Production of a Phytotoxic Compound, 3-Phenylpropionic Acid by a Bacterial Endophyte, *Arthrobacter humicola* YC6002 Isolated from the Root of *Zoysia japonica*

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An endophytic bacterial strain, *Arthrobacter humicola* YC6002, was isolated from a surface sterilized root of Korean turf grass (*Zoysia japonica*) collected from Jinju, Korea. This strain showed inhibitory effect on germination and shoot growth of radish. The inhibition of germination and shoot growth of radish seeds varied depending on the age of culture and the temperature at which it was incubated. The culture filtrate of 1/10-strength Tryptic Soy Broth medium, incubated for 48 hours at 30°C, showed the highest inhibitory effect on radish seed germination and shoot growth (92% inhibition as compared to control). The active compound with seed germination and shoot growth inhibition was purified and identified as 3-phenylpropionic acid. The purified compound had 53% and 93% inhibitory effect on seed germination and shoot growth of radish for 500 and 1000 ppm solutions, respectively.

Keywords : endophyte, phytotoxic, *Arthrobacter humicola*, 3-phenylpropionic acid

Many endophytic bacteria inhabiting in the tissue of host plants have been studied for their beneficial effects on growth and plant yield of host plant, maintenance and protection of plant health, pollution control and phytoremediation (Brooks et al., 1994; Miller et al., 1998; Ryan et al., 2008; Siciliano et al., 2001). These symbiotic interactions of endophytes with host plants are due to their ability to secrete a wide range of secondary metabolites (Ryan et al., 2008; Strobel and Daisy, 2003) including antibiotics (Strobel et al., 2004), volatile organic compounds (Ryu et al., 2003), antifungal (Beck et al., 2003; Strobel et al., 2004), and insecticidal agents (Azevedo et al., 2000). Endophytic bacteria with antifungal activity have been considered as biocontrol agents against phytopathogens (Lee et al., 2008; Liu et al., 2007; Rosenblueth and Martínez-Romero, 2006). Recently, it

was found that bacteria such as *Burkholderia* spp., and *Pseudomonas* spp. demonstrate antimicrobial activity as well as cytotoxic activity by producing both antifungal and phytotoxic metabolites (Andreote et al., 2009; Chung et al., 2008; Duijff et al., 1997; Patel et al., 2009; Ramasamy et al., 2009).

The endophytic strain of *Pseudomonas brassicacearum* YC5480 produces antifungal and phytotoxic compounds identified as 2,4-diacetylphloroglucinol (DAPG) and 2,4,6-trihydroxyacetophenone (THA) (Chung et al., 2008). The THA and DAPG toxic to plant growth produced by endophytic bacteria could be used in the eco-friendly management of parasitic weeds by preventing early growth or seed germination of the weeds (Chung et al., 2008; Vurro et al., 2009). The isolates of *Pseudomonas syringae* also produced phytotoxic compounds such as syringomycin and syringopeptin of which mode of action, regulation and biosynthesis by peptide and polyketide synthetases were elucidated (Bender et al., 1999). Some metabolites with phytotoxic activity produced by rhizobacteria or fungi reduced the number of normal seedlings of lettuce and a novel toxin such as phyllostictine inhibited germ tube elongation and seed germination of a hemp broomrape *Orobancha ramosa* and field dodder *Cuscuta campestris* (Carvalho et al., 2007; Vurro et al., 2009).

In this study, we isolated a strain of *Arthrobacter* sp. YC6002 with phytotoxic activity and identified it based on morphological, biochemical characteristics and 16S rRNA gene sequence analysis. The structure and activity of a phytotoxic substance produced by the strain was also determined.

Materials and Methods

Isolation of endophytic bacteria. Endophytic bacteria were isolated from roots of various plant samples, collected from Jinju and Sacheon area, Korea (Table 1) (Chung et al., 2008). The roots were rinsed with tap water and surface sterilized with 1% NaOCl for 10 min followed by washing

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Table 1. Endophytic bacterial strains isolated from various plant roots and inhibitory activity of radish seed germination

