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Article

Isolation and In Vitro Antimicrobial Activity of Low Molecular Phenolic Compounds from *Burkholderia* sp. MP-1

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An antagonistic strain, *Burkholderia* MP-1, showed antimicrobial activity against various filamentous plant pathogenic fungi, yeasts and food borne bacteria (Gram-positive and Gram-negative). The nucleotide sequence of the 16S rRNA gene (1491 pb) of strain MP-1 exhibited close similarity (99-100%) with other *Burkholderia* 16S rRNA genes. Isolation of the antibiotic substances from culture broth was fractionated by ethyl acetate (EtOAc) solvent and EtOAc-soluble acidic fraction. The antibiotic substances were purified through a silica gel, Sephadex LH-20, ODS column chromatography, and high performance liquid chromatography, respectively. Four active substances were identified as phenylacetic acid, hydrocinnamic acid, 4-hydroxyphenylacetic acid and 4-hydroxyphenylacetate methyl ester by gas chromatographic mass spectrum analysis. The minimum inhibition of concentration (MIC) of each active compound inhibited the growth of the microorganisms tested at 250 to 2500 μg ml^{-1}. The antimicrobial activity of crude acidic fraction at 1 mg of dry weight per 6 mm paper disc was more effective than authentic standard mixture (four active substances were mixed with the same ratio as acidic fraction) over a wide range of bacterial test.

Key words : *Burkholderia* sp. MP-1, Phenylacetic acid, Hydrocinnamic acid, 4-hydroxyphenylacetic acid, 4-hydroxyphenylacetate methyl ester, Acidic fraction, Authentic standard mixture

Introduction

Many kinds of antimicrobial compounds have been reported for plant pathogenic bacteria and suggestions have been made for their function and application. Some species of microorganisms are known to have beneficial effect on plant growth through the production of plant growth promoting regulators and antibiotic substances. For example, *Burkholderia* (previously *Pseudomonas*) cepacia was first described in 1950 as the phytopathogen responsible for bacterial rot of onion bulbs (Burkholder, 1950). In the 1960s, seminal taxonomic studies by Stanier and colleagues (Stanier et al., 1966) highlighted the organism's extraordinary metabolic ability to degrade a wide range of organic compounds. In 1992, the new genus *Burkholderia* was introduced with Burkholderia cepacia named as the type species (Yabuuchi et al., 1992). *B. cepacia* occurs as an opportunistic pathogen in plants, animals and man. The common host plant of *B. cepacia* is Allium cepa, but the bacterium also causes soft-rot symtptoms in *Allium sativum, Lycopersicon esculentum* and *Agaricus bitorquis*, and a leaf-spot disease on a number of orchids (*Cymbidium* spp., *Dendrobium* sp. and *Paphiopedium* spp.) (Saddler 1994). Other noteworthy characteristics of *B. cepacia* strains are their broad resistance to antibiotics (Smirnov et al., 1982); their ability to synthesize new compounds (Rasmay et al., 1989); to bring about the biodegradation of recalcitrant molecules (Folsom etal., 1990; Haugland et al., 1990); and cause the regression of some pathogens in soil and the rhizosphere of crop plants by the production of antibiotics, volatile antifungal compounds and siderophores (Cartwright and Benson 1995). Numerous bacterial strains that produce antibiotic in vitro have been isolated from different soils and plants (O'Sullivan and O'Gara 1992; Thomashow and Weller 1996). Recently, there has been an increasing interest among researchers in the field of pest management to exploit beneficial microorganisms that protect plants from phytopathogens

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(Fravel 1988; Weller 1988).

Thus, this study concentrates on antimicrobial compounds, which occur in ecology of bacterial growth. Therefore, the aim of this investigation was: i) to isolate and identify an antagonistic bacterial strain from the rhizoshere; ii) the extraction, purification and characterization of four antimicrobial compounds from strain MP-1 are reported and their biological activity is described; and iii) to investigate the morphological hyphae of *Phytophthora capsici* affected by isolated substances under a light microscope.

Materials and Methods

Selection of antagonistic bacteria Bacterial strains were isolated from soils in Naju area, South Korea. One g of each soil sample collected at different locations was diluted with 90 ml distilled water individually and then shaken on a rotary plate at 150 rpm for 30 min at 25℃. The solution was diluted again with distilled water and sprayed on agar plates containing (g L^{-1}): potato dextrose broth, 24; agar, 15; and pH 7. The plates were incubated at 27℃ for 3 days. Colonies were isolated and retained for subsequent screening. A strain MP-1 having good antibiotic characteristic against mycelial growth of *Phytophthora capsici* KACC 40483 was selected for basic studies and then it was subcultured in 500 ml Erlenmeyer flask containing 200 ml LB broth (Luria Bertani, Detroit, MI, U.S.A.) on a rotary shaker. After incubation of 5 days, the broth culture was cryopreserved at -75 ℃ in 50% (v/v) glycerin until used.

