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Bacterial Degradation of Fungicide Captan

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The phthalimide fungicide captan has been widely used to control plant pathogenic fungi. A strain of *Bacillus circulans* utilized the fungicide captan as sole source of carbon and energy. The organism degraded captan by a pathway involving its initial hydrolysis to yield *cis*-1,2,3,6-tetrahydrophthalimide, a compound without fungicidal activity. The formation of this compound was confirmed by HPLC, IR, NMR, and mass spectral analysis. The results also revealed that *cis*-1,2,3,6-tetrahydrophthalimide was further degraded to *o*-phthalic acid by a protocatechuate pathway. These findings indicated that there was a complete mineralization of fungicide captan by *B. circulans*.

KEYWORDS: Biodegradation; captan; *Bacillus circulans*; protocatechuic acid

INTRODUCTION

The phthalimide fungicides such as captan, captafol, and folpet are the important surface protectants used to control plant pathogenic fungi (*1*, *2*). Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) is one of the most widely used surface fungicides to inhibit the growth of fungi on seeds, fruits, vegetables, and ornamental plants (*3*−*5*). Captan is used as an agricultural fungicide to control diseases caused by *Botrytis*, *Fusarium*, *Fusicoccum*, *Pythium*, and other fungal species (*6*). Captan is also used as an industrial fungicide in paints, plastics, leather, paper, cosmetics, textiles, and plaster (*7*−*10*) to prevent fungal growth. Thus, its widespread applications can cause long-term exposure of humans. Captan has an oral LD_{50} of 9000 mg/kg in rats (*11*, *12*). Sheep and cattle are susceptible to captan poisoning. Captan is highly toxic to fish and aquatic life (*5*, *13*). The U.S. Environmental Protection Agency has assigned captan a carcinogenicity classification of B2, a probable human carcinogen (*5*). Captan is a potent mutagen in both prokaryotic and eukaryotic cellular systems producing DNA damages (*14*−*18*). This fungicide has shown an adverse effect on soil microflora. For example, captan is toxic to *Rhizobium* sp. and to symbiotic N_2-fixation process (*19*−*22*). The use of this fungicide results in the loss of soil fertility due to destruction of the microbial communities responsible for capturing nitrogen, phosphate, and other nutrients (*23*). Thus the widespread use and release of such toxic fungicide lead to environmental pollution and loss of soil fertility, which is a growing cause of global concern. Hence, sustainable agriculture has become a priority issue in the 21st century (*24*), and the remediation of contaminated soils and aquatic bodies is most essential for the protection of the environment and conservation of resources. The microbial detoxification has been considered to be the most cost-effective, safe, and promising method for the removal of organic pollutants from the environment. However, much less information is available on the microbial degradation of fungicide captan. Buyanovsky et al. (*25*) have studied the degradation of captan in soils under laboratory conditions and have shown the transformation of captan to tetrahydrophthalimide by soil microbes. But the further metabolism of tetrahydrophthalimide has not been reported. Previously, we have reported the isolation and characterization of a bacterial strain *Bacillus circulans* that degrades the herbicide pendimethalin (*26*). Interestingly, this organism was also capable of utilizing the fungicide captan as sole carbon source for its growth. Hence, the present study was undertaken to investigate the biodegradative pathway of captan by *B. circulans*.

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MATERIALS AND METHODS

Chemicals. Captan was obtained from Rallis Agrochemicals India Ltd., Mumbai, India. *cis*-1,2,3,6-Tetrahydrophthalimide, *cis*-1,2,3,6-tetrahydrophthalamidic acid, *o*-phthalic acid, protocatechuic acid, salicylic acid, gentisic acid, and catechol were obtained from Sigma-Aldrich, USA. All other chemicals used were of analytical grade.

Organism and Growth Conditions. The bacterial strain *B. circulans* used in this study was previously isolated and identified in our laboratory from the pesticide-contaminated soil samples (*26*). The organism was grown on mineral salt medium (MSM) containing 0.1% (w/v) captan as sole source of carbon as described earlier (*26*). Growth was measured turbidometrically at 600 nm using a photoelectric colorimeter (Systronics, India). The cultures were maintained on mineral salt agar slants whose surfaces were coated with captan.