Strains	Locality	Host plant	Inhibition of radish germination ^a
			1/2 TSB
YC5956	Sacheon	<i>Glycine max</i>	–
YC5957	Sacheon	<i>Glycine max</i>	++
YC5958	Sacheon	<i>Glycine max</i>	+
YC5965	Sacheon	<i>Glycine max</i>	++
YC5975	Jinju	<i>Zea mays</i>	–
YC5982	Jinju	<i>Brassica pekinensis</i>	–
YC5983	Jinju	<i>Brassica pekinensis</i>	–
YC6001	Jinju	<i>Zoysia japonica</i>	–
YC6002	Jinju	<i>Zoysia japonica</i>	+++
YC6006	Jinju	<i>Zoysia japonica</i>	+

^a The culture filtrate was used to test the radish seed germination inhibitory activity. Five seeds of radish in three replications were placed on a piece of sterile Kim-Wipe tissue (2×2 cm) in a plastic plate loaded with 0.5 ml culture filtrate for 5 days under fluorescent light. –, 0–40% inhibition, +; 40–60% inhibition, ++; 60–80% inhibition, +++; 80–100% inhibition. 1/2 TSB; 1/2-strength Tryptic Soy Broth.

with 70% ethanol for about 10 seconds. Root pieces were rinsed again in sterile distilled water (SDW), blotted dry on a sterile filter paper, placed on 1/10-strength Tryptic Soy Agar (TSA, Difco, USA) and incubated at 28°C for bacterial growth to check the surface contamination. After confirmation of the surface sterility of root segments, 1.0 g of dried plant root was ground in 9.0 ml of phosphate buffer (pH 7.2) using pre-sterilized mortar and pestle under aseptic condition. The extract was serially diluted 10³–10⁵ times using phosphate buffer and 100 µl of diluted solution was applied on the 1/10 TSA plates. After incubation at 28°C for 3 days, bacterial strains with inhibitory activity on radish (*Raphanus sativus*, Nongwoo Bio Co. Ltd., Korea) seed germination were selected and purified strains were maintained on 1/10 TSA at 4°C for further studies.

Seed germination inhibitory activity. For the test of seed germination inhibitory activity, the culture filtrate of bacterial strains prepared by centrifugation (×9000 g, 10 min) was applied to radish seeds surface-sterilized with 1% NaOCl for 5 min. Five seeds of radish were placed on a piece of sterile Kim-Wipe tissue (2×2 cm) soaked with 0.5 ml culture filtrate in a plastic plate. The plates in 3 replications were incubated for 5 days (120 h) at 25°C under the fluorescent light (65 Lux) and the percent germination of radish seeds and growth of sprouts were investigated. Concentration for active compound dissolved in 1 ml of methanol was loaded on a piece of sterile filter paper (2×2 cm). After drying completely, five seeds of radish were

placed on the piece of sterile filter paper soaked with 1 ml of distilled water in a plastic plate. The plates in 3 replications were incubated for 5 days at 25°C under fluorescent light. Methanol and sterile water were used as untreated control.

Mass production of seed germination inhibitory compounds. To standardize mass production of the active compound, following culture media were tested: 1/10-strength Tryptic Soy Broth (TSB), minimal chitin medium (colloidal chitin 5.0 g, KH₂PO₄ 0.5 g, K₂HPO₄ 0.5 g, ZnSO₄ 0.5 g, FeSO₄·7H₂O 0.01 g in 1 L DW) (Singh et al., 1999), Czapek-dox broth (CDB, Difco), R2A broth (yeast extract 0.5 g, proteose peptone NO. 3 0.5 g, casamino acid 0.5 g, dextrose 0.5 g, soluble starch 0.5 g, sodium pyruvate 0.3 g, K₂HPO₄ 0.3 g, MgSO₄ 0.05 g in 1 L DW), glucose starch broth (GSB) (soluble starch 5.0 g, glucose 5.0 g, aspartic acid 0.5 g, K₂HPO₄ 0.5 g, MgSO₄ 0.5 g and FeSO₄ 0.01 g in 1 L DW), soybean meal flour medium (SMF) (yeast extract 4.0 g, beef extract 1.0 g, soluble starch 20.0 g, soybean meal 25.0 g, glucose 5.0 g, K₂HPO₄ 0.1 g, NaCl 2.0 g in 1 L DW), soytone glucose broth (SGB) (glucose 15.0 g, soytone 15.0 g, yeast extract 5.0 g, casamino acid 1.0 g, K₂HPO₄ 0.1 g, NaCl 2.0 g and FeSO₄·(NH₄)₂SO₄·6H₂O 0.05 g and MgSO₄·7H₂O 0.2 g in 1 L DW) and M523 broth (sucrose 1.0 g, casamino acid 8.0 g, yeast extract 4.0 g and MgSO₄ 0.3 g in 1 L DW) (Chung et al., 2008). Each medium (500 ml) was incubated with the 5 ml one-day old bacterial culture for 3 days at 30°C on a rotary shaker (approximately 180 rpm). Among these culture media, the culture filtrate of 1/10 TSB had shown the strongest inhibitory effect on radish seed germination. Therefore, 1/10 TSB medium was used for mass production of the active metabolite.

