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Characterization of *Bacillus luciferensis* Strain KJ2C12 from Pepper Root, a Biocontrol Agent of Phytophthora Blight of Pepper

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In this study, we characterized the bacterial strain KJ2C12 in relation with its biocontrol activity against *Phytophthora capsici* on pepper, and identified this strain using morphological, physiological, biochemical, fatty acid methyl ester, and 16S rRNA gene sequence analyses. Strain KJ2C12 significantly (P=0.05) reduced both final disease severity and areas under the disease progress curves of 5-week-old pepper plants inoculated with *P. capsici* compared to buffer-treated controls. As for the production of antibiotics, biofilms, biosurfactant, extracellular enzyme, HCN, and swarming activity, strain KJ2C12 produced an extracellular enzyme with protease activity, but no other productions or swarming activity. However, *Escherichia coli* produced weak biofilm only. Strain KJ2C12 could colonize pepper roots more effectively in a gnotobiotic system using sterile quartz sand compared to *E. coli* over 4 weeks after treatments. However, no bacterial populations were detected in 10 mM $MgSO_4$ buffer-treated controls. Strain KJ2C12 produced significantly higher microbial activity than the $MgSO_4$-treated control or *E. coli* over 4 weeks after treatments. Bacterial strain KJ2C12 was identified as *Bacillus luciferensis* based on morphological, physiological, and biochemical characteristics as well as FAME and 16S rRNA gene sequence analyses. In addition, these results suggested that *B. luciferensis* strain KJ2C12 could reduce Phytophthora blight of pepper by protecting infection courts through enhanced effective root colonization with protease production and an increase of soil microbial activity.

Keywords : Antagonistic bacteria, *Bacillus luciferensis*, Biological control, *Phytophthora capsici*

Bacillus spp. are gram-positive bacteria and ubiquitous in agricultural systems (McSpadden Gardener, 2004), and are well known to control various plant diseases. Strains of *Bacillus megaterium*, *B. cereus*, and *B. subtilis* have been used for the biocontrol purpose (Idris et al., 2008; Kildea et al., 2008), and Serenade, EcoGuard, Kodiak, Yield Shield, and BioYield are the common commercial biocontrol products. These products could be achieved from the intrinsic properties of *Bacillus*, such as formation of oval endospores that remain dormant for a long period under unfavorable environmental conditions (Fritze, 2004; Schisler et al., 2004).

The characterization of biocontrol agents with activity against soilborne diseases is important to achieve successful control activity due to the complex and dynamic environmental conditions in the rhizosphere (Handelsman and Stabb, 1996). Generally, biocontrol ability by antagonistic bacteria involves either competition (Elad and Chet, 1987) or production of bacterial metabolites such as siderophores, hydrogen cyanide, antibiotics, or extracellular enzymes for antagonism against plant pathogens (Han et al., 2005; Kamilova et al., 2005; Lee et al., 2004; Sang et al., 2006; Yamaguchi et al., 2001). Moreover, colonization and population dynamics of antagonistic bacteria in the rhizosphere or on roots are considered as important aspects of biocontrol and have been studied in details (Kamilova et al., 2005; Landa et al., 2003; Sang et al., 2008). It has been reported that *Bacillus* spp. contain various biocontrol characteristics including secondary metabolites, colonizing potential, and production of competitors (Schmidt et al., 2004; Yoshida et al., 2001).

Phytophthora blight of pepper caused by *Phytophthora capsici* is known to be difficult to manage due to the production of motile zoospores and overwintering oospores (Ristaino and Johnston, 1999). In our previous study, we obtained an effective strain KJ2C12 antagonistic against Phytophthora blight of pepper by a sequential selection procedure including radicle, seedling, plant, and field tests (Kim et al., 2008). Thus, this selected strain KJ2C12 was identified and characterized for root colonization as well as productions of antibiotics, hydrogen cyanide, or extracellular enzyme to determine its biocontrol activity. In this

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study, the objectives were to characterize strain KJ2C12 in relation with its biocontrol activity against *P. capsici* on pepper, and identify this strain using morphological, physiological, and biochemical characteristics as well as fatty acid methyl ester (FAME) and 16S rRNA gene sequence analyses.