16S rRNA sequencing An analysis of the gene sequence of 16S rRNA was performed directly using single whole colony of the strain MP-1, according to the procedure described by Di Cello et al. (1997). The 16S rRNA was enzymatically amplified by Taq DNA polymerase using oligonucleotide primer. The forward primer was 5'-TGGCTCAGAAGCAACGCTGGCGGC-3' and the reverse primer 5'-CCCACTGCTGCCTCCCG TAGGAGT-3'. The temperature cycle was at 94℃ for 30 sec, 55℃ for 1 min, and 72℃ for 1 min 30 sec for 30 cycles and 5 min at 72℃ for extension. The polymerase chain reaction (PCR) product was cloned using pGEM®-T easy vector (Promega Co., Madison, WI, U.S.A.). The nucleotide sequence of the 16S rRNA gene was determined by Biodye™ Terminator cycle sequencing kit and compared with published 16S rRNA sequences using Blast search at Gene Bank Data base of NCBI (NCBI, Bethesda, MO, U.S.A.).

Extraction and purification of antimicrobial substances MP-1 was grown in LB broth at 30ºC for 120 h. Cells were removed from 13.5L of broth by centrifugation at 5000 rpm for 10 min, and the filtrates were adjusted to pH 3 with 1 N HCl and extracted with ethyl acetate (EtOAc, 13.5-L three times). The ethyl acetate phase was concentrated *in vacuo* and partitioned into a buffer solution of 5% $NaHCO_3$ solution at pH 8.0. The aqueous phase was adjusted to pH 3 with 1 N HCl and extracted with EtOAc to obtain the soluble acidic fraction (Lee et al., 2004). Each fraction was concentrated during the purification process, and tested for antimicrobial activity against *Staphylococcus aureus* KCTC 1928 and *Escherichia coli* KCTC 2593 by the paper disc-agar diffusion method. The EtOAc-soluble acidic fraction was purified by using the following sequence of column: 1) silica gel (Kieselgel 60, 70-230 mesh, Merk, Darmstadt, Germany, 35 g, 1.5 × 49 cm) with stepwise elution on an increasing concentration of EtOAc in hexane (14:0, 12:2, 10:4, 8:6, 6:8, 4:10, 2:12, and 0:14, vol/vol). 2) Sephadex LH-20 (1.8 × 89 cm, MeOH/$CHCl_3$ (4:1, v/v), 25-100 mesh, Pharmacia Fine Chemicals, Uppsala, Sweden) at a flow rate of a 1.0 ml min^{-1}. 3) Octadecylsilane (ODS, YMC GEL, 70-230 mesh, YMC Co, Kyoto, Japan, 4.4 g, 1.0 × 14 cm) by eluting with MeOH H_2O stepwise of 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0 (v/v). Each active fraction with antimicrobial activity was further purified by high performance liquid chromatographic (HPLC) system with a C_{18} reversed phase column (μBondapak C_{18}, 10 μm, 7.8 × 300 mm, Waters). Active fraction AI from ODS column was eluted using 50% methanol with a flow rate of 1.0 ml min^{-1} under the UV absorbance of 210 nm, and active fraction AII from Sephadex LH-20 was eluted using 40% methanol with a flow rate of 1.5 ml min^{-1} under the UV absorbance of 254 nm.

Identification and structural elucidation of antimicrobial compounds After purification from HPLC, all active compounds were identified by GC-MS (Hullet Packard, product I.D, city, U.S.A.). To confirm the property and structure of the isolated substance exactly, each compound was reacted with trimethylsilate (TMS) and then was performed by GC-EI-MS. Operating condition for analysis was HP-SMS capillary

column (0.25 mm x 30 m x 0.25 mm), carrier gas He at a flow rate of (1ml min^{-1}), split ratio 50:1, injector temperature 280℃, interface temperature 280℃, and oven temperature programmed at 40℃ (2 min) and ramped to 280℃ at 5℃ min^{-1}. Electron impact mass spectrometry (EI-MS) method with 70 eV was used for simple ionization.

Microorganisms Yeast and food borne bacteria (Gram positive and Gram-negative) used for antimicrobial assay with isolated compounds in this study were *Saccharomyces cerevisiae* KCTC 7904, *Bacillus subtilis* KCTC 1021, *Staphylococcus epidermidis* KCTC 1917, *Pseudomonas aeruginosa* KCTC 2513, *Streptococcus mutans* KCTC 3065, *Streptococcus pyogens* KCTC 3096, *Enterococcus faecalis* KCTC 3195, *Micrococcus luteus* KCTC 3523, *Staphylococcus aureus* KCTC 1928 and *Escherichia coli* KCTC 2593. These strains were purchased from Korean Collection for Type Cultures (KCTC, South Korea).

Paper disk diffusion assay The antimicrobial assay was conducted on a sterile petri plates (90 x 15 mm) containing 10 ml of nutrient agar (1.5%) (Lam et al., 2000). A three milliliters aliquot of warm nutrient agar (0.7%) containing the bacterium (10^8 cfu ml^{-1}) was poured into the plate. A sterile blank paper disc (6 mm in diameter) was placed on the agar. Then a test sample (10 μl of a 0.1 g ml^{-1} solution) in methanol was added to the disk. Only methanol (10 μl) was added to the control disk. The plate was incubated at 30℃ or 37℃ depending on typically bacteria for 20-24 h. Benzoic acid (20 μl of 10 mg ml^{-1} in methanol) served as a positive control. A transparent ring around the paper disk signified antibacterial activity (Wang et al., 2001). Three replicates were run simultaneously.

