indicating the significant PGP effects of the GIC41 treatment on the shoot growth of spinach plants under glasshouse conditions.

In contrast, nitrogen and carbon contents (%) in the shoot tissues of GIC41-treated plants were not significantly different from those of control plants (Table 3).

### Table 3. Effects of the GIC41 treatment on carbon and nitrogen contents in spinach shoots

| Treatment | Carbon content (%) | Nitrogen content (%) |
|-----------|--------------------|----------------------|
| Control   | 34.5±1.6 a         | 4.3±1.3 a            |
| GIC41     | 35.1±1.6 a         | 3.8±1.1 a            |

Data represent the mean±standard deviation of three replicates. Means were analyzed for significant differences using the Student’s t-test. Values in columns followed by the same letters are not significantly different at *P*<0.05.

**PGP effects of GIC41 at different fertilization levels**

The PGP effects of strain GIC41 were tested on spinach plants fertilized with three different concentrations of Hyponex solution (0.05, 0.1, and 0.2%). The results obtained revealed that irrespective of the fertilizer concentration, the GIC41 treatment promoted shoot and root growth to varying degrees (Fig. 5 and S1). However, significant growth promotion by the GIC41 treatment was only observed in plants that were fertilized with 0.2% (the concentration recommended by the supplier) of Hyponex (Fig. 5 and 6), indicating that strain GIC41 requires a particular level of soil nutrients to fully exert its PGP effects.

**Discussion**

The present study aimed to identify *Lysinibacillus* strains with the potential to function as plant biostimulants. We screened 10 *Lysinibacillus* strains, previously isolated from soil and the plant rhizosphere, for their PGP effects on spinach plants. Accordingly, strain GIC41, isolated from paddy field soil, was selected as the best candidate strain because of its consistent performance across screening experiments.

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**Fig. 4.** Growth-promoting effects of strain GIC41 on spinach plants under glasshouse conditions. A) Representative examples of control (upper) and GIC41-treated plants (lower) grown for 6 weeks. B) A forest plot of a meta-analysis comparing the GIC41 treatment and control treatment for shoot dry weight (g). Shoot dry weight data obtained from three independent trials were analyzed by a meta-analysis. Mean, SD, and MD values represent the mean shoot dry weight (g), standard deviation, and mean difference, respectively. Gray boxes indicate the mean difference for each trial, and horizontal bars show the corresponding 95% confidence interval (95% CI). The diamond indicates pooled mean difference across trials. A fixed effects model was used.
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Fig. 5. Effects of the GIC41 treatment on the leaf area (A), shoot dry weight (B), and root dry weight (C) of spinach plants fertilized with three different concentrations (0.05, 0.1, and 0.2%) of Hyponex solution. Spinach plants were sampled 20 days after the treatment. Bars represent the mean±standard deviation of three independent experiments. Significant differences between the treatments are indicated by different letters (Tukey's multiple comparison of means, *P*<0.05).

Fig. 6. Enhancements induced in lateral root growth by the GIC41 treatment. Spinach plants were fertilized with 0.2% Hyponex solution using spinach seedlings grown in a controlled environmental chamber (Fig. 2). Moreover, the drench treatment with strain GIC41 on the day of transplanting promoted spinach growth and significantly increased shoot biomass 6 weeks after transplantation under glasshouse conditions (Fig. 4), indicating the potential of this strain as a plant biostimulant.

Previous studies reported PGP effects following an inoculation with Lysinibacillus species, such as L. sphaericus, L. fusiformis, and L. chungkukjangi (Sharma and Saharan, 2015; Yu et al., 2016; Sahu et al., 2018; Borah et al., 2019; Ahsan and Shimizu, 2021). According to the phylogenetic analysis of 16S rRNA gene sequences, strain GIC41 was identified as L. xylanilyticus (Fig. 1). The species L. xylanilyticus was initially discovered from forest humus as a xylan-degrading bacterium and its validity was confirmed by Lee et al. (2010). Since then, several L. xylanilyticus strains have been isolated from soil, the rhizosphere, and plant tissues (Esmaeili et al., 2013; Verma et al., 2014; Tan et al., 2015). Moreover, L. xylanilyticus strains have been shown to exhibit certain PGP-associated traits, such as IAA production and phosphate solubilization (Verma et al., 2016; De Mandal et al., 2018). However, to the best of our knowledge, its actual capacity to enhance plant growth remains unknown. Therefore, the present study is the first to report the PGP capability of L. xylanilyticus.

Many PGPB promote plant growth by increasing the bioavailability of soil nutrients through the solubilization of key mineral nutrients, such as phosphorus and iron (Rawat et al., 2018; Kenneth et al., 2019). However, strain GIC41 lacked the ability to solubilize phosphate or produce siderophores, which are iron-solubilizing agents (Table 2). Furthermore, a simple in vitro assay demonstrated that this strain did not exhibit the ability to fix nitrogen, another important trait of many PGPB that promote plant growth (Table 2). Previous studies reported that an inoculation with nitrogen-fixing PGPB increased the plant biomass and also enhanced the nitrogen content in plants (Rokhzadi and Toashih, 2011; Islam et al., 2013). However, an inoculation with GIC41 did not affect the nitrogen content of spinach plants (Table 3), suggesting that the PGP effects of this strain are not attributable to nitrogen fixation.