Materials and Methods

Bacterial strain and biocontrol efficacy. The bacterial strain KJ2C12 antagonistic against Phytophthora blight of pepper was isolated from roots of pepper plants grown in the fields of Kwangju, Korea in 2001 (Kim et al., 2008). The bacterial strain has been deposited as *Bacillus luciferensis* KJ2C12 (KACC 13031) in the Korean Agricultural Culture Collection (KACC) of the National Institute of Agricultural Biotechnology, Suwon, Korea. Biocontrol efficacy of strain KJ2C12 against *P. capsici* was evaluated in 5-week-old pepper plants (cv. Nockwang) as described by Kim et al. (2008). Disease severity was evaluated using a scale of 0 (symptomless) ~ 5 (plants dead) for 11 and 18 days after inoculation for experiments 1 and 2, respectively. Areas under the disease progress curves (AUDPC) were also determined for experiments 1 and 2 based on disease severity for eight and 12 observations over 11 and 18 days following inoculation, respectively (Shaner and Finney, 1977). This experiment was conducted twice with 15 plants each.

Strain characteristics with biocontrol activity. Production of antibiotics, biofilms, biosurfactant, extracellular enzymes, HCN, and swarming activity were evaluated to characterize the antagonistic activity of strain KJ2C12 against *P. capsici*. *Escherichia coli* was used as a control in these tests. For antibiotics production, strain KJ2C12 was tested for inhibition of fungal growth against various plant pathogens such as *Alternaria mali*, *Colletotrichum orbiculare*, *Fusarium oxysporum* f. sp. *lycopersici*, *Magnaporthe grisea*, *P. capsici*, and *Rhizoctonia solani* on V8 juice agar as described by Chang et al. (2000). Biofilm formation of the strain KJ2C12 was performed using 96-well PVC microtiter plates (Fischer Scientific, Pittsburgh, USA) as described by O'Toole et al. (1999). Biosurfactant production was determined using the drop-collapse method described by Bodour and Miller-Maier (1998). Extracellular enzyme production was detected as diffusion-clearing zones on milk agar for proteolytic activity and on chitin oatmeal agar for chitinase activity as described by Boer et al. (1998). HCN production was examined qualitatively on tryptic soy agar (TSA) using the method described by Castric and Castric (1983). Swarmming ability was tested on M9 salt medium supplemented with 0.2% glucose, 2 mM $MgSO_4$, 0.5% agar, and without NH_4Cl (Köhler et al., 2000).

Root colonization. Root colonization by the bacterial strain was conducted to detect its ability to colonize fungal infection courts using a gnotobiotic system (Simons et al., 1996). Sterile pepper seeds (cv. Nockwang) treated with 1% NaOCl were immersed in bacterial suspensions (10^8 cfu/ml) of strain KJ2C12 or *E. coli* (positive control) for 3 hrs. Seeds treated with 10 mM $MgSO_4$ buffer served as a negative control. These treated seeds were placed in bottles (5 cm diameter × 10 cm high) containing sterile quartz sand with 10% (v/w) plant nutrient solution (Hoffland et al., 1989). These bottles were kept in a growth chamber (20°C, 70% relative humidity, 16 hrs daylight) for 4 weeks. Pepper roots were sampled 2 and 4 weeks after seeding and cut off to generate three fragments (root tip, mid-root, and root base). Root fragments were placed into 10 ml of sterile water and shaken at 160 rpm at 28°C for 30 min. These suspensions were cultured at 28°C for 48 hrs on nutrient agar (NA, Difco, Detroit, USA). Bacterial colonies were counted 2 days after incubation and expressed as cfu per cm length of root fragments. This experiment was conducted twice with five replicates each.

Total microbial activity. Total microbial activity was determined by measuring hydrolysis of fluorescein diacetate (FDA, Sigma, St. Louis, USA) using the method described by Schnürer and Rosswall (1982). Three-week-old pepper (cv. Nockwang) seedlings were transplanted into 10-cm-diameter pots with saucers containing steam-sterilized soil. A week later, 25 ml of the bacterial suspensions (10^8 cfu/ml) of strain KJ2C12 or *E. coli* (positive control) were added to the pots. Pepper plants treated with 25 ml of 10 mM $MgSO_4$ buffer served as a negative control. Soils (5 g) were sampled 0, 1, 2, 3, and 4 weeks after treatment (WAT) by adding 20 ml of 60 mM sodium phosphate buffer (pH 7.6). FDA (400 μg) was added to the samples and agitated on a rotary shaker at 100 rpm for 30 min. This reaction was stopped by adding 20 ml of acetone. As a blank, one of the replicates was treated with 20 ml of acetone as soon as the FDA was added, thereby terminating the reaction. Soil from the mixtures was removed by filtration through a filter paper (Whatman No. 1). The amount of hydrolyzed FDA was determined by measuring absorbance at 490 nm with a spectrophotometer (Ultrospec® 500/1100 *pro*, Piscataway, USA). Absorbances of all samples were within the linear range of standard curves with FDA. Total microbial activity was expressed as μg hydrolyzed FDA per min per g dry weight of soil. This experiment was conducted twice with three replicates each.

