JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Sphingomonas sp. Strain SB5 Degrades Carbofuran to a New Metabolite by Hydrolysis at the Furanyl Ring

IN SEON KIM,*[,†] JI YOUNG RYU,[‡] HOR GIL HUR,[‡] MAN BOCK GU,[‡] SANG DON KIM,[‡] AND JAE HAN SHIM[†]

Division of Applied Bioscience and Biotechnology, Institute of Agricultural Science and Technology, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, South Korea, and Department of Environmental Science and Engineering, Kwangju Institute of Science and Technology (K-JIST), Gwangju 500-712, South Korea

Microorganisms capable of degrading carbofuran were isolated from soils and examined for the degradation of this pesticide at ring structure. An isolate that could degrade carbofuran and carbofuran-7-phenol was selected for further studies. The 16S rRNA analysis results showed that the isolate belongs to the genus of *Sphingomonas*, close to dioxin and dicamba degraders, and is named *Sphingomonas* sp. SB5. SB5 did not show any similarity of 16S rRNA to known carbofuran degraders. When time−course degradation of carbofuran by SB5 was examined by solvent extraction combined with liquid chromatographic analysis, almost complete disappearance of carbofuran was observed within 12 h, giving several accumulative metabolites. Bacterial cultures incubated with carbofuran-7-phenol suggested that the accumulated metabolites were derived from carbofuran-7-phenol. The control without SB5 and kanamycin-treated SB5 did not show any metabolite, suggesting a biological involvement in the degradation of carbofuran. GC/MS and LC/MS analyses identified 2-hydroxy-3-(3-methylpropan-2-ol) phenol as one of the accumulated metabolites, suggesting that the strain SB5 could degrade carbofuran-7-phenol by hydrolysis at the furanyl ring. This is the first report to identify 2-hydroxy-3-(3-methylpropan-2-ol) phenol as a new product derived biologically from carbofuran-7-phenol.

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methylcarbamate) is one of the pesticides belonging to the *N*-methylcarbamate class used extensively in the agricultural business. In Korea, this pesticide has been used to control brown hoppers, green rice leaf hoppers, and rice stem borers in rice paddies since 1975. Carbofuran is known to exhibit extreme mammalian toxicity (*1*); thus, it has been classified as highly hazardous. The intensive use of carbofuran could increase the possibility of environmental exposure to this pesticide. The potential major route of the exposure to carbofuran is drinking water from both groundwater and surface water sources because this pesticide is very water soluble and mobile in soil environments (*2*, *3*). Carbofuran has been shown to be one of major compounds that accelerated degradation occurs in a wide rage of soils, suggesting a possibility that its metabolites may accumulate in the soils. Although there is relatively little information in the open literature on environmental concern of carbofuran metabolites such as carbofuran-7-phenol and hydroxylated carbofuran or carbofuran-7-phenol, they are needed to properly degrade, considering that fresh and clean soil environments are based on continuous cycling of soil elements. The soil elements include pesticides and their metabolites that accumulate in soil. The environmental concern of carbofuran or its metabolites, therefore, has prompted studies on biodegradation of the chemicals.

A number of bacteria capable of degrading carbofuran, including strains such as *Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, and *Arthrobacter*, have been isolated and characterized in an effort to better understand the bacterial role to remove carbofuran from the environment (*4*−*10*). An initial step for the bacterial degradation of carbofuran has been generally shown to occur via hydrolysis at the moiety of *N*-methylcarbamate linkage, giving carbofuran-7-phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol) and methylamine as the resultant degradation products, which has been well-documented in bacteria capable of growth on carbofuran as a sole source of carbon and nitrogen (*10*). Many studies reported involvement of plasmids in mineralization of carbofuran, focusing mostly on only its methylcarbamate moiety (*11*−*13*). A preliminary study on plasmid-mediated catabolism of carbofuran at ring structure was first reported by Head et al. (*12*). A more detailed characterization of plasmids encoding mineralization of carbofuran at the ring structure has been described in *Sphingomonas* sp. strain CF06 (*14*, *15*).

* To whom correspondence should be addressed. Tel: +82-62-530-2131. Fax: +82-62-530-2139. E-mail: mindzero@chonnam.ac.kr.
† Chonnam National University.
‡ Kwangju Institute of Science and Technology (K-JIST).

10.1021/jf035502l CCC: $27.50 © 2004 American Chemical Society
Published on Web 03/27/2004

To date, however, a metabolic pathway for the degradation of carbofuran at ring structure is still unclear, since little study on the identification of metabolic intermediates that could arise from the degradation of carbofuran at the ring structure has been performed. In the present study, we report for first time a new hydrolytic metabolite in the degradation of carbofuran-7-phenol by a yellow-pigmented isolate *Sphingomonas* sp. strain SB5. The strain SB5 could degrade carbofuran-7-phenol to a hydrolytic product by hydrolysis at the furanyl ring. Unidentified red metabolites that accumulated in the degradation of carbofuran and carbofuran-7-phenol are discussed.