In comparisons with the control treatment, the GIC41 treatment promoted lateral root development (Fig. 6), which resulted in an increase in the root biomass (Fig. 5C). The lateral roots contribute to nutrient and water absorption from the soil by increasing the overall surface area of the root system (Vessey, 2003). Therefore, enhanced shoot growth in GIC41-treated spinach plants may be attributed to an increase in the uptake of nutrients through the lateral roots. Several PGPR have also been shown to increase the number and/or length of lateral roots, consequently enhancing the growth of the whole plant (Cao et al., 2020; Grover et al., 2021).

Lateral root formation is regulated by the phytohormone auxin and exogenous auxin promotes the production of lateral roots (Fukaki and Tasaka, 2009; Moriwaki et al., 2011; Waidmann et al., 2020). In the present study, in vitro assays revealed that strain GIC41 exhibited the ability to produce IAA (Table 2), implying that IAA secreted by this strain plays a key role in the promotion of lateral root growth. The enhancements induced in shoot and root growth by the GIC41 treatment were more prominent under moderately fertilized conditions (fertilized with 0.2% Hyponex solution, the concentration recommended by the supplier)
than under less-fertilized conditions (fertilized with 0.1 and 0.05% Hyponex solution) (Fig. 5). This result is consistent with the findings reported by Lamont et al. (2014) showing that Viminaria juncea inoculated with an IAA-producing Bacillus megaterium strain generated a larger root system under moderate nitrogen conditions than under less nitrogen conditions. IAA production by bacteria has also been shown to depend on the availability of nitrogen (Thuler et al., 2003; Tamaki and Mercier, 2007; Shokri and Emtiyazi, 2010). Therefore, the soil nutrient status, particularly the content of nitrogen, is a key factor for strain GIC41 to exert its PGP effects.

Although the other Lysinibacillus strains examined in the present study were also capable of producing IAA, their PGP effects were inferior to those of strain GIC41, which may be attributed to several factors. Bacteria exhibit different responses to environmental conditions, and these variations affect the production of IAA (Sasirekha et al., 2012; Bharucha et al., 2013; Chandra et al., 2018). Mohite (2013) reported that the quantity of IAA produced by rhizospheric Bacillus and Lactobacillus strains significantly varied depending on temperature, pH, and types of carbon and nitrogen sources in culture medium. Therefore, environmental conditions, particularly the rhizospheric conditions of spinach plants, may be more favorable for strain GIC41 to synthesize IAA than for the other strains. Another factor is the involvement of PGP substances other than IAA. Among the five major PGP-associated traits (i.e., IAA production, siderophore production, ACC deaminase production, phosphate solubilization, and nitrogen fixation), the Lysinibacillus strains examined in the present study only exhibited the ability to produce IAA (Table 2). However, previous studies reported that the production of plant hormones other than IAA, namely, cytokinins and gibberellins, also contributes to the PGP effects of PGPB (Ortiz-Castro et al., 2008; Kang et al., 2014). Moreover, recent studies revealed that volatile organic compounds produced by PGPR activate phytohormone signaling pathways and enhance plant growth (Jishma et al., 2017; Tahir et al., 2017). Therefore, in addition to IAA, strain GIC41 may produce cytokinins, gibberellins, and/or VOCs, and the coordinated effects of these compounds may be responsible for its superior PGP effects. Another possible explanation for this result is differences in the colonization capacities of the strains examined. Successful root colonization is an essential step for PGPR to exert their beneficial effects on plants (Ansari and Ahmad, 2018; Gamez et al., 2019). Although the root-colonizing capacity of Lysinibacillus strains was not investigated in the present study, strain GIC41 may have colonized the spinach roots more efficiently than the other strains and continuously secreted IAA into the rhizosphere; therefore, this strain exerted stronger and more stable PGP effects than the other strains. Moreover, previous studies reported that nutrients released from the dead cells of introduced PGPB were taken up by plants and increased their biomass (Macedo-Raygoza et al., 2019; Seerat et al., 2019). Therefore, in addition to the direct growth stimulation via IAA production, a certain proportion of cells may die as GIC41 continues to proliferate around spinach roots, and spinach plants utilize the nutrients released from these dead cells, which results in a significant increase in shoot biomass.

Further studies are needed to elucidate the mechanisms underlying the PGP effects of strain GIC41. We also plan to examine the PGP effects of this strain on various crops in the future.

In conclusion, the results of the present study demonstrated that L. xylanilyticus strain GIC41 effectively promoted the growth of spinach plants, and, thus, may contribute to the development of new plant biofertilizers.

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