Utilization of Captan and Other Compounds by *B. circulans*. The ability of the *B. circulans* to utilize fungicide captan and other compounds such as *cis*-1,2,3,6-tetrahydrophthalimide, *cis*-1,2,3,6-tetrahydrophthalamidic acid, *o*-phthalic acid, and protocatechuic acid as sole carbon source was determined by measurement of growth in MSM containing 0.1% (w/v) of the compounds. Utilization of captan during growth of *B. circulans* was determined at different incubation periods by HPLC analysis as described in Isolation and Identification of Metabolites. Uninoculated controls were used to determine any transformation of captan (0.1% w/v) affected by physical factors.

Isolation and Identification of Metabolites. The metabolites were isolated from the culture filtrates of *B. circulans* after 24 and 72 h of growth on 0.1% (w/v) captan by extraction with ethyl acetate before and after

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acidification to pH 2.0 with 2 N HCl. The residues obtained were dissolved in methanol and analyzed for metabolites by thin-layer chromatography on silica gel G plates using the following solvent systems: (A) benzene−acetic acid (8:2 v/v), (B) benzene−dioxane−acetic acid (74:2:2 v/v), and (C) toluene−dioxane−acetic acid (90:20:4 v/v). The metabolites were visualized under UV light at 254 nm or by exposure to iodine vapors and also by spraying with Folin−Ciocalteau reagent or diazotized *p*-nitroaniline followed by 10% Na_2CO_3. *o*-Dihydroxy compounds were detected by spraying with Arnow's reagent (*27*). Aldehydes were detected by spraying with a solution of 2,4-dinitrophenylhydrazine (0.1%) in 2 M HCl.

The mass spectra were recorded with a Jeol, MS-DS 303 spectrometer operated at 70 eV. Metabolites were analyzed by reversed-phase HPLC (Shimadzu, Japan) equipped with UV detector using a 5 μm Spherisorb-ODS (C_{18}) column (25 cm × 4.6 mm) acetonitrile−phosphate buffer (0.05 M, pH 7.0) in the ratio 70:30 (v/v) as the mobile phase at a flow rate of 1 mL min^{-1}. Peaks were detected at 254 nm or scanned at 220−400 nm in a spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded using a 300 MHz spectrometer (Bruker AMX) of metabolites with tetramethylsilane (TMS) as internal standard at room temperature. UV−visible absorbance spectra were recorded with a Hitachi 3100 spectrophotometer. The infrared (IR) spectra were recorded with a Nicolet Impact 410 FT-IR spectrometer (Thermo Electron Corp., Madison, WI) at room temperature. The IR spectral range was 4000 to 400 cm^{-1}. Chloride ions released during captan degradation was estimated spectrophotometrically by using sodium chloride as standard (*28*). The silver nitrate test for chloride, sulfide test for H_2S, and limewater test for CO_2 were carried out according to standard protocols (*29*).

Oxygen Uptake Studies. The oxygen uptake by whole cells of *B. circulans* grown on captan was performed in an Oxygraph fitted with a Clark-type oxygen electrode (Hansatech, Germany). The cells were harvested in the early logarithmic phase by centrifugation at 12000*g* for 20 min and washed twice with 0.05 M phosphate buffer (pH 7.0). Oxygen uptake rates are expressed as nanomoles of O_2 consumed per minute per milligram of dry cells. The values were corrected for endogenous respiration.

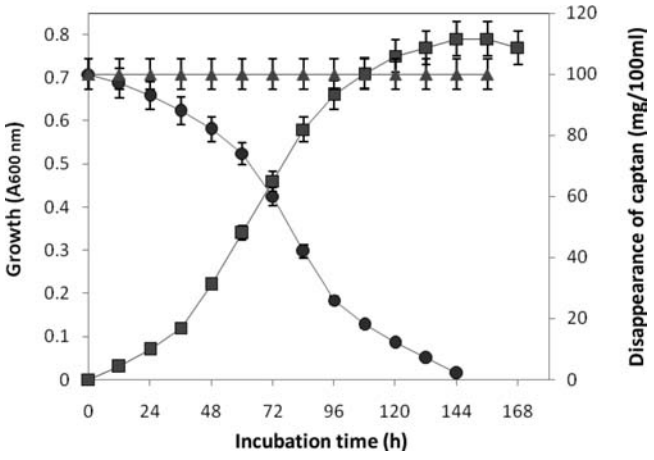

Figure 1. Utilization of fungicide captan (●) during growth (■) of *B. circulans*. Uninoculated controls (▲) in the mineral salt medium containing fungicide captan (0.1%). Error bars represent the deviation calculated from the mean of triplicates.