MATERIALS AND METHODS

Chemicals. Carbofuran (purity, 98%) and carbofuran-7-phenol (purity, 99%) were purchased from Aldrich (Milwaukee, WI). The solvents used in this study were of HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals were of analytical grade and commercially available, unless otherwise stated.

Bacterial Isolation and Media. Carbofuran-degrading microorganisms were isolated by enrichment culture techniques using an agricultural soil that had been exposed to carbofuran for at least 5 years. A 2 g amount of soil samples was suspended in 100 mL of mineral salt medium (MSM) containing 200 mg of carbofuran as a sole source of carbon and nitrogen and the following constituents (in grams per liter, pH 7.2): K_2HPO_4, 7.5; KH_2PO_4, 2.0; NaCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.02; 10 mL of trace elements containing 20 mg of $Na_2MoO_4{\cdot}2H_2O$, 50 mg of H_2BO_3, 30 mg of $ZnCl_2$, 10 mg of $CuCl_2$, and 20 mg of $FeCl_3$ per liter. The suspension was incubated at 27 °C for 7 days on a shaker at 200 rpm and then transferred to a fresh MSM. After five transfers weekly, the cultures were diluted serially with the MSM and plated onto MSM agar plates containing 0.91 mM of carbofuran. Carbofuran−MSM agar plates were prepared by adding 200 mg of carbofuran dissolved in dimethyl sulfoxide (DMSO, 1 mL per liter) into the MSM that had been previously autoclaved and cooled to approximately 55 °C. Only DMSO was added to the MSM for control plates. Following bacterial growth on the plates, visible colonies were obtained and tested for carbofuran degradation.

Identification of Carbofuran Degraders. Bacterial identifications were carried out by 16S rRNA sequence analysis. For sequence analysis, cells grown on Luria−Bertani (LB) medium containing 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter (pH 7.0) were harvested, and genomic DNA was prepared using a QIAamp DNA Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. A 1431 base segment of 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 27f (5′-AGAGTTTGATCMTGGCTCAG) and 1492r (5′-TACGGYTACCTTGTTACGACTT). PCR products containing the amplified 16S rRNA gene fragment were purified with a QIAquick spin column (QIAGEN) and cloned into a Promega pGEM T Easy vector. The cloned vector was transformed into *Escherichia coli* DH5α, and plasmids were obtained by using a Wizard Plus Miniprep DNA Purification Kit for nucleotide sequencing. The sequencing was carried out by a model ABI3700 Automated DNA Sequencer. Computer analysis for 16S rRNA sequence was performed with DDBJ software packages. The phylogenetic analysis of 16S rRNA sequence of isolates was performed by comparing with other 16S rRNA sequences available on BLAST search of the DDBJ database.

Carbofuran Degradation and Bacterial Growth. Time−course carbofuran degradation and bacterial cell growth were examined concurrently in the same culture flasks. For carbofuran degradation, bacterial cells grown overnight on LB medium were washed twice with the MSM, adjusted to optical density (OD) of approximately 1.2 at 600 nm (A_{600}), and inoculated at 1% (v/v) inoculum level into triplicate flasks containing 100 mL of the MSM with carbofuran (0.91 mM) as a sole source of carbon and nitrogen. The cultures were then incubated on a rotary shaker as described above. Controls were carbofuran-free cultures, culture-free carbofuran, and carbofuran cultures treated with kanamycin (100 μg/mL). The culture flasks containing cells and medium were taken periodically and extracted twice with 100 and 50 mL of