Dilution test This test was used for minimal inhibitory concentration (MIC) determination. Each isolated compound was tested by two fold dilution method (Murray et al., 1995). The bacterial strains were cultivated for 24 h, and two-fold serial dilution method was followed as below. The isolated compound was first dissolved in 50% methanol solvent. This solution was further diluted with the same solvent and was added to growth media (Nutrient, Brain Heart Infusion, and YM broth) to final concentration of 0, 100, 250, 350, 500, 650, 800, 1000, 1250, 1500, 2000, 2500 μg ml^{-1}. The test tubes containing these broth media were allowed to stand overnight in sterile hood to remove the solvent completely. The bacterial suspensions of test strains were inoculated in the 20 ml of cap tube in broth medium and incubated for 24 h at their respective optimum temperatures. The minimum concentration at which no visible growth was observed in the tube was defined as minimum inhibitory concentration, which expressed in μg ml^{-1}. A set of tubes containing only seeded liquid medium was kept as control and 50% methanol solvent control was also maintained. All the tests for minimum inhibitory concentration determinations were performed in triplicate.

Preparation of fungal spores *Phytophothora capsici* KACC 40483 was obtained from Korean Agricural Culture Collection (KACC, Suwon, Republic of Korea) and then grown on the V8 juice agar under the dark condition at 30℃ for 3 days. A chopped medium containing hyphae of the fungus was flooded with sterile de-ionized water and incubated under continuous fluorescent light for 5 days at 30℃ for sporangial production. After 5 days of incubation, it was chilled at 4 ℃ for 30 min to release zoospores. Mycelia and sporangial debris were removed from zoospore suspension by filtration through sterile cheesecloth thoroughly and the filtrate was diluted with sterilized water at 2 x 10^4 zoospores ml^{-1} (Kim et al., 1997).

Hyphal morphology affected by the active compound To observe the fungal growth inhibition by isolated substances, the inoculum of liquid cultures of *Phytophthora capsici* spores was prepared by the conventional method to a final concentration approximately 2 x 10^4 spores ml^{-1}. The fungal spores (20 μl) were grown in each well containing 180 μl of potato dextrose broth. The dry matter of antimicrobial compound was first dissolved in 50% methanol solvent. This solution was further diluted with the same solvent and was added to growth media. One mg of each acidic fraction and authentic standard mixture (four active substances which are mixed with the same ratio as found in the acidic fraction) was tested. One mg of the total mass authentic standard mixture by compositional separation of each compound as phenylacetic acid (0.36 mg), hydrocinnamic acid (0.01 mg), 4- hydroxyphenylacetic acid (0.58 mg) and 4 -hydroxyphenylacetate methyl ester (0.05 mg) was added into each well broth individually 24 h after

inoculation of fungal spores. A set of wells containing only seeded liquid medium was poured 20 μl of the same solvent as control. The resultant solution was incubated with shaking 150 rpm at 30℃ and the mycelia were observed under a light microscope at various interval. All the tests for observations of mycelial morphology of *P. capsici* were done in triplicate.

Results and Discussion

Identification and growth of bacterial strain MP-1 About 150 microorganisms were isolated from the soil samples. Among them, strain MP-1 showed the strongest inhibition against mycelial growth of *Phytophthora capsici,* which was selected for basic study in this case. To identify bacterial isolate MP-1, the genomic RNA was amplified with universal primers and 16S rRNA sequence was analyzed. The isolate MP-1 was Gram negative and rod shaped. The bacterium was confirmed as *Burkholderia* sp. by the 16S rRNA sequence analysis. The partial 16S rRNA sequence of isolate MP-1 was deposited in GenBank under accession number DQ094147. The partial 16S rRNA sequence of strain MP-1 showed 99-100% identity with these of *B.* sp. strains (Genbank accession number AJ491304, AF219125, AY769902, and AY769904) and *B. cepacia* strains (Genbank accession number AY741352, AY741351, AY741350, AY741355, AF311971, and AY946011). From observation on strain MP-1 growth in LB broth, population was accumulated at the highest level (7.2 x 10^9 cfu ml^{-1}) and optical density increased to 2.6 at 5 days after incubation (Fig. 1). The optimal condition was found 5 days for cultivation at 30℃ with shaking 150 rpm.

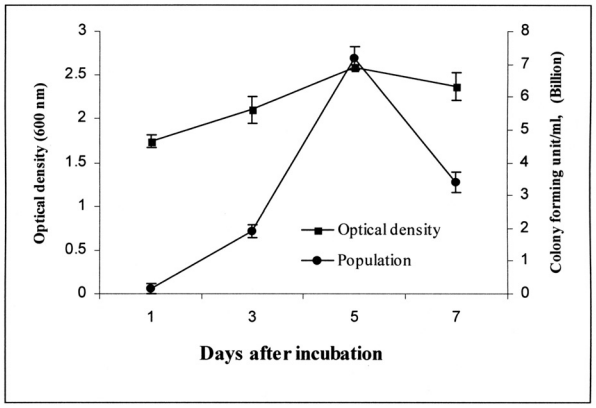

Fig. 1. Formation of population and optical density of *Burkholderia* sp. MP-1 changed in LB broth at 30℃.

