J. Microbiol. Biotechnol. (2015), 25(9), 1467–1475
http://dx.doi.org/10.4014/jmb.1502.02011

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Enterococcus faecium LKE12 Cell-Free Extract Accelerates Host Plant Growth *via* Gibberellin and Indole-3-Acetic Acid Secretion S

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Received: February 4, 2015
Revised: April 9, 2015
Accepted: April 22, 2015

First published online
April 23, 2015

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Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

The use of microbial extracts containing plant hormones is a promising technique to improve crop growth. Little is known about the effect of bacterial cell-free extracts on plant growth promotion. This study, based on phytohormonal analyses, aimed at exploring the potential mechanisms by which *Enterococcus faecium* LKE12 enhances plant growth in oriental melon. A bacterial strain, LKE12, was isolated from soil, and further identified as *E. faecium* by 16S rDNA sequencing and phylogenetic analysis. The plant growth-promoting ability of an LKE12 bacterial culture was tested in a gibberellin (GA)-deficient rice dwarf mutant (*waito-C*) and a normal GA biosynthesis rice cultivar (Hwayongbyeo). *E. faecium* LKE12 significantly improved the length and biomass of rice shoots in both normal and dwarf cultivars through the secretion of an array of gibberellins (GA_1, GA_3, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, and GA_{53}), as well as indole-3-acetic acid (IAA). To the best of our knowledge, this is the first study indicating that *E. faecium* can produce GAs. Increases in shoot and root lengths, plant fresh weight, and chlorophyll content promoted by *E. faecium* LKE12 and its cell-free extract inoculated in oriental melon plants revealed a favorable interaction of *E. faecium* LKE12 with plants. Higher plant growth rates and nutrient contents of magnesium, calcium, sodium, iron, manganese, silicon, zinc, and nitrogen were found in cell-free extract-treated plants than in control plants. The results of the current study suggest that *E. faecium* LKE12 promotes plant growth by producing GAs and IAA; interestingly, the exogenous application of its cell-free culture extract can be a potential strategy to accelerate plant growth.

Keywords: *Enterococcus faecium*, gibberellins, yellow melon, *waito-C* rice

Introduction

Soil microorganisms can produce plant growth-promoting metabolites, solubilize insoluble phosphates, and survive under various environmental conditions. Plants cannot always meet their demand for mineral uptake in natural soils owing to the low availability of most of the essential nutrients [29]; moreover, the frequent use of agricultural lands for vegetable and fruit production also limits nutrient uptake. In order to enhance crop growth, the biostimulators produced naturally by bacteria, fungi, algae and protozoa can be used in agricultural lands [26]. The application of non-pathogenic soil bacteria as biofertilizers can be environmentally safer for improvements in crop productivity and soil fertility owing to their ability to produce cytokinins, gibberellins, and auxin [6]. Exogenous hormonal treatments supporting plant growth are widely reported. Recently, we found that the gibberellin-producing bacteria *Acinetobacter*

calcoaceticus and *Pseudomonas putida* increased the growth of soybean, crown daisy, Chinese cabbage, and cucumber [19, 21]. Some of the phytohormones-secreting bacteria cause diseases to plants and inhibit plant growth. A well-known rhizobacterium, *Agrobacterium tumefaciens*, produces cytokinins and induces crown gall disease. Alternatively, the use of a bacterium cell-free extract with phytohormones could represent an effective environmentally friendly approach to enhance plant productivity.

The cell-free extract of antagonistic bacteria, which contains lytic enzymes and secondary metabolites, including volatile organic compounds, seems to control pathogen growth and reduces the associated infection effects on plants. For example, the chitinase and β-glucanase activities of *Bacillus*, *Lysinibacillus*, *Enterobacter*, and *Serratia* spp. against *Fusarium*, *Phytophthora*, and *Rhizoctonia* spp. [23], as well as the production of siderophores, antibiotics, and volatile compounds by *Bacillus* and *Pseudomonas* spp. against *Sclerotium* infection [37], suggest that the application of cell-free extracts of antagonistic microorganisms can protect plants from diseases. Few studies have examined the mechanisms by which bacterial cell-free extracts accelerate plant growth and yield. Moreover, Yandigeri *et al*. [39] recently reported that application of cell-free extracts of drought-tolerant actinobacteria containing phytohormones increased wheat growth and yield under drought conditions.