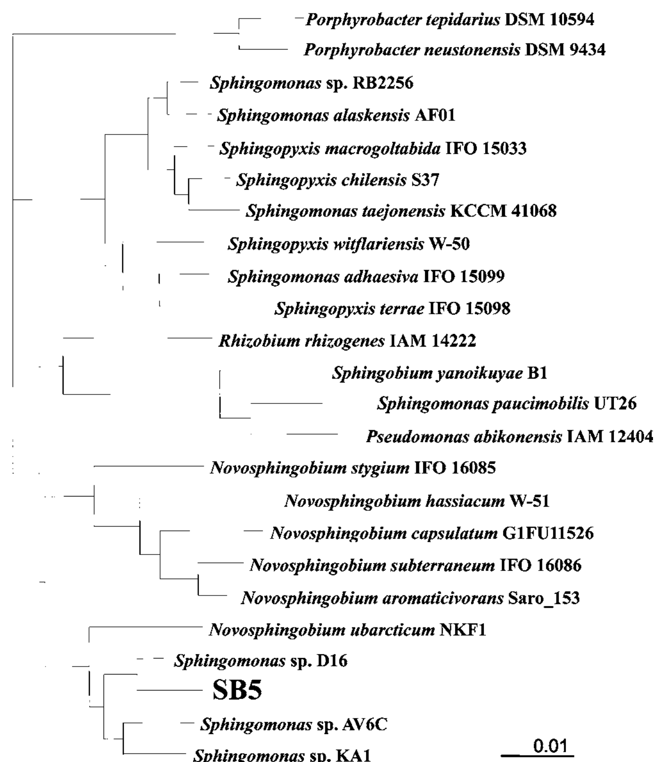

Figure 1. Phylogenetic tree of *Sphingomonas* sp. strain SB5 based on 16S rRNA analysis.

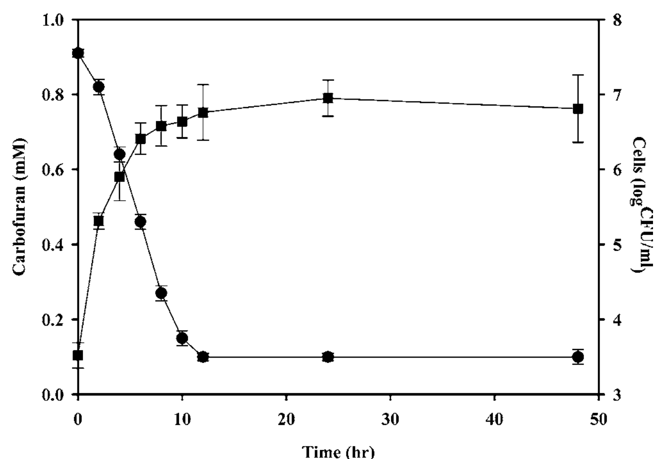

Figure 2. Degradation of carbofuran by *Sphingomonas* sp. strain SB5 (●) and bacterial growth based on CFU counting (■). The data given are the means of three measurements ± SD.

dichloromethane after adding an internal standard. 2,3-Dihydrobenzofuran (Aldrich, Milwaukee, WI) was used as an internal standard because it did not show any interference in the chromatogram analysis of interest. The organic extracts obtained above were collected, dried over anhydrous sodium sulfate, and evaporated at 40 °C by a vacuum rotary evaporator. The residue was dissolved again in 5 mL of methanol, and an aliquot of the residue in a 10 μL sample size was injected into a high-performance liquid chromatograph (HPLC). For the bacterial growth assay, an aliquot (100 μL) of the cultures incubated with carbofuran was plated onto the carbofuran−MSM agar plates and their colonies that appeared in 3 days of incubation were counted.

Analytical Methods. The HPLC was a Varian 230 dual pump (Walnut Creek, CA) equipped with a Varian model 410 autosampler. Carbofuran was detected by using a photodiode array detector (Varian model 330) at 280 nm. The HPLC column was a Waters OD2 stainless column (4.6 mm × 250 mm in length, 5 μm film thickness). The HPLC mobile phase consisted of acetonitrile (solvent A) and water (solvent B) acidified with 1% (v/v) formic acid, respectively. Isocratic elution

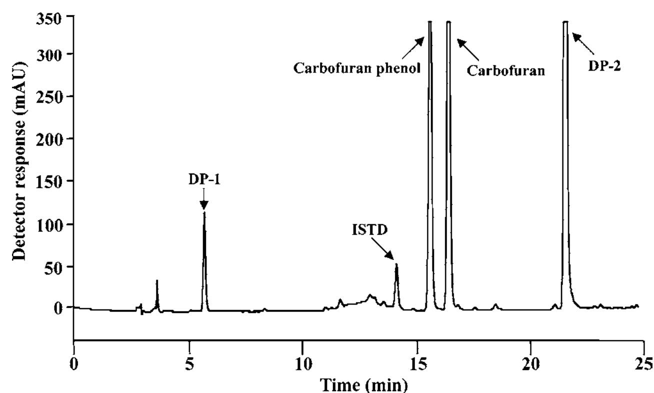

Figure 3. Typical HPLC chromatograms of the culture extracts obtained from *Sphingomonas* sp. strain SB5 incubated with carbofuran. The culture flasks incubated for 6 h were extracted with dichloromethane as described in the Materials and Methods section, and the resultant extracts were dissolved in methanol for HPLC analysis. ISTD represents the internal standard, 2,3-dihydrobenzofuran.