Enzyme Assays. Cell-free extracts were prepared from the washed cells suspended in three volumes of 0.05 M phosphate buffer, pH 7.0, by sonication (ultrasonic processor model XL2010) for 5 min and centrifugation at 12000*g* for 40 min at 4 °C. The clear supernatant was used as crude extract for enzyme assays.

Captan hydrolase activity was assayed colorimetrically by measurement of hydrolysis of captan. The reaction mixture (1 mL) containing 0.01 M phosphate buffer, pH 6.0 (0.6 mL), 1 μM captan (0.2 mL), and cell-free extract (0.2 mL) incubated at 30 °C for 10 min. The reaction was stopped by the addition of 0.2 mL of 50% trichloroacetic acid (TCA) and centrifuged. The amount of captan hydrolyzed was estimated according to the method of Bhattacharjee and Gupta (*30*). *cis*-1,2,3,6-Tetrahydrophthalimide hydrolase activity was determined spectrophotometrically by measuring the disappearance of *cis*-1,2,3,6-tetrahydrophthalimide at 245 nm. Protocatechuate 2,3-dioxygenase activity was assayed according to Crawford (*31*). Protocatechuate 3,4-dioxygenase activity was assayed according to Fujisawa and Hayaishi (*32*). Protocatechuate 4,5-dioxygenase activity was assayed according to Dagley et al. (*33*). The protein was determined by the method of Lowry et al. (*34*) using bovine serum albumin (BSA) as the standard. One unit of enzyme activity was defined as the amount required to catalyze the formation or consumption of 1 μmol of product or substrate per minute under the specified assay conditions. Specific activities were expressed as units per milligram of protein.

Statistical Analysis. Experiments were carried out in triplicate. All results are presented as mean ± standard deviation (SD). The level of statistical significance (95%) was determined by analysis of variances using one-way ANOVA (SPSS 7.5 version) followed by Duncan's multiple range test (*35*) to determine the significant differences among the means.

RESULTS

Utilization of Captan and Other Compounds. *B. circulans* utilized the fungicide captan as sole source of carbon and energy for its growth. The growth of *B. circulans* in the mineral salt medium (MSM) containing 0.1% captan as sole carbon source is shown in **Figure 1**. There was complete utilization of captan at 1 g/L by the organism within 6 days of growth. The cells of *B. circulans* grown on captan utilized *cis*-1,2,3,6-tetrahydrophthalimide, *cis*-1,2,3,6-trahydrophthalamidic acid, *o*-phthalic acid, and protocatechuic acid as carbon source.

Identification of Metabolites of Captan Degradation. The analysis of the 24-h-old culture filtrate of *B. circulans* grown on captan by thin-layer chromatography (TLC) revealed the presence of a single compound **I**, and analysis of 72 h culture filtrate showed three compounds (**II**, **III**, and **IV**) whose R_f values corresponded well with those of authentic compounds (**Table 1**). These metabolites were purified by preparative TLC and analyzed by HPLC. The mass spectrum of isolated compound **I** (**Figure 2**) showed a molecular peak M^+ at *m*/*z* 151 with empirical formula $C_8H_9NO_2$. The fragmentation pattern showed a base peak at *m*/*z* 123 ($M^+ - CO$), *m*/*z* 136 ($M^+ - NH$), and the base ion peak at *m*/*z* 79.

Table 1. Chromatographic and Spectral Properties of Metabolites of Captan by *B. circulans*

property	isolated compd **I**	authentic *cis*-1,2,3,6-tetrahydrophthalimide	isolated compd **II**	authentic *cis*-1,2,3,6-tetrahydrophthalamidic acid	isolated compd **III**	authentic *o*-phthalic acid	isolated compd **IV**	authentic protocatechuic acid
TLC: R_f values in different solvents[a]								
A	0.66	0.66	0.71	0.71	0.47	0.47	0.31	0.31
B	0.35	0.35	0.25	0.25	0.13	0.13	0.10	0.10
C	0.56	0.56	0.39	0.39	0.30	0.30	0.25	0.25
HPLC:[b] retention time (min)	1.99	1.99	1.95	1.95	2.06	2.06	2.17	2.17
UV absorption λ_{max} in methanol (nm)	223, 245	223, 245	232, 264	232, 264	225, 275	225, 275	250, 290	250, 290

[a] Solvent systems A, B, and C are as described in Materials and Methods. [b] HPLC, high-performance liquid chromatography.