Enterococci are ubiquitous microflora in the gastrointestinal and genitourinary tracts of humans and animals, as well as in soil, water, and a variety of plant products. Whereas some have been reported as casual agents of nosocomical infections, including urinary tract, wood, and pelvic infections, some other species have been used in the fermentation of food products [10, 25, 28]. The European Food Safety Authority recognized some of the *E. faecium* strains as feed additives [8]. Pessione *et al*. [32] observed that an *E. faecium* strain had the potential to produce enantioselective lactic acid, the building block to produce polylactides from agro-industrial waste. *Enterococcus* strains can produce peptidoglycan hydrolases, which could be useful for food preservation [11]. Studies on the effect of *Enterococcus* spp. in plant growth are scarce. Furthermore, the ability of *E. faecium* to produce phytohormones has still to be reported. In the current study, the phytohormones produced by *E. faecium* LKE12 were identified, and the effect of its cell-free extract on the growth of oriental melon was evaluated. The main objective of this study was to elucidate the key compounds present in the cell-free extract of *E. faecium* LKE12 that are known to trigger host plant growth during plant-microbe interaction.

Materials and Methods

Enterococcus faecium LKE12: Isolation and Identification

Soil samples were collected from the rhizosphere of oriental melon (*Cucumis melo* L.) cultivated in a greenhouse located in Gyeongbuk Province, Republic of Korea. Approximately, 10 g of soil was transferred to 100 ml of 0.85% sterile saline solution. The soil suspensions were serially diluted, and 0.1 ml aliquots were inoculated on tryptic soy agar (TSA; Merck Co., Germany) medium (tryptone (15 g/l), soytone (5 g/l), NaCl (5 g/l), and agar (15 g/l)) for bacterial culture and incubated for 48 h at 30°C. The bacterial colonies were identified by their colony morphology and pigmentation, and further cultured separately on fresh TSA medium. The pure cultured bacterial isolates were stored in 50% glycerol at −80°C for long-term preservation.

The LKE12 bacterial strain was identified on the basis of 16S ribosomal DNA (rDNA) sequencing. The genomic DNA was extracted from the LKE12 bacterial strain and the 16S rDNA sequence was amplified using the 27F primer (5′-AGAGTTTGA TC(AC)TGGCTCAG-3′) and 1492R primer (5′-CGG (CT) TACCTT GTTACGACTT-3′), which were complementary to the 5′ end and 3′ end of the prokaryotic 16S rDNA, respectively. The amplification reaction was performed as previously described by Adachi *et al.* [1]. To compare the nucleotide sequence homology of the LKE12 strain, the BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/) was used in the analysis. The relatively similar sequences were aligned by ClustalW using MEGA ver. 5.0 software, and the neighbor-joining tree was generated using the same software. Bootstrap replication (1,000 replications) was used for more robust statistical support of the resulting nodes in the phylogenetic tree.

Gibberellin Mutant Rice and *E. faecium* LKE12 Interaction

The plant growth-promoting effect of *E. faecium* LKE12 was analyzed on a gibberellin-deficient rice mutant (*waito-C*) with dwarf phenotype as well as in a normal gibberellin biosynthesis rice cultivar, Hwayongbyeo. The pre-cleaned rice seeds were surface-sterilized with 2.5% sodium hypochlorite for 30 min and later rinsed with sterile distilled water. Uniconazole (20 ppm) was added to the sterilized seeds and kept on an incubator for 24 h to obtain equally germinated seeds [14]. The germinated seeds of *waito-C* and Hwayongbyeo were shifted to sterilized 0.8% agar medium and cultivated in a growth chamber (day/night cycle: 14 h, 28°C/10 h, 18°C). *E. faecium* LKE12 culture filtrate (20 μl) was applied on the apex of rice seedlings at the two-leaf stage. The shoot length, root length, fresh weight, and chlorophyll content of both the normal cultivar and the GA-deficient mutant were measured after one week of culture filtrate (CF) treatment.

Gibberellins and Indole-3-Acetic Acid (IAA) Production by *E. faecium* LKE12

GAs were obtained from the pure culture of *E. faecium* LKE12 after incubation in LB medium for 3 days. The culture medium