Isolation, identification and elucidation of antimicrobial compounds By the activity-guided bioassay, the antimicrobial substances were found in the acidic fraction. The acidic fraction (5.1 g) was analyzed by silica gel column chromatography using solvent system, hexane-ethyl acetate. Two active subfractions (AI, 1.9 g and AII, 0.9 g) with the ratio of 12:2 and 10:4 to 8:6 (v/v) respectively were separately rechromatographed on Sephadex LH-20 with solvent MeOH-$CHCl_3$. In particular, chromatography on Sephadex LH-20 of AI showed antimicrobial activity with a ratio for Ve/Vt (elution volume/total volume) of 0.74-0.84 (1062 mg). This active fraction was purified by ODS column chromatography with MeOH-H_2O as a stepwise mobile phase, gave an active fraction at 60% MeOH elution (627 mg). In case of AII, bioassay test of Sephadex LH-20 column chromatography with the same solvent system was obtained at value of Ve/Vt: 0.86-1.00 (560 mg). The active fraction of AI from an ODS column was purified by HPLC and was divided into two active compounds (C1 and C2) with single peaks at the retention time (t_R) of 10.8 min and 17.9 min. Active fraction AII from Sephadex LH-20 column was also divided into two active compounds (C3 and C4) with single peaks at (t_R) of 8.9 min and 21.6 min, respectively. Then, each compound (C1, C2, C3 and C4) was identified by GC EI MS as phenylacetic acid (C1), hydrocinnamic acid (C2), 4 hydroxyphenylacetic acid (C3) and 4 hydroxyphenylacetate methyl ester (C4), respectively. All isolated substances were reconfirmed by trimethylsilation and GC-EI-MS (Fig. 2).

This study is the first example of four isolated compounds being produced from *Burkholderia* sp. isolated from soil. Among of these compounds, C1 was isolated from other microorganisms such as *Enterobacter cloacae* S11:T:07 (Burkhead et al., 1998), *Glomerella cingulata* (Hirota et al., 1992), *Pseudomonas* sp. (Jae et al., 1999), *Streptomyces humidus* (Byung et al., 2001) and *Bacillus licheniformis* (Yoon et al., 2004). C1 is known as plant growth regulator (Wightman and Lighty 1982; Camirand et al., 1983) and phytotoxic (Hirota et al., 1992). C1 produced by *Bacillus subtilis* strains had *in vitro* toxic activity against the pine wood nematode *Barsaphelenchus xylophilusi* (Kawazu et al., 1996). On the other hand, 3 phenylpropionic (C2) acid supports the growth of some soil microorganisms and it is found amongst the products of putrefaction of proteins in soil (Dagley et al., 1965). There are information about C2, C3

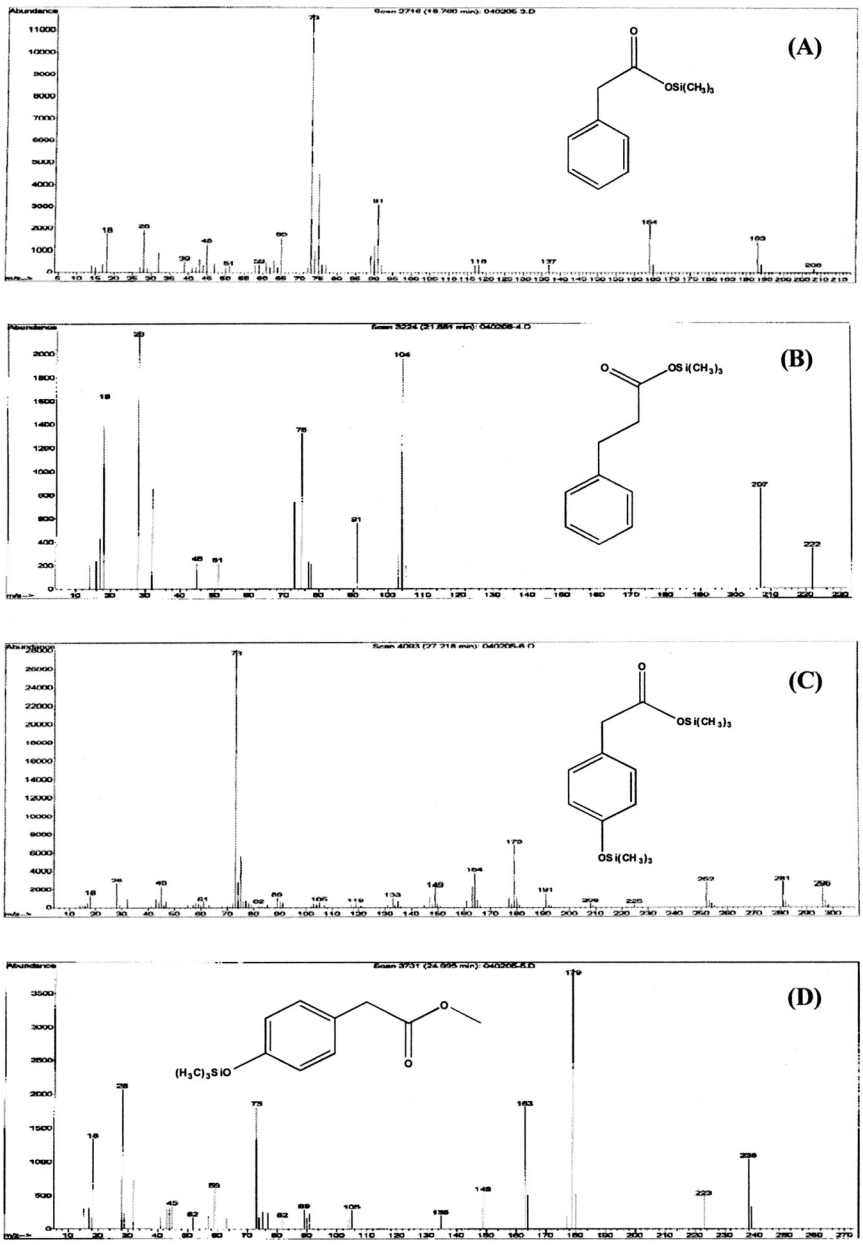

Fig. 2. EI-MS spectra of trimethylsilation and structures of the active compounds C1 (A), C2 (B), C3 (C) and C4 (D).