of samples was obtained with 20% solvent A and 80% solvent B for 5 min. Linear gradient elution was obtained with 50% solvent A for 10 min and 95% solvent A for 10 min, followed by isocratic elution with 95% solvent A for 15 min. The mobile phase was eluted at 1 mL/min. Metabolite identifications were carried out based on gas chromatography/mass spectrometry (GC/MS) and HPLC/mass spectrometry (HPLC/MS) analysis. GC/MS analysis was performed using an Agilent model 5973N system with HP-5MS capillary column (0.25 mm i.d. × 30 m, 1.0 μm film thickness). The carrier gas was helium with a flow rate of 1 mL/min. Injections were done in split mode of 20:1. The column temperature programmed was 2 min at 100 °C, followed by a ramp rate of 10 °C/min to 280 °C. Mass spectroscopy was performed in the electron ionization (EI) mode at 70 eV. The injector temperature was 280 °C. The source and inlet temperatures were held at 200 °C. Mass values were scanned from 50 to 500 amu at 2.94 s per scan. For further identification of metabolites, a Hewlett-Packard model 1100 HPLC coupled with a VG Quattro LC triple quadrupole tandem mass spectrometer (Micromass, Mancgester, U.K.), equipped with an electrospray source, was used. LC/MS spectra were acquired with the positive ion mode.

RESULTS AND DISCUSSION

Five bacterial strains capable of growth on carbofuran as the sole carbon and energy sources were isolated. Among them, a strain that showed degradation of carbofuran-7-phenol ("carbofuran phenol" hereafter) was designated strain SB5 and selected for further studies. The 16S rRNA sequence analysis of SB5 showed most similarity of 98% to the dicamba-degrading *Sphingomonas* sp. strain D16 with the accession number AF025352 in the GenBank database (**Figure 1**). The strain SB5 showed the similarities of 97% to *Sphingomonas* sp. AV6C that could degrade monocyclic aromatic acids (*16*). SB5, however, did not show any similarity of 16S rRNA sequence to *Sphingomonas* sp. strain CF06 that could degrade carbofuran and carbofuran phenol (*14*). The 16S rRNA sequence analysis of SB5 showed the similarities of 96% to *Sphingomonas* sp. KA1 capable of degrading chlorinated dibenzo-*p*-dioxins (*17*). SB5 could degrade carbofuran and carbofuran phenol. However, SB5 could not degrade dicamba. It was probably due to its chemical structure different to carbofuran. Dicamba does not have a carbamate moiety and furanyl ring structure. On the basis of 16S rRNA sequence analysis, SB5 did not show any similarity of the sequence to known carbofuran degrader. These observations suggest that SB5 is a newly isolated carbofuran degrader placed in the genus *Sphingomonas* as a member of the α-group of Proteobacteria. However, more study is required to characterize SB5 in more detail because this microorganism degrades carbofuran to carbofuran phenol and methylamine, as does other carbofuran degraders. SB5 is now deposited in the GeneBank database under accession number AY245435.

Utilization of carbofuran by SB5 was examined on the basis of the bacterial growth, as determined by colony forming unit (CFU) counting, in the MSM containing carbofuran as a sole source of carbon and nitrogen. When SB5 was incubated with carbofuran, the growth of SB5 was accompanied by the disappearance of carbofuran, giving almost complete degradation of the added carbofuran after incubation for 12 h (**Figure 2**). The growth of SB5 reached a maximum in 24 h of incubation and afterward decreased slightly. SB5 could utilize methylamine