Isolation and purification of a seed germination inhibitory compound. Mass culture of strain YC6002 was carried out in a 500 L fermentor (KF-500, KoBioTech, Korea) with a working volume of 100 L. The specific culture conditions (30°C, pH 7.0 and 0.6 volume/medium volume/min aeration) and impeller speed (300 rpm) was maintained throughout the incubation. After 2 days culture, the broth culture (54 liters) was centrifuged using continuous centrifugation system at 9000 g for 10 min to remove the bacterial cells. The cell-free supernatant was concentrated to 1 liter under reduced pressure and the residual solution was extracted twice with an equal volume of ethyl acetate (EtOAc). The extracts combined were concentrated under reduced pressure to give 1.5 g of brown oil. This brown oil was then separated into 5 fractions by a silica gel column using different proportions of CH₂Cl₂, acetone and MeOH. The bioactive fraction 2, eluted with CH₂Cl₂ and acetone (95:5), was again fractionated by silica gel column using a step

Fig. 1. Separation procedure of the inhibitory compound from the culture filtrate of *Arthrobacter humicola* YC6002.

gradient elution of different n-hexane and EtOAc combinations. The bioactive fraction 7 (380 mg), eluted with n-hexane-EtOAc 4:1, was further purified by a Sephadex LH20 column chromatography (using CH_2Cl_2 and MeOH in 1:4 ratio as mobile phase) to obtain 55 mg of compound 1 (Fig. 1).

Characterization of the phytotoxic compound. UV spectrum was recorded on a AGILENT 8453 UV-Visible spectrophotometer and IR spectrum on a JASCO FTIR-4100 spectrometer. Low and high resolution EIMS were recorded on a MICROMASS AUTOSPEC mass spectrometer equipped with a OPUS data system. NMR data, including COSY, DEPT, HMQC, and HMBC, were taken on a VARIAN UNITY 500 spectrometer working at 500 MHz for proton and 125 MHz for carbon. Chemical shifts are reported in ppm relative to the solvent (CDCl_3 , δ_{H} 7.24, δ_{C} 77.0).

Biochemical and physiological characterization of a bacterial strain. Biochemical, physiological and molecular characteristics for identification of the bacteria were investigated according to “Current protocols in molecular biology” and “Chemical methods in prokaryotic systems” (Ausubel et al., 1995; Goodfellow and O’Donell, 1994). Gram staining was performed using a bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology, flagella and gliding motility were studied using phase-contrast microscopy and transmission electron

microscopy (H-600, Hitachi). The morphology of the strain was studied using a scanning electron microscopy (XL30 S FEG; Philips) (Kageyama et al., 2008; Ludmila et al., 2004). The physiological characteristics of the strain was examined by growing the isolate on 1/10 TSA agar at different temperatures (5–50°C at 5°C intervals) and in 1/10 TSB adjusted at different pH values (5.0–10.0 at 0.5 pH unit intervals). Hydrolysis of casein, Tween 20, Tween 80, esculin, urea, tyrosine, starch and nitrate reduction were studied on 1/10 TSA agar after a 7-day incubation at 30°C according to standard methods (Lanyi, 1987; Smibert and Krieg, 1994). Enzymatic activities and biochemical features were determined using API ZYM and API ID32E kits as recommended by the manufacturer (bioMérieux) except that kits were incubated for 4 hours and 2 days at 30°C, respectively. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.). Analyses of polar lipids and isoprenoid quinones were carried out using the standard methods (Komagata & Suzuki, 1987; Minnikin et al., 1984). The DNA G+C content was determined using a HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE, GROM) (Tamaoka and Komagata, 1984).