Scanning electron microscopy. Morphological characteristics of strain KJ2C12 including shape, size of bacterial

cell, and existence of flagella were examined using scanning electron microscopy (SEM). To prepare specimens for SEM, strain KJ2C12 was grown on NA for 2 days at 28°C. Agar sections (2.5-3.0 cm) were first fixed with Karnovsky's fixative for 1 day, and then washed three times with 0.05 M cacodylate buffer. These agar sections were then treated with 1% osmic acid for 2 hrs and washed three times with 0.05 M cacodylate buffer. The sections were preceded through a graded alcohol dehydration series. The specimens were processed in a critical point dryer after dehydrated, then mounted and placed in a gold coater. After gold coating was complete, specimens were observed with a scanning electron microscope (LED 440 1420vp, LEO Electron Microscopy Ltd., Cambridge, England).

Biochemical and physiological identification. Phenotypic tests such as Gram staining, and hydrolysis of casein, gelatin, and starch were conducted with strain KJ2C12 and other reference species of *Bacillus* such as *B. luciferensis*, *B. funiculus*, *B. thuringiensis*, and *B. mycoides* as described by Williams et al. (1989). Measurements of the hydrolysis of aesculin, DNA, and tween 80 was conducted by the methods of Gerhardt et al. (1994). Motility was determined on motility test medium with 2,3,5-triphenyltetrazolium chloride (TTC) (Ball and Sellers, 1966). Additional biochemical tests were conducted including nitrate reduction by Grisess-Ilosvay regents I and II, and acid production from lactose, D-glucose, D-mannitol, D-mannose, and D-xylose. Bacterial growth on D-galactose, D-cellobiose, sucrose and maltose were also tested (Vries and Stouthamer, 1968).

FAME analysis. Total cellular FAME analysis of strain KJ2C12 was conducted by gas chromatography using the MIDI system (Microbial Identification System, Inc., Newark, USA) according to the manufacturer's instruction. The bacterial culture (40 mg) grown on BBL trypicase soy agar (Becton, Dickinson and Company, Sparks, USA) at 28°C for 24 hr was harvested and processed as the method described by Stead (1989). FAMEs were extracted into methyl-*tert* butyl ether/hexane (1:1, v:v) and injected into a gas-liquid chromatograph (Hewlett-Packard 5898A, GC system, Avondale, USA). Separated peaks were detected by flame ionization and identified by a Hewlett-Packard 5898A Microbial Identification System. For each peak generated by a FAME, the peak area was weighted against a known calibration mix and normalized to give 100% for all peaks in the run. An equivalent carbon chain length was calculated by the linear interpolation of the retention time between reference peaks of the two saturated-straight chain fatty acids (methyl ester).

16S rRNA gene sequencing. DNA was extracted from strain KJ2C12 using a QIAGEN Genomic-tip system (Qiagen GmbhH, Hilden, Germany) according to manufacturer's instructions. A 1,545-bp DNA fragment encompassing the 16S rRNA gene was amplified from gDNA of strain KJ2C12 using the universal primer fD1 (5'-AGAGTTTG-ATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTG-TTACGACTT-3') (Weisburg et al., 1991). A PCR reaction (100 μl) contained 5U of *Taq* DNA polymerase (Roche, Mannheim, Germany), 10 μl (10 ng/μl) of template DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 250 μM dNTP, and 0.4 μM of each primer. The sample was first incubated for 5 min at 95°C. Next, 35 cycles were performed as follows: 4 min at 95°C, 1 min at 58°C, and 2 min at 72°C. Lastly, the reaction was incubated at 72°C for 8 min. PCR products were purified using the QIAquick spin PCR purification kit (Qiagen GmbhH, Hilden, Germany). DNA sequence analysis was performed using BLAST network services at the NCBI (http://www.ncbi.nlm.nih.gov). A phylogenetic tree was constructed with the neighbor-joining method using MEGA3.1 (Kumar et al., 2004), and bootstrap analysis was also conducted using the same program. The 16S rRNA gene sequence of strain KJ2C12 has been deposited in GenBank under accession number AY514023.