was separated from the culture and used for GA analysis. The CF was partitioned with ethyl acetate, and the organic layer was vacuum dried. Next, 60% methanol (MeOH) was added, the pH was increased to 8.0 ± 0.3 by adding 2N NH_4OH, and deuterated GA internal standards were also added. The Lee *et al*. [27] method was followed to determine final GA concentrations. The prepared extracts were dissolved in 60% MeOH, and passed through a Davisil C18 column (90–130 μm; Alltech, Deerfield, IL, USA); 5 g of C18 powder was used as the solid phase, and 60% MeOH was used as the mobile phase. The eluent was collected and concentrated at 40°C in vacuum. The sample was then dried onto Celite and loaded onto a SiO_2 partitioning column to separate the GAs from the more polar impurities. GA extracts were eluted with 95:5 (v/v) ethyl acetate (EtOAc):hexane saturated with formic acid. This solution was dried at 40°C in vacuum, re-dissolved in EtOAc, and partitioned three times against 0.1 M phosphate buffer (pH 8.0). Next, 2N NaOH was required during the first partitioning to neutralize residual formic acid. Polyvinylpolypyrrolidone was added to the combined aqueous phases, and the resulting mixture was stirred for 1 h. The pH was reduced to 2.5 and the extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum, and the residue was dissolved in 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (AES 2000, Madrid, Spain). The CF extract was subjected to HPLC using a Bondapak C18 column (Table S1) to prepare the GA fractions. The fractions were then prepared and injected into a gas chromatography and mass spectrometry (GC-MS) with a selected ion monitoring (SIM) system (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, Palo Alto, CA, USA). Each fraction (1 ml) was injected into a 30 m × 0.25 mm i.d., 0.25 mm film thickness DB-1 capillary column (J & W Scientific Co., Folsom, CA, USA). The GC oven temperature was programmed for a 1-min hold at 60°C, and then a constant raise of 15°C/min until 200°C, followed by an increment of 5°C/min until 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a mass selective detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV, and a dwell time of 100 min. Full-scan mode (the first trial) and three major ions of the supplemented [$^2H_2$] GA internal standards and the bacterial GAs were monitored simultaneously. The retention time was determined using hydrocarbon standards to calculate the KRI (Kovats retention index) value of standard GAs. The quantity of GA_1, GA_3, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, and GA_{53} were calculated from the peak area ratios of 506/508, 504/506, 222/224, 594/596, 298/230, 300/302, 434/436, 418/420, 314/316, and 448/450, respectively.

E. faecium LKE12 was cultured in TSB medium (tryptone (15 g/l), soytone (5 g/l), and NaCl (5 g/l)) with 0.5 g/l tryptophan and incubated for 1 week at 28 ± 2°C to detect IAA in the culture medium. Thereafter, the bacteria were removed from the culture medium and the pH of the culture filtrate was adjusted to 2.8–3.0, and ethyl acetate was added. The organic layer obtained was vacuum evaporated and the extracts were resuspended in 0.1 M acetic acid and transferred to a reverse-phase C18 column. The extract was eluted using stepwise elution with a methanol gradient (30%, 50%, and 100%). The collected samples were dried. Methyl esters of the samples were prepared by dissolving the residues in MeOH and adding ethereal diazomethane; the methyl esters were then re-dissolved in ethyl acetate and analyzed using GC-MS with SIM. The quantity of IAA was measured using a known standard peak area.

Preparation of Cell-Free Extract of *E. faecium* LKE12

E. faecium LKE12 was cultured in LB medium for 3 days, and the resulting bacterial culture was centrifuged at 6,000 rpm. The supernatant (cell-free extract) and pellets thus obtained were separated. The supernatant was further filtered and diluted with sterile distilled water at 10 times, and the pellet was dissolved in sterile distilled water (1 g/l).

***E. faecium* LKE12 and Host Plant Growth**

Oriental melon seeds (*Cucumis melo* L.) were surface-sterilized with 5% sodium hypochlorite and rinsed with sterile distilled water. The sterilized seeds were sown in autoclaved plastic pots containing an artificial soil mixture (peat moss (13–18% (w/v)), perlite (7–11% (w/v)), coco-peat (63–68% (w/v)), and zeolite (6–8% (w/v)), plus other nutrients (~90 mg/kg NH_4^+, ~205 mg/kg NO_3^-, ~350 mg/kg P_2O_5, and ~100 mg/kg K_2O), and cultivated in a greenhouse at 30 ± 2°C. The prepared cell-free extract and LKE12 pellet were applied to soil samples containing 13-day-old oriental melon seedlings, and subsequent additional treatments were applied 15 days later. The shoot length, root length, fresh weight, and chlorophyll content were measured (by chlorophyll meter; Minolta Co., Ltd, Japan) in 30-day-old plants. The freeze-dried plant samples were ground and used to determine mineral contents.

Nutrient Uptake in Cucumber Plants

Cucumber plant samples (500 mg) were digested with $HClO_4$ and H_2SO_4, heated up to complete digestion, and allowed to cool at room temperature. The digested samples were diluted by distilled water and filtered through 0.5 μm filters. Macro and micronutrients, such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), boron (B), copper (Cu), aluminum (Al), silicon (Si), cobalt (Co), and zinc (Zn), were determined by injecting digested samples to inductively coupled plasma mass spectrometry (ICP-MS). Moreover, in order to determine nitrogen (N) contents, 1 g of sample was digested with H_2SO_4 and 50% $HClO_4$, and then heated at 200°C for 12 h. It was later filtered through Whatman No. 2 filter paper and diluted with distilled water. The N content was quantified with the Kjeldahl method [24], whereas the phosphorus (P) content was determined with the vanadate method [15]. Ammonium meta vanadate was added to 5 ml of digested solution. This mixture stood for 15 min at 30°C and was measured

at 470 nm. The P content was calculated using standard values.