and C4 isolated from *Burkholderia* sp. yet. The three of four isolated compounds are carboxylic acids and C4 is an ester. We speculate that C4 may be appeared by an esterification of C3 with methanol solvent during purification of crude acidic fraction.

Antimicrobial activity of the four isolated compounds In this experiment, the antimicrobial activities of all isolated compounds, acidic fraction (AF) and authentic standard mixture (AS) were tested against various microorganisms *S. cerevisiae, B. subtilis, S. epidermidis, P. aeruginosa, S. mutans, S. pyogens, E. faecalis, M. luteus, S. aureus* and *E. coli*. The biological activity of all active compounds against with food-born pathogenic bacteria and yeast was investigated by inhibition zones and minimum inhibitory concentration values. According to the results given in Table 1 and 2, C1, C2, C3 and C4 showed different potential of antimicrobial activity against the strains tested. C1, C2 and C3 are typical carboxylic acids, which are stronger effective than an ester C4. As shown in Table 1, C4 was not inhibited by all microorganisms at 0.1 mg of dry weight mass. C1 is already known as an antifungal and antibacterial compound (Burkhead et al., 1998; Jae et al., 1999; Byung et al., 2001; Yoon et al., 2004), but there is no information about their antimicrobial activities of the

Table 1. The zone of inhibition of phenylacetic acid (C1), hydrocinamic acid (C2), 4-hydroxyphenylacetic acid (C3), 4-hydroxyphenylacetate methyl ester (C4), acidic fraction (AF) and authentic standards mixture (AS) which contains four isolated compounds with the same ratio as found in acidic fraction

Microorganisms	Zone of inhibition (mm)†					
	C1	C2	C3	C4	AF	AS
	---------- 0.1 mg ----------				-------- 1.0 mg --------	
Staphylococcus aureus KCTC 1928	10.5 ± 0.5	10.5 ± 0.5	9.2 ± 0.4	nd‡	16.5 ± 0.5	14.3 ± 0.5
Escherichia coli KCTC 2593	11.2 ± 0.3	8.8 ± 0.5	8.1 ± 0.4	nd	16.8 ± 0.4	14.8 ± 0.4
Baccillus subtilis KCTC 1021	8.3 ± 0.5	8.3 ± 0.5	7.1 ± 0.2	nd	20.5 ± 0.5	19.6 ± 0.4
Staphylococcus epidermidis KCTC 1917	7.3 ± 0.4	7.8 ± 0.5	6.8 ± 0.2	nd	15.8 ± 0.2	14.5 ± 0.2
Pseudomonas aeruginosa KCTC 2513	7.8 ± 0.5	9.1 ± 0.2	7.5 ± 0.2	nd	15.3 ± 0.5	13.5 ± 0.5
Streptococcus mutans KCTC 3065	nd	nd	nd	nd	9.3 ± 0.4	8.5 ± 0.5
Streptococcus pyogens KCTC 3096	nd	nd	nd	nd	9.5 ± 0.2	8.8 ± 0.2
Enterococcus faecalis KCTC 3195	nd	nd	nd	nd	8.5 ± 0.2	8.1 ± 0.2
Micrococcus luteus KCTC 3523	8.5 ± 0.5	7.8 ± 0.5	nd	nd	18.6 ± 0.5	13.1 ± 0.5
Saccharomyces cerevisiae KCTC 7904	nd	8.3 ± 0.5	nd	nd	10.7 ± 0.5	9.3 ± 0.5

† Each value is the mean ± S.D (n=3).

‡ nd means no detection of antimicrobial activity.

other isolated compounds from culture broth of MP-1. The minimum inhibition of concentration (MIC) of each active compound inhibited the growth of microorganisms at 2000 to 2500 μg ml^{-1} for C4 and from 250 to 800 μg ml^{-1} for C1, C2 and C3 except strains as *S. mutans, S. pyogens* and *E. faecalis*. The significance and impact of this study, *S. cerevisiae* and *B. subtilis* were not inhibited by C3 and C4, respectively (Table 2). *B. capacia* is known to produce a wide range of secondary metabolites such as pyrrolnitrin, phenazine, cepabactin, and other unidentified volatile or nonvolatile compounds (Roitman et al., 1990; Cartwright et al., 1995; Meyer et al., 1989). The pure amounts (261 mg, 10 mg, 420 mg, and 36 mg) of C1, C2, C3 and C4, respectively were isolated from the culture broth and we calculated the composition of acidic fraction containing C1 (35.9%), C2 (1.38%), C3

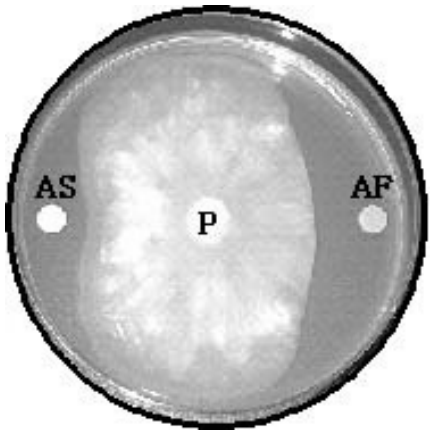

Fig. 3. Inhibition zone of each 1 mg dry weight of acidic fraction (AF) and authentic standard (AS) containing four isolated compounds which are mixed with the same ratio as acidic fraction against *Phytophthora capsici* KACC 40483 (P) on the potato dextrose agar after 5 day incubation at 30℃.