Statistical analysis. Statistical analysis of data was conducted using the Statistical Analysis Systems (SAS Institute, 1988). Analysis of variance was determined using the general linear model procedures and means were separated with the least significant difference (LSD) at P=0.05.

Results

Biocontrol efficacy and characteristics of strain KJ2C12. Strain KJ2C12 significantly (P=0.05) reduced both final disease severity and AUDPC of 5-week-old pepper plants inoculated with *P. capsici* compared to buffer-treated controls (Fig. 1). As for the production of antibiotics, biofilms, biosurfactant, extracellular enzyme, HCN, and swarming activity, strain K2C12 produced an extracellular enzyme with weak protease activity, but no other productions or swarming activity (Table 1). However, *E. coli* (control) produced weak biofilm only (Table 1).

Root colonization and total microbial activity by strain KJ2C12. The antagonistic strain KJ2C12 could effectively colonize pepper roots (root tip, mid-root, root base) in a gnotobiotic system compared to the positive control *E. coli* over 4 WAT (Fig. 2). The numbers of colonies of strain KJ2C12 ranged from $\log_{10}$ 4.50 to $\log_{10}$ 5.33 and those of *E. coli* from $\log_{10}$ 2.98 to $\log_{10}$ 3.74 2 WAT while KJ2C12 ranged from $\log_{10}$ 3.83 to $\log_{10}$ 4.50 and those of *E. coli*

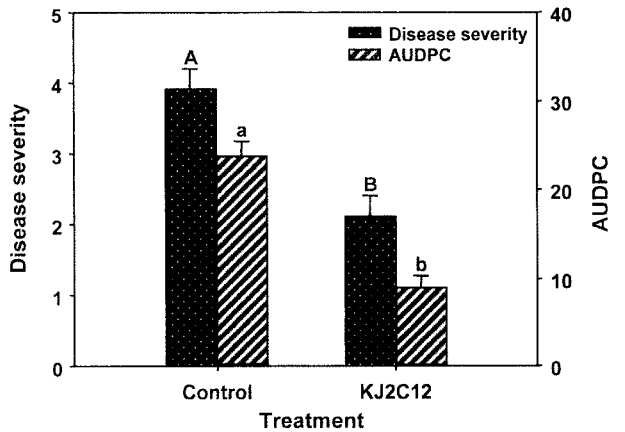

Fig. 1. Biocontrol activity of antagonistic bacterial strain KJ2C12 and 10 mM $MgSO_4$ buffer (control) on final disease severity [0 (symptomless) ~ 5 (plants dead)] and areas under the disease progress curve (AUDPC) caused by *Phytophthora capsici* in 5-week-old pepper plants (cv. Nockwang). Vertical bars on columns indicate standard errors of means of 30 replicates from combined data of two experiments. Different capital and small letters on columns indicate significant difference between treatments at P=0.05.

Table 1. Characteristics of *Bacillus luciferensis* strain KJ2C12 associated with its biocontrol activity compared to *Escherichia coli*

Character	Strain KJ2C12	*Escherichia coli*
Antibiotics	--[a]	–
Biofilm	–	+w
Biosurfactant	–	–
Extracellular enzymes		
Chitinase activity	–	–
Proteolytic activity	+w	–
HCN	–	–
Swarming	–	–

[a]Symbols: +, positive reaction; +w, weakly positive reaction; –, negative reaction.