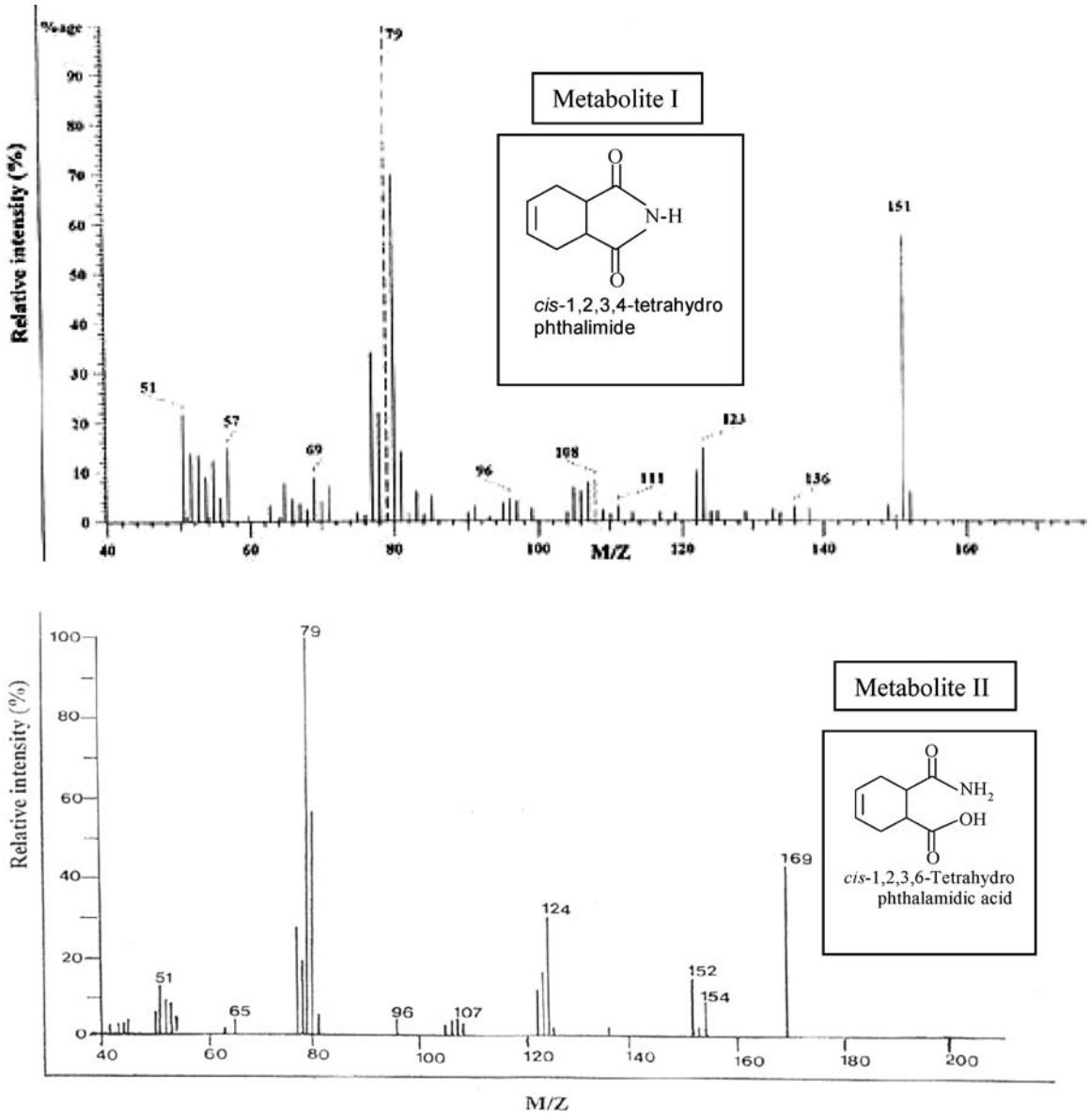

Figure 2. Mass spectra of isolated metabolites **I** and **II** of captan degradation by *B. circulans*.

The infrared spectrum of compound **I** showed characteristic absorption bands of −NH stretching at 3197 cm^{-1}, −CH stretching at 2916 cm^{-1}, and C═O stretching at 1706 cm^{-1}. The proton magnetic resonance spectrum of compound **I** showed −NH proton appeared at δ 8.8 ppm, two $-CH_2$ protons at δ 3.1 ppm, two −CH protons at δ 2.6 ppm, and two protons at δ 2.2 ppm. These spectral data (mass, IR, and NMR) of the isolated compound **I** were found to be identical to that of authentic *cis*-1,2,3,6-tetrahydrophthalimide.