Table 2. Minimum inhibitory concentration (MIC) of phenylacetic acid (C1), hydrocinamic acid (C2), 4-hydroxyphenylacetic acid (C3), 4-hydroxyphenylacetate methyl ester (C4)

Microorganisms	MIC (μg/ml)†			
	C1	C2	C3	C4
Staphylococcus aureus KCTC 1928 (+)	650	650	500	2,500
Escherichia coli KCTC 2593 (-)	650	650	500	2,000
Baccillus subtilis KCTC 1021 (+)	350	250	500	nd
Staphylococcus epidermidis KCTC 1917 (+)	650	650	650	2,500
Pseudomonas aeruginosa KCTC 2513 (-)	650	650	350	2,500
Streptococcus mutans KCTC 3065	2,000	2,000	1,500	2,500
Streptococcus pyogens KCTC 3096	2,000	2,000	1,500	2,000
Enterococcus faecalis KCTC 3195 (+)	2,500	2,000	2,000	2,500
Micrococcus luteus KCTC 3523 (+)	650	650	650	2,500
Saccharomyces cerevisiae KCTC 7904 (y)	800	500	nd	2,500

† MIC and nd mean minimum inhibitory concentration and no detection of antimicrobial activity, respectively.

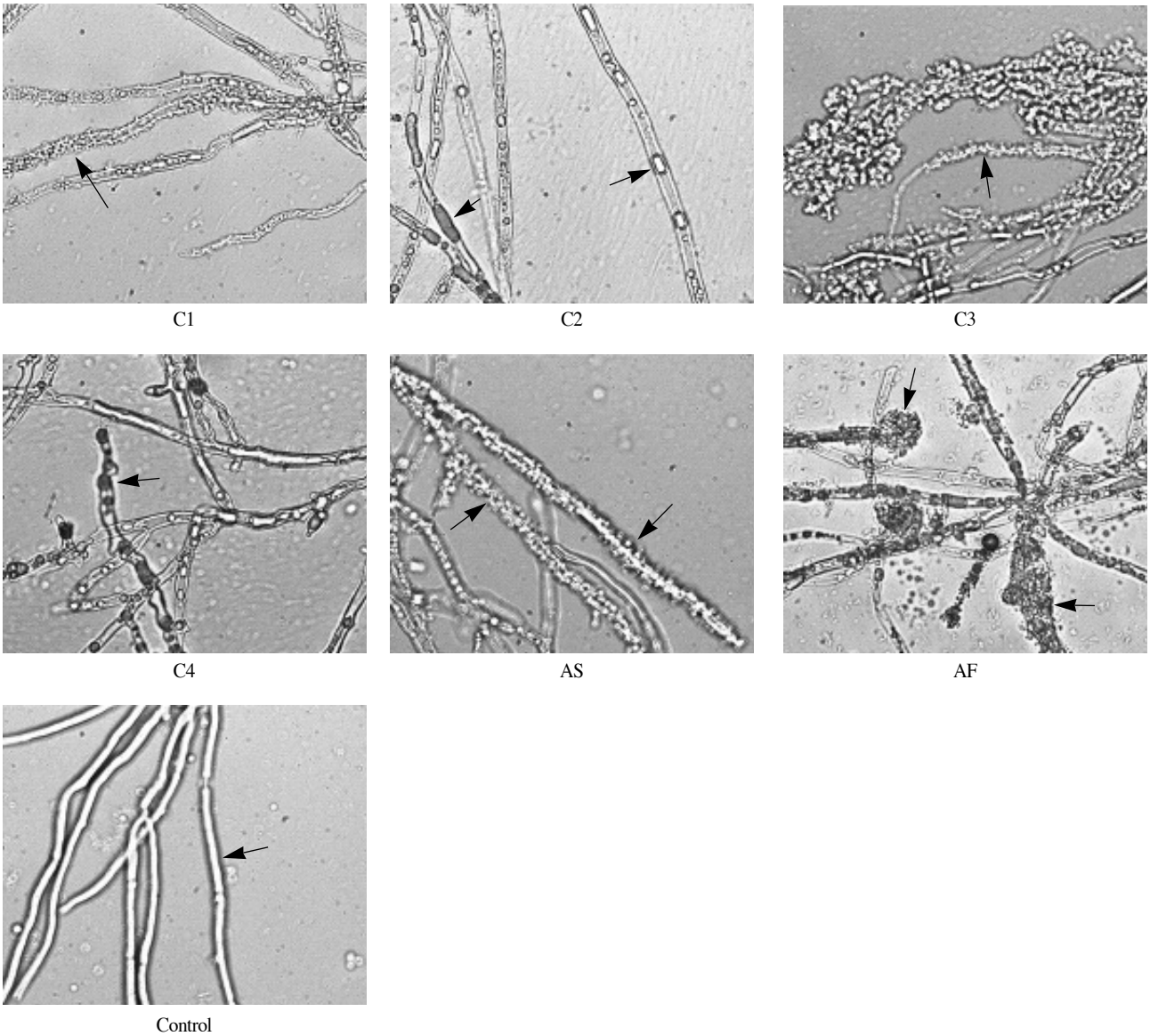

Fig. 4. Morphological hyphae of *Phytophothora capcisi* grown in potato dextrose broth supplemented with phenylacetic acid (C1, 0.36 mg); hydrocinamic acid (C2, 0.01 mg); 4-hydroxyphenylacetic acid (C3, 0.58 mg); 4-hydroxyphenylacetate methyl ester (C4, 0.05 mg); authentic standard mixture (AS, 1 mg); acidic fraction (AF, 1 mg) and control (20 μl of 50% MeOH) individually after incubation for 24 h at 30℃.

(57.77%), and C4 (4.95%). According to these percentages, the authentic standard mixture could be made the same ratio as found in acidic fraction. When the proportion of each isolated compound, acidic fraction and authentic standards were treated to *Phytophthora capsici* individually. The mycelia were deformed and destroyed as illustrated by light microscopy (Fig. 4). We found that the antimicrobial activity at the same concentration of crude acidic fraction is more effective than authentic standard mixture either wide range microorganisms tested (Table 1) or mycelial growth of *P. capsici* (Fig. 3 and 4(SA and AF)). This reason may have two cases in acidic fraction as minor antimicrobial volatile compounds not isolated but active and their synergistic effect lost after purification.

Conclusion

At least four antimicrobial compounds were isolated from the culture broth of *Burkholderia* sp. MP-1. Owing to property of chemical compound and concentration, different inhibitions of antimicrobial activities were shown in Table 1 and 2. Isolated compound C1, C2, C3 and C4 were identified as low molecular phenolic compounds and the potential of antimicrobial identities. Thus, this is a new report that several antimicrobial compounds can be isolated simultaneously from cultural broth of *Burkholderia* sp. Taken from experimental data,