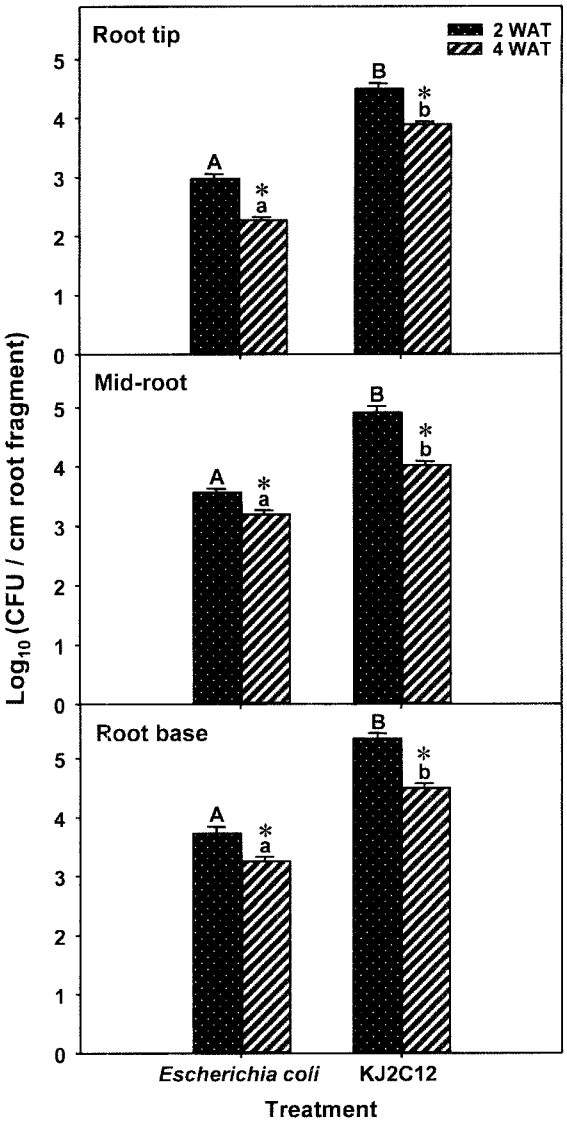

Fig. 2. Temporal and spatial changes in the colonization of germinated pepper (cv. Nockwang) roots (root tip, mid-root, and root base) treated with the antagonistic bacterial strain KJ2C12 and *Escherichia coli* (positive control) 2 and 4 wks after treatment (WAT). No bacterial strains were observed in 10 mM $MgSO_4$ buffer-treated controls. Different capital and small letters, or asterisks on columns indicate significant difference between treatments or WAT at P=0.05, respectively. Vertical bars are standard errors of means of 10 replicates from combined data of two experiments.

from $\log_{10}$ 2.27 to $\log_{10}$ 3.25 4 WAT. Strain KJ2C12 effectively colonized basal roots better than root middles or tips. The bacterial populations of roots were reduced significantly (P=0.05) over time regardless bacterial treatments. However, no bacterial populations were detected in 10 mM $MgSO_4$ buffer-treated controls (Fig. 2). Microbial activities of strain K2C12 and *E. coli* were reduced gradually over time as shown in Figure 3. Strain KJ2C12 produced significantly higher microbial activity than the untreated control or *E. coli* over 4 WAT. However, the total microbial activity of buffer-treated controls proved the lowest among treatments, and remained the same level over time (Fig. 3).

Identification of strain KJ2C12. Bacterial strain KJ2C12, which was antagonistic to *P. capsici*, was identified as *B. luciferensis* based on morphological, biochemical, and physiological characteristics as well as 16S rDNA sequence analysis (Tables 2 and 3; Figs. 4 and 5). The strain was gram-positive aerobic rods (0.8-0.9 μm × 1.3-1.6 μm) with no flagella, and colonies were white on TSA (Table 2 and Fig. 4). Strain KJ2C12 was positive for hydrolysis of casein, aesculin and starch, nitrate reduction, acid from lactose, D-glucose, D-mannitol and D-mannose, and utilization of D-

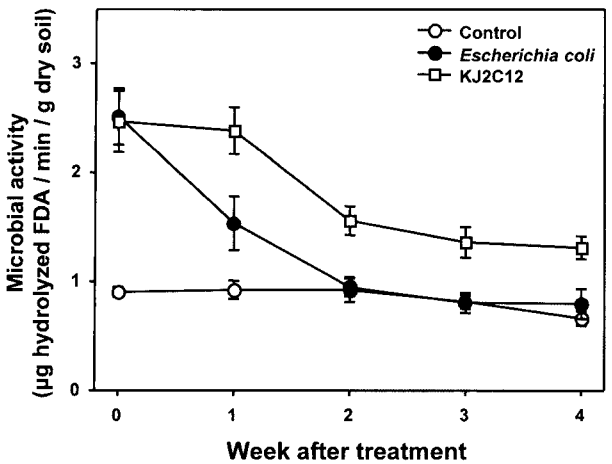

Fig. 3. Total microbial activity measured by hydrolysis of fluorescein diacetate (FDA) in pot soils grown with 4-week-old pepper plants (cv. Nockwang) that were drenched with antagonistic bacterial strain KJ2C12, *Escherichia coli* (positive control), or 10 mM $MgSO_4$ buffer-treated control. Vertical bars indicate standard errors of means of six replications from combined data of two experiments.