Phylogenetic analysis of 16S rRNA gene sequence. The 16S rRNA gene sequences was amplified from the purified genomic DNA by using a set of primers 27F (5'-AGAGTT-TGATCMTGGCTCAAG-3') and 1492R (5'-GGYTACC-TTGTTACGACTT-3') (Lane, 1991). The purified PCR product was sequenced by GenoTech Inc. (Daejeon, Korea). The 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR) and that was compared with the sequences of related type strains, obtained from the GenBank database. The sequences were aligned using CLUSTAL_X program (Thompson et al., 1997) and phylogenetic trees were constructed by neighbor-joining distance method using Mega4 program with bootstrap values based on 1000 replications (Felsenstein, 1985; Saitou and Nei, 1987; Tamura et al., 2007). Gaps were edited using the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983).

Results

Isolation and culture of the bacteria with seed germination inhibitory activity. Among 300 bacterial strains tested for inhibition of radish germination, the culture filtrate of five strains had the inhibitory activity and the strain YC6002 isolated from the root of Korean turf grass had over 80% of inhibitory activity (Table 1). To select

Table 2. Effect of culture filtrate of *Arthrobacter humicola* YC6002 with different culture period in 1/10 TSB on the germination inhibition of radish seeds

Culture period (hrs)	Inhibition of radish seed germination (%) ^a				
	Days				
	1	2	3	4	5
0	0	20.0	13.3±11.5	6.6±11.5	0
24	0	66.6±11.5	40.0	20.0	20.0
48	0	66.6±11.5	66.6±11.5	60.0	60.0
60	0	46.6±11.5	33.3±23.0	13.3±11.5	13.3±11.5
72	0	60.0±20.0	46.6±11.5	46.6±11.5	46.6±11.5

^aThe culture filtrate was used to test the radish seed germination inhibitory activity. Five seeds of radish in three replications were placed on a piece of sterile Kim-Wipe tissue (2×2 cm) in a plastic plate loaded with 0.5 ml culture filtrate for 5 days under fluorescent light.

culture media for mass production of the germination inhibitory compound, six different culture media were tested. The rate of germination of radish seeds in the culture filtrate of 1/10 TSB was higher than any other culture filtrate of TSB, Czapek-dox broth, but the filtrate of M523, MMC and R2A had no inhibition activity (data not shown). The strain YC6002 was cultured in 1/10 TSB for 3 days and the seed germination inhibitory activity was investigated by using culture filtrates of different time period (24 h, 48 h, 60 h and 72 h). Results suggested that culture filtrate of 2-5 day old bacterial strain had over 60% inhibition of seed germination, the 48 hours old culture filtrate, however, revealed the most inhibition towards seed

germination. However, the inhibition ratio was decreased below 40% after 3 days in the culture filtrate of 24 h and 60 h. The culture filtrates of 72 h showed less activity comparing to that of 48 hr (Table 2). Effect of different culture temperatures of the strain YC6002 on the inhibition of radish seed germination was investigated. The ratio of germination inhibition was over 70% in both culture broth and filtrate of the strain at 20 °C, 25 °C and 30 °C. There was no significant difference in the inhibition activity of culture broth containing bacterial cells cultured at 20 °C, 25 °C and 30 °C with the rates of 92%, 95% and 88%, respectively. However, in case of culture filtrate, the inhibition rate was highest in the culture filtrate incubated at 30 °C for 72 h with the rate of 92% (Fig. 2).

Characterization of the phytotoxic compound. The phytotoxic compound (1) 55 mg was purified from the culture filtrate fraction YC-11 (380 mg) prepared with mass cultured broth (54 L) of the strain YC6002 (Fig. 1). The structure of active compound KS1 was determined to have the molecular formula $C_9H_{10}O_2$ based on high resolution EI-MS data. This compound showed two methylene, one carbonyl, and six aromatic methine carbon signals in the DEPT spectra. Five protons at low field of 1H NMR in compound 1 were assigned as one monosubstituted benzene by the 1H - 1H COSY. Physico-chemical properties of compound 1 are summarized in Table 3. These data were well consistent with those of authentic 3-phenylpropionic acid (hydrocinnamic acid) (Fig. 3). The active compound purified from the culture filtrate of the strain YC6002 showed seed germination inhibition by 53%, 93% and 100% at 500 ppm, 1,000 ppm and 10,000 ppm, respectively (Table 4).