Statistical Analysis

The experiment was set in a randomized block design and the subsequent statistical analysis was performed using the SigmaPlot software. Mean values were compared by Duncan's multiple range test at $p < 0.05$.

Results

Effect of *E. faecium* LKE12 on Growth of the GA Rice Mutant *waito-C*

E. faecium LKE12 was isolated from the rhizosphere of oriental melon and identified by phylogenetic analysis (Fig. 1). The result of the BLAST search showed that 16S rDNA sequences of the LKE12 strain had full similarity (100%) with *Enterococcus* species (Fig. 1). The phylogenetic analysis with other related and unrelated 16S rDNA bacterial sequences established that the LKE12 strain belonged to *E. faecium* (97% sequence homology). The sequence of the target strain was submitted to GenBank, receiving the accession number KJ956040. The plant growth-promoting ability of *E. faecium* LKE12 was tested in dwarf (*waito-C*) and normal rice (cv. Hwayongbyeo). A stunted growth, mainly associated with reduced shoot length, shoot fresh and dry weights, and chlorophyll content, was noted for dwarf rice plants when compared with Hwayongbyeo (normal phenotype) (Fig. 2). The shoot length of *waito-C* and Hwayongbyeo was significantly higher for the LKE12 bacterial treatment than in bacterium-untreated control plants. The bacterial association also influenced the biomass of rice plants. A remarkable increase of fresh and dry weights for both *waito-C* and Hwayongbyeo was observed in LKE12-associated plants. In addition, a photosynthetic pigment, chlorophyll, increased slightly owing to the effect of the LKE12 strain in both the dwarf and the normal rice plants. The medium components used for bacterial culture did not play a significant role in rice growth as proved *via* separate treatments for LB medium.

Phytohormone Production in *E. faecium* LKE12 Culture

Plant growth-supporting hormones such as GAs and IAA were detected in the *E. faecium* LKE12 culture medium. An array of GAs was found in diverse quantities for the culture medium of *E. faecium* LKE12 (Fig. 3). GA_1, GA_3, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, and GA_{53} were found in a range between 0.32 and 4.04 ng/100 ml. Among them, GA_7 was higher (4.04 ng/100 ml) in the culture

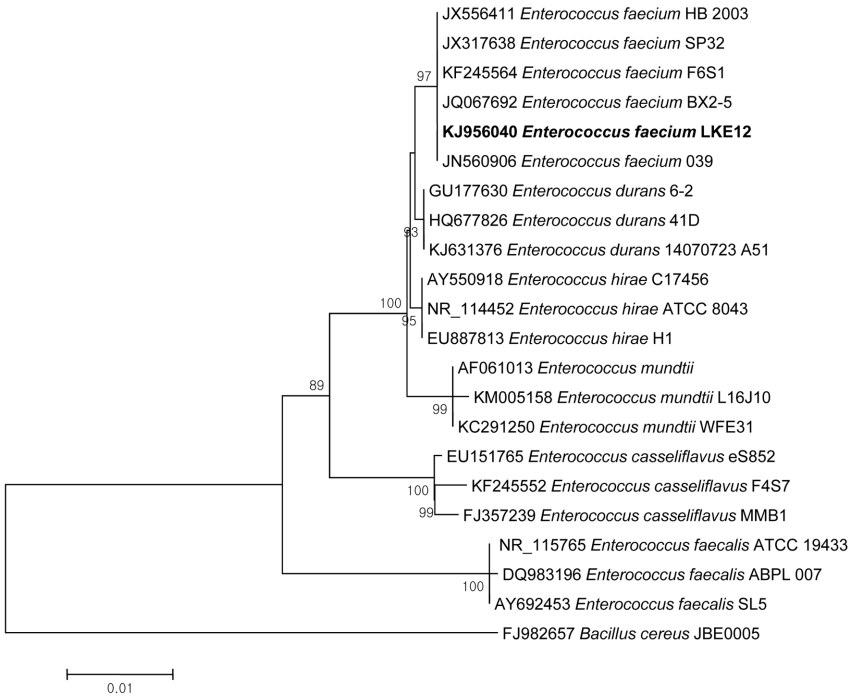

Fig. 1. Phylogenetic tree of the bacterial isolate *Enterococcus faecium* LKE12 (KJ956040), based on the 16S rDNA sequences of LKE12 and related bacteria.

Bacillus cereus was used as an out-group.