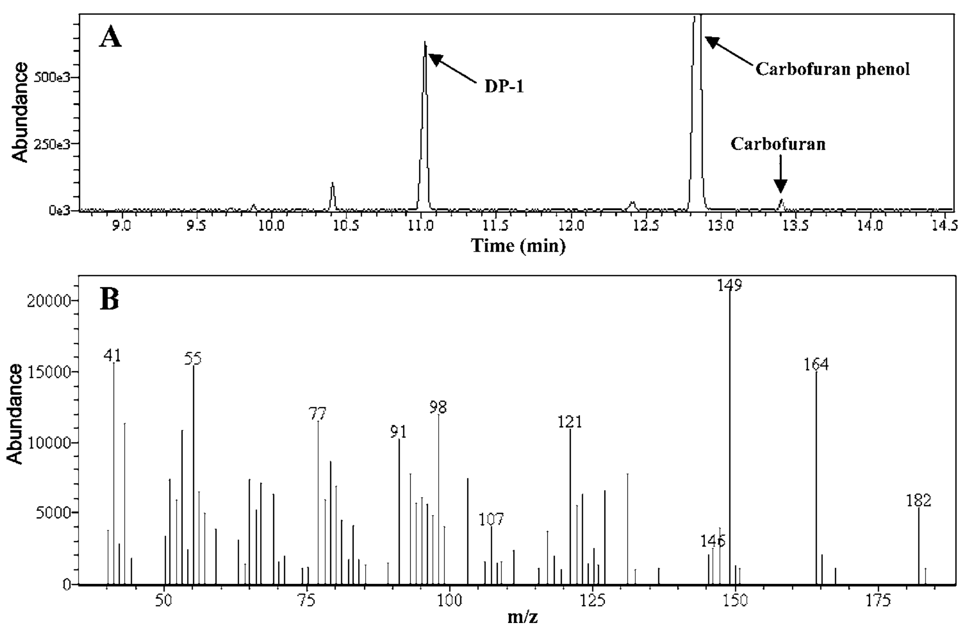

Figure 4. Total ion chromatogram and GC/MS spectra of the culture extracts obtained from *Sphingomonas* sp. strain SB5 incubated with carbofuran. The culture flasks incubated for 12 h were extracted with dichloromethane as described in the Materials and Methods section, and the resultant extracts were dissolved in methanol for GC/MS analysis.

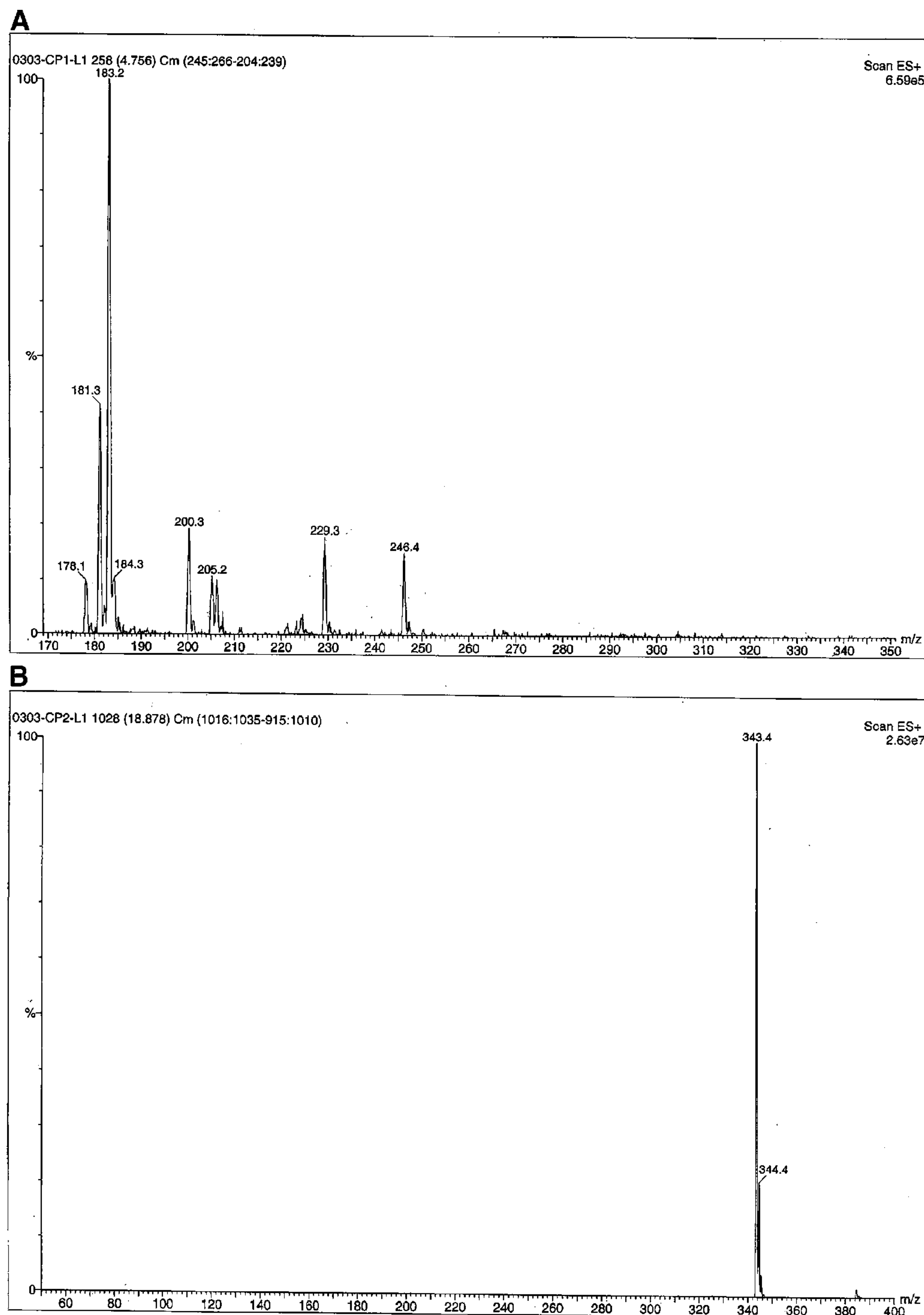

Figure 5. (**A**) Positive ion LC/MS spectra of DP-1. (**B**) Positive ion LC/MS spectra of DP-2.

as a sole source of carbon, suggesting that the strain used the methylcarbamate moiety of carbofuran.