The mass spectrum of isolated compound **II** (**Figure 2**) showed a molecular peak M^+ at m/z 169 with empirical formula $C_8H_{11}NO_3$. The fragmentation pattern showed the base peak at m/z 154 ($M^+ - NH_2$), m/z 152 ($M^+ - OH$), and the base peak at m/z 79. The infrared spectrum of compound **II** showed characteristic absorption bands of −OH stretching at 3221 cm^{-1}, −NH stretching at 3085 cm^{-1}, −CH stretching at 2937 cm^{-1}, and C═O stretching at 1909 cm^{-1}. The proton magnetic resonance spectrum of compound **II** showed acidic −OH proton appeared at δ 11.25 ppm, $-NH_2$ proton appeared at δ 8.1 ppm, two $-CH_2$ protons appeared at δ 3.1 ppm, two −CH protons at δ 2.5 ppm, and two protons at δ 2.2 ppm. These spectral data (mass, IR, and NMR) of the isolated compound **II** were found to be identical to that of authentic *cis*-1,2,3,6-tetrahydrophthalamidic acid. The mass spectrum of isolated compound **III** (**Figure 3**) showed a molecular peak M^+ at m/z 166 with empirical formula $C_8H_8O_4$. The fragmentation pattern showed the base peak at m/z 149 ($M^+ - OH$), m/z 121 ($M^+ - COOH$), and base peak at 76. The infrared spectrum of compound **III** showed characteristic absorption of −OH stretching at 3086 cm^{-1}, C═O stretching at 1609 cm^{-1}, and aromatic −CH stretching at 2894 cm^{-1}. The proton magnetic resonance spectrum of compound **III** showed four protons of aromatic ring appeared at δ 7.7 ppm and acidic hydroxyl proton appeared as one singlet at δ 13.1 ppm. These spectral data (mass, IR, and NMR) of the isolated compound **III** were found to be identical to that of authentic *o*-phthalic acid.

The mass spectrum of isolated compound **IV** (**Figure 3**) showed a molecular peak M^+ at m/z 154 with empirical formula $C_7H_6O_4$. The fragmentation pattern showed a base peak at m/z 137 ($M^+ -$ OH). The other important fragments observed are m/z 119 ($M^+ -$ 2OH) and m/z 109 ($M^+ -$ COOH). The infrared spectrum of compound **IV** showed characteristic absorption of −OH stretching at 3376 cm^{-1}, C═O stretching at 1678 cm^{-1}, C−O stretching at 1250 cm^{-1}, and C═C stretching at 1659 cm^{-1}. The proton magnetic resonance spectrum of compound **IV** showed acidic