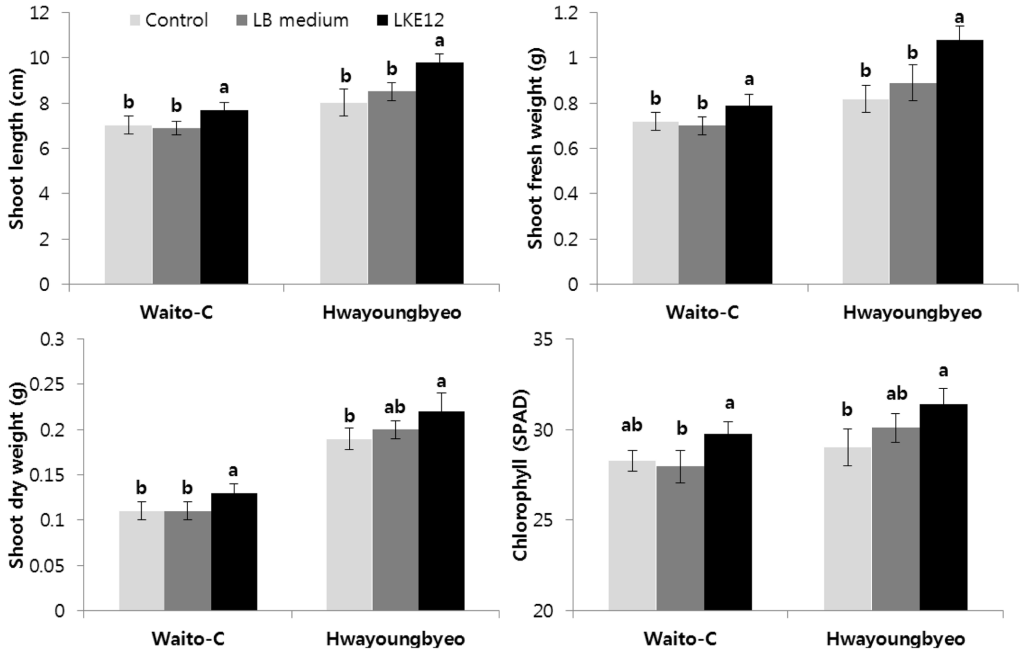

Fig. 2. Effect of *E. faecium* LKE12 on the growth of the GA-deficient rice mutant *waito-C* and a normal rice cultivar (Hwayongbyeo).
Values represent the mean ± standard error (n = 15). Means followed by the same letter are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

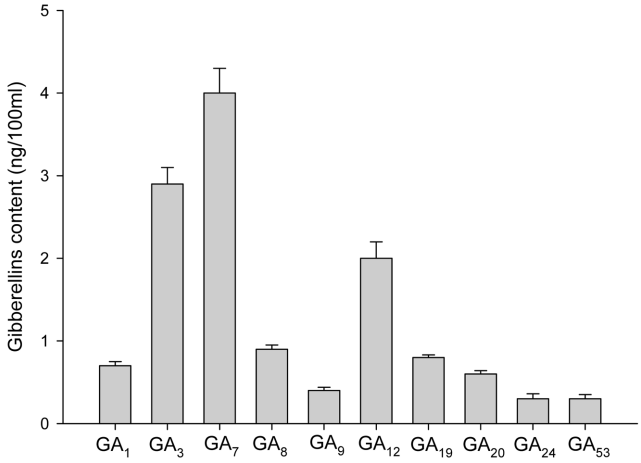

Fig. 3. Secretion of exogenous gibberellins from *E. faecium* LKE12 into the culture medium.
Bars represent means ± standard error (n = 3).

medium (Fig. S1), followed by GA_3 (2.94 ng/100 ml) and GA_{12} (1.97 ng/100 ml). Strain LKE12 produced GA_8 (0.92 ng/100 ml), GA_{19} (0.77 ng/100 ml), GA_1 (0.70 ng/100 ml), GA_{20} (0.59 ng/100 ml), GA_9 (0.38 ng/100 ml), GA_{53} (0.33 ng/100 ml), and GA_{24} (0.31 ng/100 ml) in trace amounts. The root-stimulating hormone IAA was found in a high concentration (4.5 μg/100 ml) in the culture medium (Fig. S2).