Table 2. Phenotypic characteristics of *Bacillus luciferensis* strain KJ2C12 that differentiate the isolates from other *Bacillus* species

Characteristics	1[a]	2	3	4	5
Motility	–[b]	–	–	–	–
Anaerobic growth	–	+	–	+	+
Gelatin hydrolysis	–	–	–	–	–
Casein hydrolysis	+	+	+	–	–
Aesculin hydrolysis	+	+	+	+	+
Starch hydrolysis	+	+w	+	–	–
Tween 80 hydrolysis	–	–	–	–	–
Nitrate reduction	+w	–	–	–	–
DNase	–	+w	–	+	+
Acid from:					
Lactose	+w	+w	–	–	–
D-Glucose	+	+	+w	+	+
D-Mannitol	+	–	–	+w	+w
D-Mannose	+	–	–	+	+
D-xylose	–	–	+w	–	–
Utilization of:	–	–	–	–	–
D-Galactose	+	+	+	–	–
D-Cellobiose	+	+	–	+	–
Sucrose	+	+	+	+	+
Maltose	+	–	+	+	–

[a]1, strain KJ2C12; 2. *B. luciferensis*; 3, *B. funiculus*; 4, *B. thuringiensis*; 5, *B. mycoides.*
[b]Symbols: +, positive reaction; +w, weakly positive or delayed response; –, negative reaction.

galactose, D-cellobiose, sucrose, and maltose. However, the strain was negative for gelatin hydrolysis or tween 80, and DNA hydrolysis, and acid from D-xylose (Table 2). In fatty acid methyl ester profiles, strain KJ2C12 composed mainly

Table 3. Fatty acid compositions of *Bacillus luciferensis* strain KJ2C12 in comparison to other *Bacillus* species

Fatty acid[a]	1[b]	2	3	4	5
Saturated					
14:0	2.1	2.7	–[c]	1.2	3.0
15:0	0.4	–	–	–	–
16:0	2.7	2.69	8.8	3.2	6.2
18:0	–	–	1.9	–	–
Saturated branch					
13:0 iso	0.5	–	–	–	7.6
14:0 iso	6.9	3.2	–	2.0	5.0
15:0 iso	30.2	24.3	18.0	19.0	20.9
15:0 anteiso	40.4	51.5	44.3	8.8	4.5
16:0 iso	9.7	4.46	3.8	2.1	10.2
17:0 iso	1.0	tr	–	–	6.9
17:0 anteiso	3.3	2.95	–	1.1	2.2
Unsaturated					
16:1 ω 11c	0.8	1.46	–	–	4.0
16:1 ω 7c alchol	1.1	3.36	–	–	4.6
18:1 ω 7c	0.6	–	–	–	–
Hydroxy					
17:0 2OH	–	–	–	–	–
17:0 iso 3OH	–	–	–	–	–

[a]The position of the double bond in unsaturated fatty acids is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*.
[b]1, strain KJ2C12; 2, *B. luciferensis*; 3, *B. funiculus*; 4, *B. thuringiensis*; 5, *B. mycoides.*
[c]Symbols: –, not detected; Tr, trace (< 0.1).