Carbofuran phenol was observed as an initial intermediate during incubation with carbofuran (**Figure 3**). The initial metabolite, carbofuran phenol, was also degraded almost completely within 48 h of incubation. Because carbofuran is known to hydrolyze to carbofuran phenol under an alkali condition, pH of the cultures was measured during the incubation. Negligible changes of the pH were observed, ranging from 7.20 to 7.26 with cell growth. This suggests that the pH changes did not affect the formation of carbofuran phenol in the degradation of carbofuran. In control samples without SB5 cultures, only carbofuran was observed without its degradation throughout the experiment, suggesting that abiotic degradation of carbofuran was negligible. HPLC analysis showed two additional main metabolites, DP-1 and DP-2, from the cultures (**Figure 3**), which was followed by the disappearance of carbofuran and carbofuran phenol. To test if the metabolites were from carbofuran phenol, cells grown on carbofuran as the sole carbon source for 12 h were obtained, washed twice with the MSM, and adjusted to $A_{600} = 1.0$. The washed cells were then incubated with carbofuran phenol (1.22 mM) for 12 h and extracted with solvent for HPLC analysis. The same metabolite profiles that had been observed in the incubation with carbofuran were observed, suggesting that the two main accumulated metabolites in carbofuran-grown cultures were derived from carbofuran phenol. The control cultures incubated with carbofuran in the presence of kanamycin and the heat-killed cultures

did not show the appearance of the accumulated metabolites. This suggests that the metabolites were due to a biological degradation of carbofuran phenol. When SB5 was incubated in the presence of carbofuran or carbofuran phenol, a red color was observed in the culture media with cell growth. The observation of the red color was faster in cells incubated with carbofuran phenol than cells incubated with carbofuran. A red metabolite that correlated with the disappearance of carbofuran and carbofuran phenol was also reported previously in other studies with *Sphingomonas* sp. strain CF06 (*14*, *15*). However, the intermediate still remained unidentified.

Figure 4 shows the GC/MS data obtained from the cultures incubated with carbofuran as a sole source of carbon and nitrogen. Carbofuran was detected at about 13.4 min, and the metabolite at about 12.8 min was identified as carbofuran phenol. The metabolite at 11.0 min was identified by M^+ peak at *m*/*z* 182 (**Figure 4B**), giving 18 mass differences from the ion spectrum of carbofuran phenol with M^+ peak at *m*/*z* 164. The GC/MS fragment ions of the metabolite detected at 11.0 min were similar to those of carbofuran phenol except the molecular ion peak at *m*/*z* 182. The fragment ion at *m*/*z* 149 was detected as a base peak, resulting from loss of CH_3 from *m*/*z* 164. This suggested that a water molecule was added to carbofuran phenol by SB5 to give 2-hydroxy-3-(3-methylpropan-2-ol) phenol as a hydrolytic product of carbofuran. The retention time of this metabolite (DP-1) on the HPLC column was the shortest among the metabolites found in this study (**Figure 3**), giving a $(M + H)^+$ peak at *m*/*z* 183.2 by LC/MS (**Figure 5A**). The appearance of this metabolite was observed within the first 6−8 h of incubation and kept appearing during incubation. The maximum UV/vis spectra (λ_{max}) of DP-1 were observed at 285.58 nm. When another carbofuran-degrading *Sphingomonas* sp. strain CF06 was used as a reference strain for a comparative study, the same metabolite was observed. *Sphingomonas* sp. strain CF06 has been reported to be capable of degrading the aromatic ring of carbofuran phenol (*15*). In the study, one of metabolites due to degradation of carbofuran phenol has been proposed to be 2-hydroxycarbofuran phenol. We should point out that this metabolite may not be 2-hydroxycarbofuran phenol, since LC/MS data of the metabolite showed a $(M + H)^+$ peak at *m*/*z* 183.2 (**Figure 5A**). If the metabolite were the 2-hydroxycarbofuran phenol, a $(M + H)^+$ peak would be observed at *m*/*z* 181. Metabolites of carbofuran by microorganisms include carbofuran phenol, 3-hydroxycarbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol, and 3-ketocarbofuran phenol (*10*, *18*, *19*). Carbofuran has been currently reported to degrade to 4-hydroxycarbofuran (*20*). However, no study has been done to identify microbial metabolites of carbofuran resulting from ring cleavage. In the present study, we identified 2-hydroxy-3-(3-methylpropan-2-ol) phenol as a new metabolite resulting from bacterial degradation of carbofuran phenol at the furanyl ring.