Effect of Cell-Free Extract of *E. faecium* LKE12 Strain on Cucumber Plant Growth

The GAs and IAA produced by *E. faecium* LKE12 were further studied for host plant growth improvement. Separate experiments were conducted for cucumber plants under cell-free extract and LKE12 bacterium treatments. In a comparison with controls, the plant shoot length was significantly increased (40%) by the application of LKE12 cell-free extract (Table 1 and Fig. 4). The bacterial interaction was also responsible for an enhancement of shoot length (36%) with respect to controls. A similar pattern for root length variation was observed in cucumber plants treated with cell-free extract and bacterial inoculation. LKE12 cell-free extract helped cucumber plants to obtain the maximum increment in root length rate (20%). The fresh weight was higher in plants associated with the LKE12 strain , followed by those treated with the cell-free extract of LKE12, and controls. In addition, low amounts of chlorophyll were found in control plants. The inoculation of LKE12 and the application of its cell-free extract contributed to higher concentrations of chlorophylls in cucumber plants.

Role of *E. faecium* LKE12 in Nutrient Uptake by Cucumber Plants

The primary nutrients, nitrogen (N), phosphorus (P), and

Table 1. Influence of *E. faecium* LKE12 and its cell-free extract on oriental melon plant growth.

Treatment	Shoot length (cm)	Root length (cm)	Plant fresh weight (g)	Chlorophyll content (SPAD)
Control	12.54 ± 1.07^{b}	14.24 ± 0.67^{c}	3.16 ± 0.36^{b}	22.74 ± 1.73^{b}
LKE12	17.06 ± 1.04^{ab}	15.8 ± 1.30^{b}	5.64 ± 0.69^{a}	27.64 ± 2.04^{a}
Cell-free extract	17.58 ± 0.76^{a}	17.02 ± 2.89^{a}	5.54 ± 0.48^{ab}	26.14 ± 1.93^{ab}

Values represent the mean ± standard error (n = 15). Means followed by the same letter are not significantly different (p < 0.05) as determined by Duncan's multiple range test.

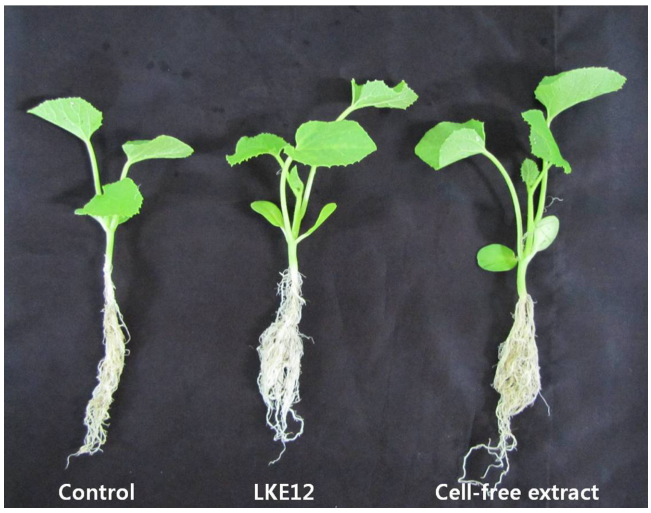

Fig. 4. Effect of *E. faecium* LKE12 and its cell-free extract on the growth of the oriental melon plant.

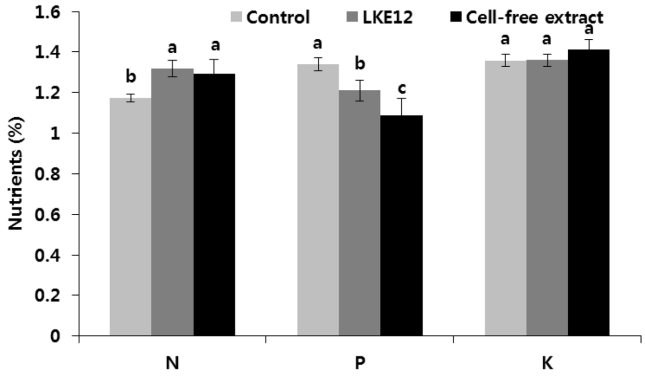

Fig. 5. Effect of *E. faecium* LKE12 and its cell-free extract on the uptake of nitrogen, phosphorus, and potassium by oriental melon plants.

Bars represent means ± standard error (n = 3). Means followed by the same letter are not significantly different (p < 0.05) as determined by Duncan's multiple range test.