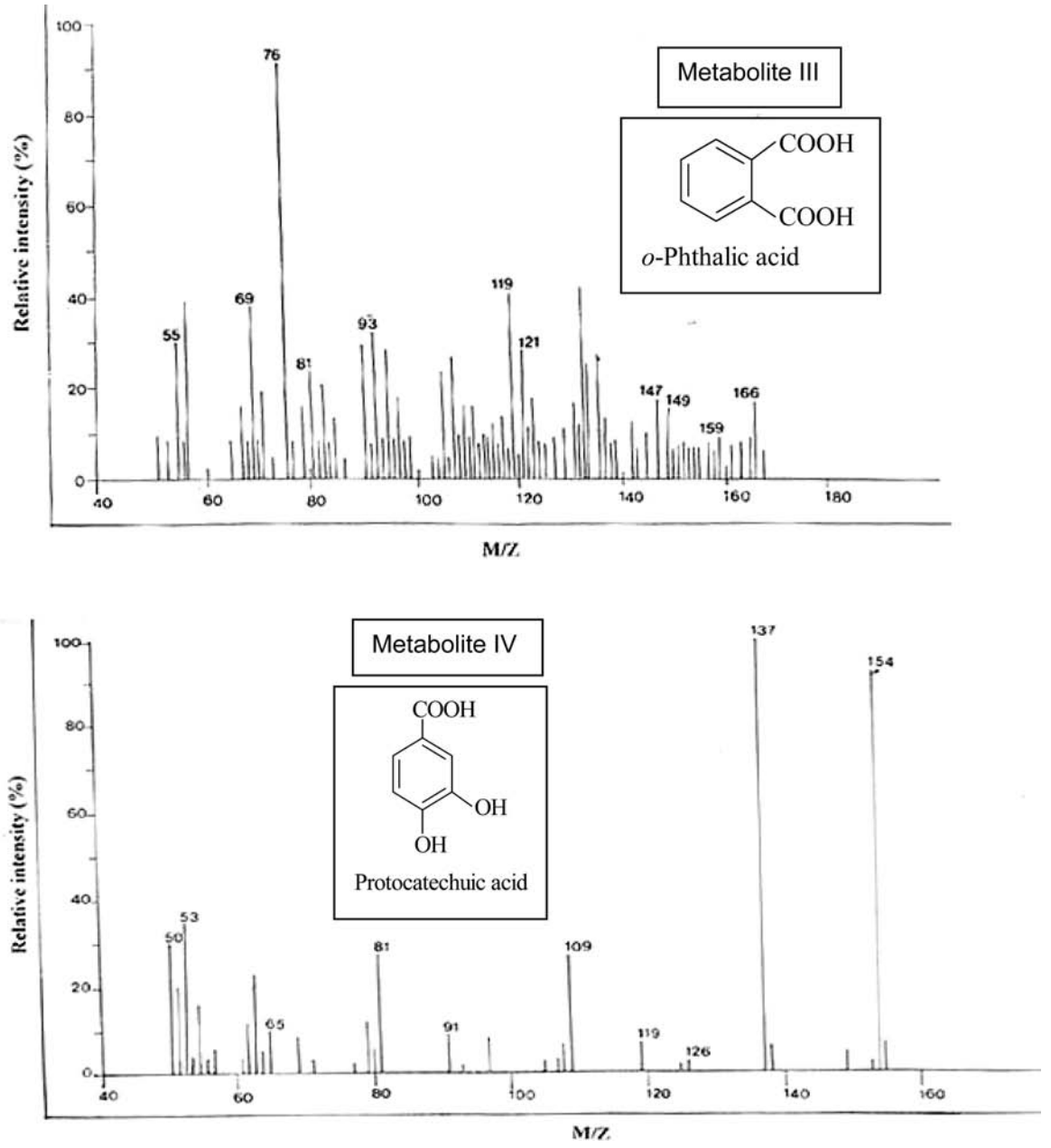

Figure 3. Mass spectra of isolated metabolites **III** and **IV** of captan degradation by *Bacillus circulans*.

Table 2. Oxidation of Various Compounds by Cells of *B. circulans* Grown on Captan

substrate (1 μmol)	oxygen uptake[a] (nmol min^{-1} (mg of dry cells)$^{-1}$)
captan	16.53
cis-1,2,3,6-tetrahydrophthalimide	21.61
cis-1,2,3,6-tetrahydrophthalamidic acid	20.08
o-phthalic acid	20.00
protocatechuic acid	21.00
salicylic acid	
gentisic acid	

[a] The values are corrected for endogenous respiration rates.

proton appeared as a singlet at δ 12.0 ppm, and hydroxyl proton appeared as a singlet at δ 9.5 ppm. Three protons of aromatic ring appeared as multiplet ranging from δ 6.5 to 7.5 ppm. These spectral data (mass, IR, and NMR) of the isolated compound **IV** were found to be identical to that of authentic protocatechuic acid. There was a release of 3.4 mM chloride ions in the culture supernatants during the degradation of captan after 3 days of incubation. The culture supernatant of the organism grown on captan gave positive tests for the presence of chloride, H_2S, and CO_2. The decrease in pH of the culture filtrate to 5.5 indicated the formation of HCl.

Oxidation of Metabolites by Whole Cells. The whole cells of *B. circulans* grown on captan readily oxidized *cis*-1,2,3,6-tetrahydrophthalimide, *cis*-1,2,3,6-tetrahydrophthalamidic acid, *o*-phthalic acid, and protocatechuic acid. But glucose-grown cells failed to oxidize any of these compounds, which indicated that the oxidation of these probable intermediates was induced in the organism by growth on captan but not on glucose. On the other hand, compounds such as salicylic acid and gentisic acid were not oxidized by the organism (**Table 2**). These results suggest that the organism degrades captan through *o*-phthalate by a protocatechuate pathway.