We observed an unusual intermediate (DP-2) in the degradation of carbofuran by SB5 (**Figure 3**). LC/MS analysis identified this unusual intermediate by a $(M + H)^+$ peak at *m*/*z* 343.4 (**Figure 5B**). This intermediate was observed as the most predominant metabolite among the metabolites of carbofuran found in this study, based on HPLC analysis. As this metabolite appeared in the cultures during the incubation, the cultures were changed to a red color. Considering the molecular weight of carbofuran is approximately 222 g/mol, this metabolite would be the result of condensation of some metabolites. For further identification of DP-2, the metabolite was purified and collected by HPLC fractioning. The fractioned red metabolite was

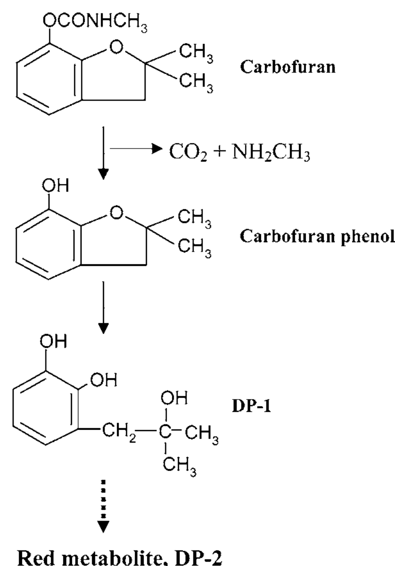

Figure 6. Proposed degradation pathway of carbofuran by *Sphingomonas* sp. strain SB5.

extracted again by dichloromethane, and the solvent was evaporated in vacuo. The metabolite was insoluble in *n*-hexane but soluble in dichloromethane. When the metabolite was dissolved again in MSM, the appearance of a red color was observed in the MSM. DP-2 was not extractable from the cultures with ethyl acetate under a neutral condition (pH 7.0) but extractable with dichloromethane or chloroform under the same condition. When the cultures were acidified to pH 2−4, the metabolite was extractable with ethyl acetate. GC/MS analysis of the red metabolites was not successful regardless of derivatization by diazomethane and trimethylsilylation (TMS). The reason is still not clear. We attempted a nuclear magnetic resonance (NMR) analysis of the purified DP-2 to investigate its chemical structure in more detail, but it also was not successful due to an unidentified reason. When the HPLC fractioned DP-2 was dissolved in dichloromethane and developed on a thin-layer chromatography (TLC, silica gel F_{254}, 20 cm × 20 cm × 200 μm thickness, Merck) plate by a solvent system of dichloromethane/ethyl acetate/acetic acid (85/15/1, v/v/v), two major red metabolites and one minor pink metabolite were detected by visual observation. When the metabolites were isolated and further purified by successive TLC analysis, they did not separate each other. Thus, more study to identify the red metabolites is required.

Taken from our observations, we could suggest a possible metabolic pathway for degradation of carbofuran by SB5, as shown in **Figure 6**. In brief, carbofuran was degraded first to carbofuran phenol and the resultant methylamine could be used as a carbon source for bacterial growth. Carbofuran phenol was degraded to give a hydrolytic product, 2-hydroxy-3-(3-methylpropan-2-ol) phenol. In the pathway, an unidentified red metabolite with a $(M + H)^+$ peak at 343.4 appeared as a main intermediate. Our study is the first to report a new hydrolytic intermediate, 2-hydroxy-3-(3-methylpropan-2-ol) phenol, in the degradation of carbofuran and carbofuran phenol by SB5. Hydrolysis of carbofuran phenol by SB5 occurred at the furanyl ring.

ACKNOWLEDGMENT

We thank Dr. A. V. Ogram for providing *Sphingomonas* sp. CF06 as a reference strain.

LITERATURE CITED

(1) Fahmg, M. A. H.; Fukuto, T. R.; Myers, R. O. The selective toxicity of *N*-phosphorothionylcarbamate esters. *J. Agric. Food Chem.* **1970**, *18*, 793−796.

(2) Sukop, M.; Cogger, C. G. Adsorption of carbofuran, metalxyl, and simazine: Koc evaluation and relation to soil transport. *J. Environ. Sci. Health* **1992**, *27B*, 565−590.

(3) Trotter, D. M.; Kent, R. A.; Wong, M. P. Aquatic fate and effect of carbofuran. *Crit. Rev. Environ. Control*. **1991**, *21*, 137−176.

(4) Chaudhry, G. R.; Ali, A. N. Bacterial metabolism of carbofuran. *Appl. Environ. Microbiol*. **1988**, *54*, 1414−1419.