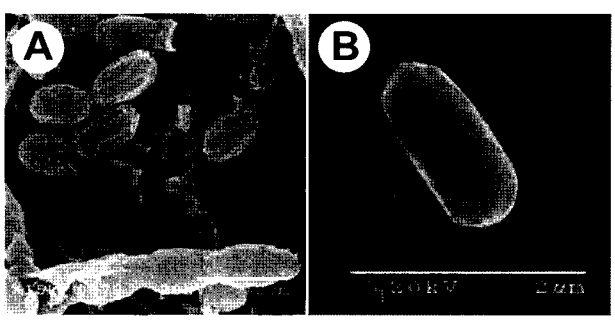

Fig. 4. Scanning electron micrographs of *Bacillus luciferensis* strain KJ2C12 grown on nutrient agar for 48 hrs. A, aggregated bacterial cells; B, single cell of strain KJ2C12.

of anteiso-15:0 and iso-15:0 and was identical to *Bacillus marinus* (similarity=0.535%) (Table 3). When the 16S rDNA gene sequence (1,545 bp) of strain KJ2C12 was compared with published sequences of species within the genus *Bacillus* and other members of the family *Bacillaceae*, strain KJ2C12 exhibited 98.2% similarity to that of *B. luciferensis* (AJ419629) (Fig. 5).