Fig. 2. Effect of culture temperatures on the inhibition of radish seed germination by *Arthrobacter humicola* YC6002 in 1/10 TSB on rotary shaker (180 rpm, 72 h). Seed germination inhibitory test was performed at 25 °C for 72 hrs. Vertical bars on columns indicate standard errors.**Table 3.** Physico-chemical properties of the active compound (1) purified from culture filtrate of *Arthrobacter humicola* YC6002 with seed germination inhibitory activity

Appearance	Colorless powder
Melting point (°C)	48-49
Molecular formula	$C_9H_{10}O_2$
Molecular weight	150 [M] ⁺
HREIMS (m/z)	Found 150.0702 Calcd. 150.0680
UV λ_{max} (MeOH) nm	212, 265
IR ν_{max} (thin film) cm^{-1}	3332, 1698, 1454, 1301, 1219
TLC (R_f) ^a	0.67
1H NMR ($CDCl_3$)	δ 10.41 (1H, brs), 7.38-7.19 (5H, m), 2.99 (2H, t), 2.69 (2H, t)
^{13}C NMR ($CDCl_3$)	δ 179.0, 139.9, 128.4 (x2), 128.1 (x2), 126.2, 35.6, 30.6

^aSilica gel 60F₂₅₄ (Merck), EtOAc-n-hexane 1:1.

Fig. 3. The chemical structure of 3-phenylpropionic acid isolated from the culture filtrate of *Arthrobacter humicola* YC6002.

Table 4. Effect of purified compound (KS1) from the culture filtrate of *Arthrobacter humicola* YC6002 on the inhibition of germination and shoot growth of radish seeds

Concentration (ppm) ^a	Inhibition of radish seed germination (%) ^b	Shoot length (mm)
0	0	15.6±0.5
100	13.3±0.6	17.3±0.3
500	53.3±2.1	4.2±0.4
1,000	93.3±1.5	0
10,000	100	0

^a Active compound dissolved in methanol (1 ml) was loaded on a piece of sterile filter paper (2×2 cm).

^b One ml of methanol dissolved with active compound was loaded on the piece of sterile filter paper (2×2 cm) and dried. Five seeds of radish in 3 replications were placed on the filter paper soaked with 1 ml of distilled water in a plastic plate for 5 days at 25 °C under fluorescent light.

Identification of the strain YC6002. The bacterial strain YC6002 with germination inhibitory activity of radish

seeds was isolated from the root of *Z. japonica* and identified. Based on biochemical, physiological characteristics and 16S rRNA gene sequence analysis, the strain YC6002 was identified as *Arthrobacter humicola* (Fig. 4). Cells were Gram-positive, non-motile and long and short rod-shaped (0.3-0.5×2.5-4.0 μm). The colonies grown on 0.1 TSA agar plates for three days were smooth, circular, and whitish very shine yellow in color, 1-2 mm in diameter. Casein, gelatin, starch and tyrosine are hydrolyzed. Tween 20 and 80, esculin, carboxymethylcellulose and urea are not hydrolysed. Nitrate is not reduced to nitrite and nitrogen. In API ZYM kit, alkaline phosphatase, leucine arylamidase and valine arylamidase activities are present; esterase (C-4), esterase lipase (C-8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-mannosidase, α-glucosidase, α-galactosidase, β-galactosidase are present; acid phosphatase, naphthol-AS-BI-phosphohydrolase, trypsin, lipase (C-14), lipase (C-14), α-fucosidase, β-glucuronidase and β-glucosidase are negative. MK-8 is the major respiratory menaquinone. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-N-methylethanolamine and ninhydrin-phosphatidylglycerol. The G+C content of the genomic DNA was 61.2 mol%. The major cellular fatty acids in strain YC6002 included C_{14:0} iso (1.0%), C_{14:0} (1.2%), C_{15:0} iso (4.7%), C_{15:0} anteiso (36.5%), C_{16:1} iso (5.0%), C_{17:0} iso (1.2%), C_{17:0} anteiso (15.0%) and C_{18:0} (8.8%). Comparative 16S rRNA gene sequence analysis showed that the strain was most closely

Fig. 4. Phylogenetic tree constructed from the comparative analysis of 16S rRNA gene sequences showing the relationships of strain YC6002 with other related species. This phylogenetic tree was constructed by using the neighbor-joining method and Jukes & Cantor evolutionary distance matrix data obtained from unambiguous aligned nucleotides. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are shown at the branch points. Bar, 1 substitution per 100 nucleotide position.

related to *A. humicola* KV-653^T (99.7%), *A. oryzae* KV-651^T (99.2%), *A. globiformis* DSM20124^T (98.5%) and *A. ramosus* DSM20546^T (98.3) (Fig. 4).