Burkholderia sp. MP-1 was suggested to be valuable source for using in the biocontrol of food, animal and plant pathogens. Further studies are needed to evaluate the possibility of these isolated compounds as antimicrobial ingredients in food industry, a plant growth regulator and fungicide.

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References

Burhead, K., Slininger, P. A., and Schisler, D. A. 1998. Biological control bacterium *Enterobacter cloacae* S11:T:07 (NRRL B-21050) produces the antifungal compound phenylacetic acid in sabouraur maltose broth culture. Soil Biol. Biochem. 30:665-667.

Burkholder, W.H. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology. 40:115-117.

Byung, K.H., Song, W.L., Beom, S.K., Jung, Y.L., and Surk, S.M. 2001. Isolation and in vitro and in vivo antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. Appl. Environ. Microbiol. 67:3739-3745.

Camirand, A., Phipps, J., and Wightman, F. 1983. Comparative metabolism of L-tyrosine and L-phenylalanine in tobacco plants in relation to the biosynthesis of phenylacetic acids. Can. J. Bot. 61:2302-2308.

Cartwright, D.K., Chilton, W.S., and Benson, D.M. 1995. Pyrrolnitrin and phenazine production by *Pseudomonas cepacia,* strain 5.5B, a biocontrol agent of *Rhizoctonia solani*. Appl. Microbiol. Biotechnol. 43:211-216.

Cartwright D.K., and Benson D.M. 1995. Optimization of biological control of *Rhizoctonia* stem rot of poinsettia by *Paecilomyces lilacinus* and *Pseudomonas cepacia*, Plant Dis. 79:301-308.

Dagley S., Chapman P.J., and Gibson D.T. 1965. The metabolism of b phenylpropionic acid by an *Achromobacter*. Biochem. J. 97: 643 -650.

Di Cello F., Bevivino A., Chiarini L., Fani R., Paffetti D., Tabacchioni S., and Dalmastri C. 1997. Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizoshere at different plant growth stages. Appl. Environ. Microbiol. 63: 4485-4493.

Folsom B.R., Chapman P.J., and Pritchard P.H. 1990. Phenol and tricholoethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates, Appl. Environ. Microbiol. 65: 1279-1285.

Fravel, D. R. 1988. Role of antibiosis in the biocontrol of plant desease. Ann. Rev. Phytopathol. 26: 75-91.

Haugland R.A., Schlemm D.J., Lyons R.P., Sferra P.R., and Chakrabarty A.M. 1990. Degradation of chlorinated phenoxyacetate herbicide 2,4-dichlorophenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid by pure and mixed bacterial cultures, Appl. Environ. Microbiol. 56: 1357-1362.

Hirota, A., Horikawa, T. and Fujiwara, A. 1992. Isolation of phenylacetic acid from a phytopathogenic fungus, *Glomerella cingulata*. Biosci. Biotech. Biochem. 57: 492.

Jae, G.K., Sun, T.K., and Kyu, Y.K. 1999. Production of the antifungal compound phenylacetic acid by antagonistic bacterium *Pseudomonas* sp. Agric. Chem. Biotechnol. 42: 197-201.