(5) Desaint, S.; Hartmann, A.; Parekh, N. R.; Fournier, J. C. Genetic diversity of carbofuran-degrading soil bacteria. *FEMS Microbiol. Ecol*. **2000**, *34*, 173−180.

(6) Karns, J. S.; Mulbry, W. W.; Nelson, J. O.; Kearney, P. C. Metabolism of carbofuran by a pure bacterial culture. *Pestic. Biochem. Physiol*. **1986**, *25*, 211−217.

(7) Parekh, N. R.; Hartmann, A.; Charnay, M. P.; Fournier, J. C. Diversity of carbofuran-degrading soil bacteria and detection of plasmid-encoded sequences homologous to the *mcd* gene. *FEMS Microbiol. Ecol*. **1995**, *17*, 149−160.

(8) Ramanand, K.; Sharmila, M.; Singh, N.; Sethunathan, N. Metabolism of carbamate insecticides by resting cells and cell-free preparations of a soil bacterium, *Arthrobacter* sp. *Bull. Environ. Contam. Toxicol*. **1991**, *46*, 380−386.

(9) Tomasek, P. H.; Karns, J. Cloning of a carbofuran hydrolase gene from *Achromonobacter* sp. strain WM111 and its expression in gram-negative bacteria. *J. Bacteriol*. **1989**, *171*, 4038−4044.

(10) Topp, E.; Hanson, R. S.; Ringelberg, D. B.; White, D. C.; Wheatcroft, R. Isolation and characterization of an *N*-methyl-carbamate insecticide-degrading methylotrophic bacterium. *Appl. Environ. Microbiol*. **1993**, *59*, 3339−3349.

(11) Feng, X.; Ou, L. T.; Ogram, A. V. Cloning and sequence analysis of a novel insertion element from plasmids harbored by the carbofuran-degrading bacterium, *Sphingomonas* sp. CF06. *Plasmid* **1997**, *37*, 169−179.

(12) Head, I. M.; Cain, R. B.; Suett, D. L. Characterization of a carbofuran-degrading bacterium and investigation of the role of plasmids in catabolism of the insecticide carbofuran. *Arch. Microbiol*. **1992**, *158*, 302−308.

(13) Parekh, N. R.; Hartmann, A.; Fournier, J. C. PCR detection of the mcd gene and evidence of sequence homology between the degradative genes and plasmids from diverse carbofuran-degrading bacteria. *Soil Biol. Biochem*. **1996**, *28*, 1797−1804.

(14) Feng, X.; Ou, L. T.; Ogram, A. V. Plasmid-mediated mineralization of carbofuran by *Sphingomonas* sp. CF06. *Appl. Environ. Microbiol*. **1997**, *63*, 1332−1337.

(15) Ogram, A. V.; Duan, Y.; Trabue, S. L.; Feng, X.; Castro, H.; Ou, L. T. Carbofuran degradation mediated by three related plasmid systems. *FEMS Microbiol. Ecol*. **2000**, *32*, 197−2003.

(16) Gioia, D. D.; Barberio, C.; Spagnesi, S.; Marchetti, L.; Fava, F. Characterization of four olive-mill-wastewater indigenous bacterial strains capable of aerobically degrading hydroxylated and methoxylated monocyclic compounds. *Arch. Microbiol*. **2002**, *178*, 208−217.

(17) Habe, H.; Ashikawa, Y.; Saiki, Y.; Ypshida, T.; Nojiri, H.; Omori, T. *Sphingomonas* sp. strain KA1, carrying a carbazole dioxygenase gene homologue, degraded chlorinated dibenzo-*p*-dioxins in soil. *FEMS Microbiol. Lett.* **2002**, *211*, 43−49.

(18) Chapalmandugu, S.; Chaudhry, G. R. Microbial and biotechnological aspects of metabolism of carbamates and organophosphates. *Crit. Rev. Biotechnol.* **1992**, *12*, 357−389.

(19) Chaudhry, G. R.; Mateen, A.; Kaskar, B.; Bloda, M.; Sheikh, R. Purification and biochemical characterization of the carbamate hydrolase from *Pseudomonas* sp. 50432. *Biotechnol. Appl. Biochem*. **2002**, *36*, 63−70.

(20) Chaudhry, G. R.; Mateen, A.; Kaskar, B.; Sardessai, M.; Bloda, M.; Bhatti, A. R.; Walia, S. K. Induction of carbofuran oxidation to 4-hydroxycarbofuran by *Pseudomonas* sp. 50432. *FEMS Microbiol. Lett.* **2002**, *214*, 171−176.

Received for review December 23, 2003. Revised manuscript received February 20, 2004. Accepted February 24, 2004. The financial supports of this work by the Biogreen 21 program of Rural Development Administration and the Brain Korea 21 program of Korea Research Foundation, Republic of Korea, are gratefully acknowledged.

JF035502L