Discussion

The endophytic bacterium, YC6002 with seed germination inhibitory activity was isolated from the root of *Z. japonica* and identified as *A. humicola*. *Arthrobacter* species are wide-spread in soil, air, ice cave, clinical sources and plant tissues (Aravind et al., 2009; Guido et al., 1996; Lee et al., 2003; Rosa et al., 2004). Some strains of *Arthrobacter* species have been reported to degrade a wide range of xenobiotic substances such as fluorene, isocarbophos and 4-fluorophenol (Casellas et al., 1997; Ferreira et al., 2008). In addition, many strains of *Arthrobacter* species were also known to inhibit plant growth and seed germination which were used for biological control of plant diseases (Barrows-Broadus et al., 1985; Chung et al., 2008; Mundt and Hinkle, 1976; Pusey, 1997). The strain YC6002 inhibited seed germination and stem growth of radish by producing a phytotoxic compound. The strain of *A. pascens* was reported to inhibit plant and root growth by producing inhibitors of auxin and gibberellin (Edmund et al., 1971). On the contrary, some strains of *A. ilicis* and *A. oxydans* were used for biological control against *Helminthosporium solani*, a causal agent of potato silver scurf (Elson et al., 1997; Michaud and Martinez, 2002). Sziderics et al. (2007) suggested that a strain of *A. oxydans* inhabited within pepper plants as endophytic bacterium produced indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) which were known to enhance plant growth and contributed to a biotic stress adaptation in host plants. Although many strains of *Arthrobacter* spp. are worth as the biological control agent, the phytotoxic activity was hardly reported. In a certain endophytic bacteria, both phytotoxic and antifungal compounds were produced (Chung et al., 2008). *A. humicola* was also isolated from a paddy soil sample but no phytotoxic activity was reported in this strain (Kageyama et al., 2008).

The chemical structure of a compound in culture filtrate of the strain YC6002 with seed germination inhibitory activity was determined to be 3-phenylpropionic acid (hydrocinnamic acid), which was rarely produced as a microbial metabolite. Phenylpropionic acid is a member of the phenylpropanoid family, comprising a wide variety of C3-C6 compounds synthesized by plants from phenylalanine and is important in the synthesis of flavonoids, insect repellents, UV protectants and signal molecules for defense mechanisms (Hahlbrock and Scheel, 1989). The phenolic compound 3-phenylpropionic acid is known to be inhibitory to germinating seeds or growing plants (Jing and

Yoshihisa, 1994; Williams and Hoagland, 1982). Moss et al. (1970) found that hydrocinnamic acid and cinnamic acid were produced from cultures of *Clostridium sporogenes* when cell suspensions exposed to l-phenylalanine. Chamkha et al. (2001) reported that the strain of *Clostridium bifermentans* isolated from olive mill wastewaters produced 3-phenylpropionic acid by conversion of cinnamic acid. The 3-phenylpropionic acid was also found in ruminal fluid by chemical reduction of dietary phenolic monomers by ruminal bacteria (Cremin et al., 1994). It has been recently reported that an antagonistic strain of *Burkholderia* spp. isolated from the Naju area produces antifungal compounds composed of phenylacetic acid, hydrocinnamic acid, 4-hydroxyphenylacetic acid and 4-hydroxyphenylacetate methyl ester (Mao et al., 2006). A strain of *Streptomyces* sp. isolated from laterite soil also has been found to produce 3-phenylpropionic acid and showed antimicrobial activity against different bacteria and *Fusarium udum* causing wilt disease in pigeon pea (Narayana et al., 2007). Recently, aromatic compounds extracted from fresh leaves and twigs of *Oxalis pes-caprae* including cinnamic acid esters have been shown to be phytotoxic to the germination and growth of lettuce. The inhibitory activity of some of these compounds was higher than that of pendimethalin, a commercial pre-emergence herbicide (DellaGreca et al., 2009). The phytotoxic compound produced by the strain of *A. humicola* YC6002, 3-phenylpropionic acid, could be developed for an environmentally safe herbicide with the further study on its chemical derivatization.

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