Kawazu K, Zhang H, and Kanzaki. 1996. Accumulation of benzoic acid in suspension of cultured cells of *Pinus thungergii* Oarl. in response to phenylacetic acid administration. Biosci Biotechnol Biochem 60: 1410-1412.

Kim K.D, Nemec S, and Musson G. 1997. Control of Phytophthora root and crown rot of bell pepper with composts and soil amendments in the greenhouse. Applied Soil Ecology. 5: 169-179.

Lam, Y. W., Wang, H.X., and Ng, T.B. 2000. A robust cysteine-deficient chitinase like antifungal protein from inner shoots of the edible chive *Allium tuberosum*. Biochem. Biophys. Res. Commun. 279: 74-80.

Lee S. J., J. Y. Cho., J. I. Cho., J. H. Moon., K. D. Park., Y. J. Lee, and K. H. Park. 2004. Isolation and characterization of antimicrobial substance macrolactin A produced from bacillus *amyloliquefaciens* CHO104 isolated from soil. J. Microbiol. Biotechnol. 14: 525-531.

Meyer, J.M., Hohnadel, D., and Halle, F. 1989. Cepabactin from *Pseudomonas cepacia*, a new type of siderophore. J.Gen. Microbiol. 135: 1479-1487.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolke, R. H. 1995. Manual of Clinical Microbiology, Vol. 6th ed. ASM, Washington, DC.

O'Sullivan, D.J. and O'Gara, F. 1992. Traits of fluorescent *Pseudomonas* ssp. Involved in suppression of plant root pathogens. Microbiol. Rev. 56: 662-676.

Rasmay B.A., Ramsay J.A., and Cooper D.G. 1989. Production of poly-β-hydroxyalkanoic acid by *Pseudomonas cepacia*. Appl. Environ. Microbiol. 55: 584-589.

Roitman, J.N., Mahoney, N.E., and Janisiewicz, W.J. 1990. Production and composition of phenylpyrrole metabolites produced by *Pseudomonas cepacia*. Appl. Microbiol. Biotechnol. 34: 381-386.

Saddler G.S. 1994. *Burkholderia cepacia,* Mycopathologia.128: 53-54.

Smirnov V.V., Garagulya A.D., and Kiprianova E.A. 1982. Antibiotic properties of *Pseudomonas cepacia*. Antibiotiki (Moscow). 27: 577-580.

Stanier, R.Y., Palleroni, N.J., and Doudoroff, M. 1966. The aerobic pseudomonas: a taxonomic study. J. Gen. Microbiol. 43: 159-271.

Thomashow, L.S., and Weller, D.M. 1996. Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Plant Microbe Interactions (Stacey, G., and Keen, N.T., Eds.) Vol. 1, pp. 187-

236. Chapman and Hall, Ltd., London.

Wang, H.X., Lui, F., and Ng,T.B. 2001. Examination of pineal indoles and 6-methoxy-2-benzoxaolinone for antioxidant and antimicrobial effects. Comp. Biochem. Physiol. C130: 379-388.

Weller D. M. 1988. Biological control of soilborne plant pathogens in the rhizoshere with bacteria. Annual Review of Phytopathology. 26: 379-407.

Wightman, F. and Lighty, D. L. 1982. Identification of phenylacetic acid as a natural auxin in the shoots of higher plants. Physiol. Plant. 55: 17-24.

Yabuuchi, E., Kosako, Y., and Oyaizu, H. 1992. Proposal of *Burkholderia* gen. Nov. and transfer of seven species of the genus *Pseudomonas* homology group II to a new genus, with the type species *Burkholderia cepacia* (Palleroni and Homes 1981) comb. Nov. Microbiology and Immunology. 36: 1251-1275.

Yoon K, Cho JY, Kuk JH, Moon JH, Cho JI, Kim YC, and Park KH. 2004. Identification and antimicrobial activity of phenylacetic acid produced by *Bacillus licheniformis* isolated from fermented soybean, Chungkook-Jang. Curr. Microbiol. 48:312-317.

Brukholderia sp. MP-1 에서의 페놀화합물의 분리와 항균활성의 측정

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길항미생물 MP-1 은 여러 종의 곰팡이와 효모 및 음식물을 부패시키는 세균 (그람양성 및 음성) 에 대하여 항균활성을 보였다. MP-1의 16S rRNA gene 염기서열은 기존에 밝혀진 *Brukholderia* sp.의 염기서열과 99-100% 유사하였다. 배양액으로부터의 항균물질은 에틸아세테이트 (EtOAc)를 사용하여 분리하였으며 EtOAc에 용해된 산성 분획은 silica gel, Sephadex LH-20, ODS 컬럼크로마토 그래피 및 HPLC를 사용하여서 정제를 실시 하였다. 최종적으로 Gas chromatographic mass 스펙트럼을 통한 분석을 통하여 4 종의 항균 활성을 나타내는 물질은 phenylacetic acid, hydrocinnamic acid, 4-hydroxyphenylacetic acid 및 4-hydroxyphenylacetate methyl ester로 확인 되었다. 각각의 항균 활성물질들의 미생물에 대한 최소저해농도(MIC) 는 250-2500 ug ml^{-1} 인 것으로 나타났다. 또한 6 mm paper disc 에서의 1mg (건물중) 산성 분획의 항균활성은 같은 비율로 혼합한 표준 혼합용액 보다 더 효과적인 것으로 나타났다.