potassium (K), as well as other macro and micronutrients such as magnesium (Mg), calcium (Ca), sodium (Na), copper (Cu), iron (Fe), manganese (Mn), boron (B), silicon (Si), cobalt (Co), zinc (Zn), and aluminum (Al), were determined in cucumber plants inoculated with LKE12 bacterium and cell-free extract. Results revealed that strain LKE12 seemed to improve N uptake by the plants, as shown by the low levels of N in untreated controls (Fig. 5). On the other hand, the rate of P uptake was reduced in the LKE12 interaction, and no significant differences in K content were observed between inoculated and un-inoculated plants. The cell-free extract containing phytohormones of LKE12 stimulated the uptake of N and K and reduced the accumulation of P in cucumber, as seen by differences between treated and control plants. In addition, a higher quantity of macronutrients such as Mg, Ca, and Na was found in plants treated with the cell-free extract (Table 2). LKE12 led to an enhancement of Ca uptake for cucumber, but was not effective for the transport of Mg and Na by the plant. The concentration of micronutrients changed drastically for LKE12 bacterial and its cell-free extract interaction. The Fe content in control plants was 69.11 mg/kg, but increased to 98.81 and 216.74 mg/kg when the plants were treated with bacterium and cell-free extract, respectively. For micronutrients, a high quantity of Mn and B was observed in cucumber plants. The LKE12 strain significantly inhibited Mn and B uptake, whereas its extract produced a positive increase in Mn content in cucumber. Cu and Al accumulation was lower in plants with the LKE12 strain than in those plants with cell-free extract. In addition, cell-free extract-treated plants had a higher amount of Si, Co, and Zn than bacterium-inoculated and un-inoculated controls. The LKE12 bacterium treatment stimulated Si and Zn transport in cucumber.

Discussion

The many potential benefits of using microorganisms for crop improvement has raised the research interest on plant-growth promoting bacteria from soil. In this study, a bacterial strain, LKE12, isolated from the rhizosphere of oriental melon and later identified as *Enterococcus faecium*, was applied to host plants to evaluate its associated mechanisms for plant growth promotion. The positive

Table 2. Role of *E. faecium* LKE12 and its cell-free extract on uptake of macro and micronutrients by oriental melon plants.

Nutrients (mg/kg)	Control	LKE12	Cell-free extract
Macronutrients			
Mg	12,556.75 ± 136.54^{b}	12,775.08 ± 141.17^{b}	13,524.08 ± 357.78^{a}
Ca	35,634.22 ± 343.86^{b}	47,945.89 ± 1417.03^{a}	42,758.22 ± 872.22^{ab}
Na	2,697.39 ± 20.50^{b}	2,228.92 ± 55.86^{c}	5,522.19 ± 163.25^{a}
Micronutrients			
Fe	69.11 ± 2.18^{c}	98.81 ± 2.22^{b}	216.74 ± 16.45^{a}
Mn	171.17 ± 1.39^{b}	55.94 ± 2.13^{c}	200.80 ± 17.14^{a}
B	363.81 ± 5.30^{a}	164.86 ± 3.93^{b}	67.99 ± 4.62^{c}
Cu	2.21 ± 0.06^{a}	0.20 ± 0.04^{c}	0.75 ± 0.16^{b}
Al	59.75 ± 0.92^{a}	37.81 ± 0.97^{b}	51.94 ± 1.41^{ab}
Si	2.74 ± 1.43^{b}	12.17 ± 2.29^{ab}	15.97 ± 4.94^{a}
Co	0.24 ± 0.11^{b}	0.26 ± 0.29^{b}	0.86 ± 0.07^{a}
Zn	35.68 ± 0.39^{c}	41.90 ± 1.11^{b}	59.49 ± 3.36^{a}

Values represent the mean ± standard error ($n = 3$). Means followed by the same letter are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

effect of *E. faecium* LKE12 was confirmed by promoting plant growth in rice. In fact, the significant increase of biomass and length for a GA-deficient rice dwarf (*waito-C*) and a normal GA-producing rice cultivar (Hwayongbyeo) through the effects of *E. faecium* LKE12 revealed that bacterial treatments could be beneficial for crop production. The C13 hydroxylation pathway, responsible for GA biosynthesis, is blocked in *waito-C* [14]. In order to recognize the importance of the phytohormones produced by *E. faecium* LKE12, the seeds of *waito-C* were subjected to an additional treatment with uniconazole, thus aiming at complete suppression of GA synthesis. In this experiment, we assumed that *E. faecium* LKE12 can segregate GAs from their active biosynthetic pathway, similar to that observed in *Gibberella fujikuroi*, which has been widely reported as a producer of different GA arrays [38].