Discussion

Bacillus spp. have been widely used for biocontrol agents

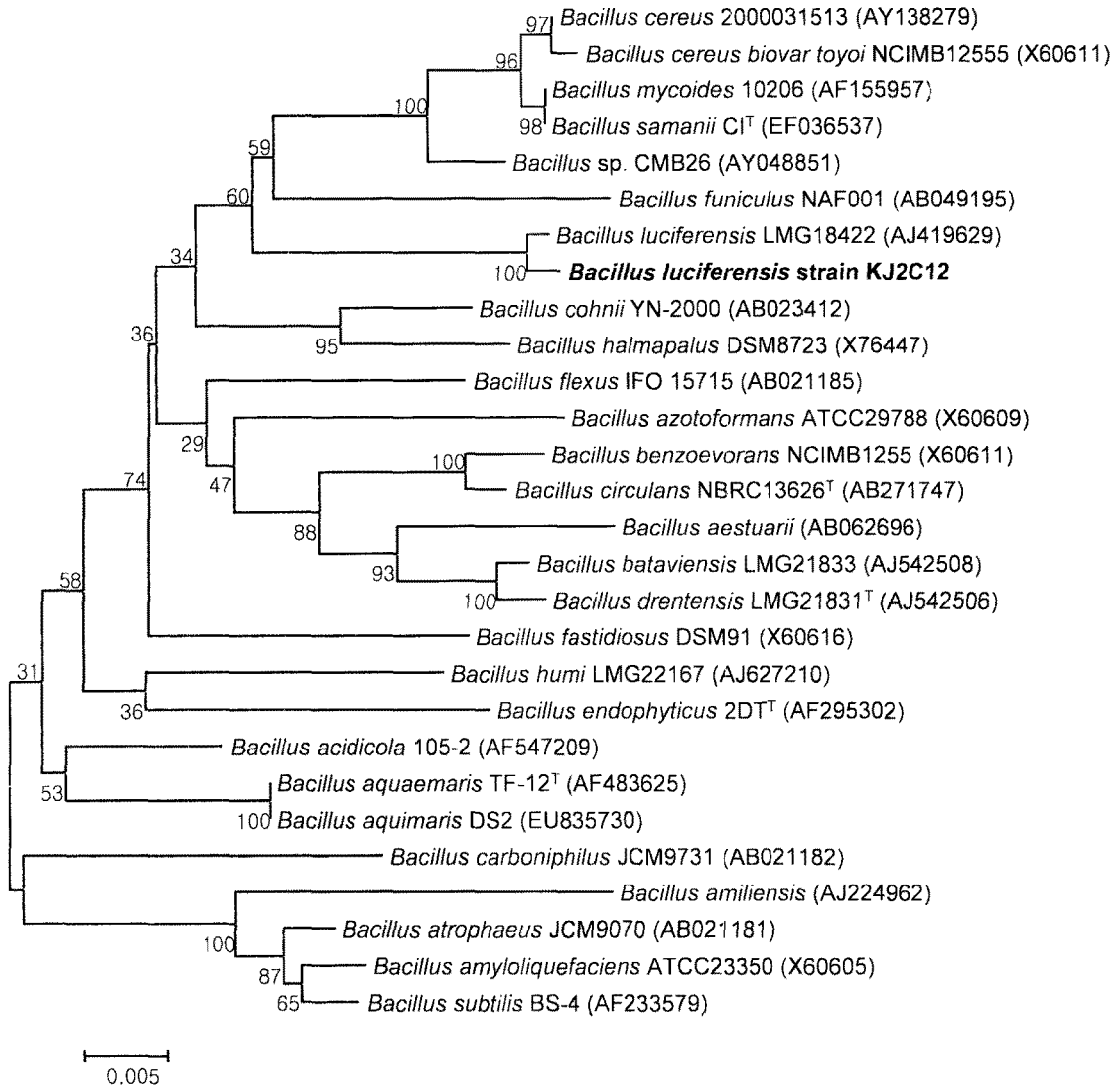

Fig. 5. A phylogenetic neighbor-joining tree showing the relationship of *Bacillus luciferensis* strain KJ2C12 with other related species in the *Bacillaceae* family based on phylogenetic analysis of 16S rRNA gene sequence. Bootstrap values of 1,000 analyses are shown at the branching points. The bar represents five nucleotide substitutions per 1,000 nucleotides of the 16S rRNA gene sequence. The type strain of the species and accession numbers are indicated as "T" and in parentheses, respectively.

against airborne or soilborne diseases such as Pythium damping-off, Phytophthora blight, and Septoria tritici blotch (Lee et al., 2008; Kildea et al., 2008; Schmidt et al., 2004). In our previous study, the bacterial strain KJ2C12 was confirmed to be an antagonist of *P. capsici* on pepper plants in the fields (Kim et al., 2008). In this study, we identified this strain to be *B. luciferensis* using various morphological, physiological, biochemical, and molecular analyses. For strain identification, 16S rRNA gene sequence analysis was more robust than other methods such as morphological, physiological, and biochemical analysis. The 16S rRNA gene sequence analysis reveals that this strain shares 98.2% similarity to that of *B. luciferensis*. Consequently, these results confirmed the strain KJ2C12 as *B. luciferensis*.

Several features of *Bacillus* spp. such as root colonization, biofilm formation, and production of secondary metabolites have been suggested as potential mechanisms for their biological control activities (Emmert and Handelsman, 1999; Yoshida et al., 2001). In this study, we found that *B. luciferensis* strain KJ2C12 could colonize root surfaces effectively and increase total soil microbial activity when it was applied to pepper plants. These results suggested that *B. luciferensis* strain KJ2C12 might alleviate the disease caused by *P. capsici* on pepper plants through induced systemic resistance and/or colonization on root surface to protect the infection courts with protease production. Many studies have shown that effective root colonization is correated to the protection of soilborne pathogen infections

with a concomitant reduction of plant diseases (Jaroszuk-Ściseł et al., 2008; Ongena and Jacques, 2008). Tombolini et al. (1999) found that *Pseudomonas chlororaphis* MA342, a potential biocontrol agent for seed-borne diseases, colonized the embryo of plant roots, co-localizing with the pathogen on plants, thereby facilitating the action of the fungistatic compounds produced by the strain. In addition, some *Bacillus* spp. are known to be good root colonizers and could protect infection courts effectively regardless of soilborne or airborne pathogens (Demoz and Korsten, 2006; Reva et al., 2004).

In addition, *B. luciferensis* strain KJ2C12 might be a rhizosphere competitor. Rhizosphere competed by biocontrol agents is potentially important for controlling plant diseases. Biocontrol agents utilize host substrates and were shown to support their activity (Kim et al., 2008). Consequently, this leads to a level of protection of plant roots from plant diseases. This is supported by Landa et al. (2003) who reported that colonization traits are caused by chemotaxis and the transfer of exudates compounds. Several studies (Caesar and Burr, 1987; Landa et al., 2003; Yuen and Schroth, 1986) demonstrated that introduction of fluorescent pseudomonads caused an alteration of rhizosphere microflora and reduced populations of major and minor pathogens. For example, when *Pseudomonas putida* W4P63 was applied to potato seedpieces, the populations of strain W4P63 ranged between 10^4 and 10^5 cfu/g root in the field. In comparison, the populations of *Erwinia carotovora* on the same roots were only 10% of that on roots without strain W4P63. Increasing the populations of biocontrol agents on a root might improve the levels of pathogen suppression. Overall, these results confirm that the strain KJ2C12, which exibits antagonistic activity against Phytophthora blight of pepper is a *B. luciferensis*. In addition, *B. luciferensis* strain KJ2C12 is a good biocontrol agent in protecting infection courts through effective root colonization, and possibly by protease production and an increase of soil microbial activity.

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