The functional role of endogenous GAs in plants was evaluated in several physiological processes, such as seed germination, shoot elongation, sex determination, flowering, fruiting, and senescence [30, 34]. Microorganisms release GAs to the rhizosphere of plant roots, representing an additional source of GAs for plant growth [20, 33]. Furthermore, limited work has been performed for the production of plant growth regulators from soil bacteria. Recently, we reported the GA-producing *Pseudomonas putida* H-2-3 enhanced soybean plant growth during salt and drought stresses [21]. Garcia-Fraile *et al.* [12] detected IAA in *Rhizobium leguminosarum* culture, which stimulated plant growth and fruit production in pepper and tomato. The culture medium of *E. faecium* LKE12 contained an array of GAs (GA_1, GA_3, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, and GA_{53}) and IAA, whose presence seemed to directly stimulate rice growth. *E. faecium* LKE12 segregated GAs and IAA that might be transported into *waito-C* rice plants through the roots, thereby accelerating the cellular processes. Root growth promoted by bacteria can be explained by auxin production, as well as other additional effects on plant growth, through GA secretion [3]. IAA is involved in apical dominance, root initiation, cell division, and elongation [35]. However, nodule formation in legume roots is mainly influenced by the bacterial IAA concentration [36]. The results of this study confirmed the significant role that *E. faecium* LKE12 plays for potential enhancements of plant growth in rice.

To evaluate and confirm the plant growth-promoting effect of *E. faecium* LKE12 and its cell-free extract, oriental melon plants were grown in a greenhouse under bacterial treatments. A remarkable increase in length and biomass for plants with a high content of chlorophyll, due to the interaction of *E. faecium* LKE12 and its cell-free extract, revealed that the bacterial association positively supports growth of the host plant. Similar reports on plant growth benefits of GA-producing bacterial isolates *Acetobacter diazotrophicus*, *Acinetobacter calcoaceticus*, *Azospirillum brasilense*, *Herbaspirillum seropedicae*, *Bacillus licheniformis*, *Bacillus macrolides*, *Bacillus pumilus*, *Burkholderia* sp. KCTC 11096BP, *Leifsonia soli* SE134, *Pseudomonas putida* H-2-3, and *Rhizobium phaseoli* have been documented by several researchers [2, 4, 13, 16–21]. GAs and IAA segregated by the bacteria might be transferred into oriental melon plants through the absorption process in the roots, additionally stimulating the biosynthesis of various metabolites such as chlorophyll. The accelerated synthesis of chlorophyll might be due to the presence of a higher amount of Mg in plants; this can be

observed when comparing the high content of Mg in the plants under the cell-free extract treatment with respect to control plants. The role of chlorophyll in photosynthesis is well known; an increase of chlorophyll content triggers photosynthesis and enhances starch production, thus leading to improved plant growth [7, 21].

The nutritional uptake efficiency of plants can be enhanced by microbial interaction and plant hormonal treatments. For example, the N-fixing and P-solubilizing properties of rhizobacteria help to store the essential nutrients in the soil, and plants can use these nutrients for their growth [5]. In addition, gibberellin-producing *A. calcoaceticus* and *B. megaterium* and *P. putida* solubilized phosphate and increased the nutrient content in plants [19, 21, 22]. In the current study, we found that *E. faecium* LKE12 and its cell-free extracts containing bio-active metabolites enhanced nutrient uptake by the plants. The cell-free extract of LKE12 significantly increased Mg, Ca, Na, Fe, Mn, Si, Zn, and N accumulation in oriental melon through the availability of phytohormones in its extract. Previously, Eid and Abou-Leila [9] reported that the exogenous application of GA increased Mg, Fe, Zn, and Mn contents in plants. The transport of macro and micronutrients in plants activates many cellular functions, since these minerals are involved in the synthesis of proteins, nucleic acids, and lipids, and also stimulate enzyme activities. The cell-free extract of *E. faecium* LKE12 increased the content of Na by 2-fold with respect to controls. A certain level of Na is desirable to maintain the osmotic potential and to absorb water; on the contrary, an excess of Na becomes toxic to plant cells [31]. The results of the current study revealed that the inoculation of *E. faecium* LKE12 and its culture extract provided significant beneficial effects on nutrient uptake for the plants.

The *E. faecium* LKE12 strain isolated from soil was found to be involved in promoting plant growth of both dwarf and normal rice cultivars, suggesting that LKE12 produces phytohormones such as GAs, which might stimulate crop growth. In this study, we identified 10 GAs as well as IAA in the bacterial culture medium. Further research on the interaction of cell-free extract from *E. faecium* LKE12 with oriental melon confirmed that the secretion of a higher amount of the plant growth-stimulating hormones GA and IAA by LKE12 is an important mechanism to promote plant growth. Comparatively, the lower growth of oriental melon under LKE12 bacterial treatment with respect to its cell-free extract revealed that bacterial growth and interaction might be affected by environmental factors, and that bacteria can produce high amounts of phytohormones in a suitable medium. Therefore, the application of a phytohormone-rich cell-free extract of the bio-inoculant *E. faecium* LKE12 can enhance crop growth, with its added value of being a safe method for sustainable agriculture.

Acknowledgments

We thank the National Research Foundation of Korea (NRF), founded by the Ministry of Science, ICT, and Future-Planning through the Basic Science Research Program (2014R1A1A1004918).

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