Enzyme Activities in Cell-Free Extracts. The cell-free extracts of the *B. circulans* grown on captan contained the activities of captan hydrolase, *cis*-1,2,3,6-tetrahydrophthalimide hydrolase,

Table 3. Specific Activities of Enzymes in the Cell-Free Extract of *B. circulans* Grown on Captan

enzyme	specific activity (units/mg of protein)
captan hydrolase	0.22[a] ± 0.01
cis-1,2,3,6-tetrahydrophthalimide hydrolase	0.34[a] ± 0.01
protocatechuate 3,4-dioxygenase	0.52[a] ± 0.02
protocatechuate 4,5-dioxygenase	0.00 ± 0.00

[a] Specific activities of enzymes are the mean ± SE of assays from triplicate cell-free extracts.

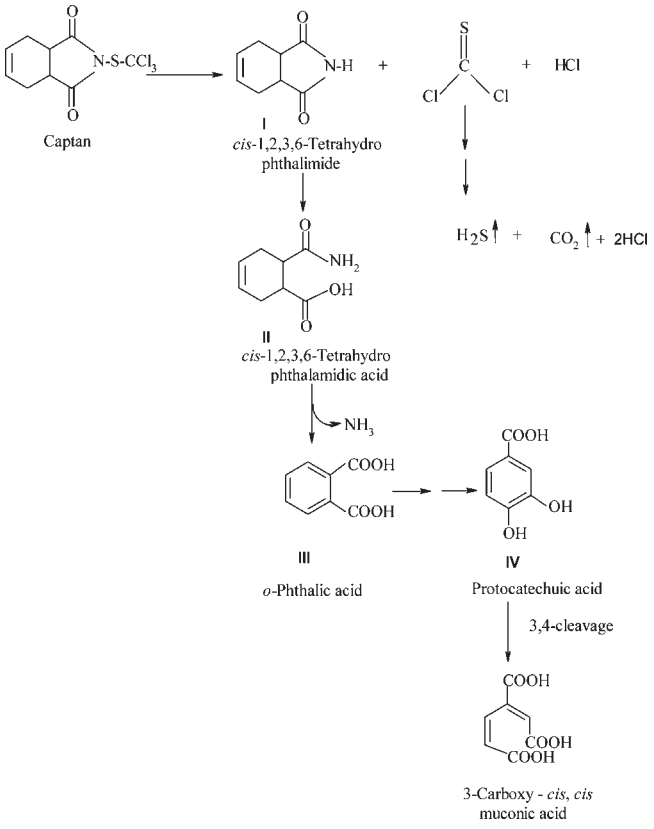

Figure 4. Proposed pathway for the degradation of fungicide captan by *B. circulans*.

and protocatechuate 3,4-dioxygenase (**Table 3**). However, *o*-phthalic acid oxidase activity could not be detected in the cell-free extract probably because of its instability (*36*−*39*). The cell-free extracts of glucose-grown cells did not contain these enzyme activities. These results have indicated that the enzymes of captan degradation were induced by growth of the organism on captan.

DISCUSSION

The bacterial strain *B. circulans* degraded the fungicide captan by initial hydrolysis to yield 1,2,3,6-tetrahydrophthalimide, which resulted in the loss of its fungicidal activity. The other products of captan hydrolysis were detected to be H_2S, CO_2, and HCl. There are reports of formation of 1,2,3,6-tetrahydrophthalimide by the hydrolysis of captan in water (*40*) and from transformation of captan by soil microbes (*25*). But the further degradation of its hydrolyzed product by microorganisms has not been studied. However, *cis*-1,2,3,6-tetrahydrophthalimide was further metabolized by the *B. circulans* with the formation of intermediates *cis*-1,2,3,6-tetrahydrophthalamidic acid, *o*-phthalic acid, and protocatechuic acid. The presence of high activities of protocatechuate 3,4-dioxygenase in the cell-free extract of *B. circulans* grown on captan suggested that protocatechuic acid was further oxidized through *ortho*-cleavage pathway. Thus there was complete degradation of fungicide captan by *B. circulans* as shown in **Figure 4**. Such bacterial strains could be potentially useful in the bioremediation of soils contaminated with toxic phthalimide fungicides, which may have adverse effects on soil microflora and especially microorganisms involved in nitrogen cycling of agricultural soils.

LITERATURE CITED

(1) Lukens, R. J.; Sisler, H. D. Chemical reactions involved in the fungitoxicity of captan. *Phytopathology* **1958**, *52*, 94.
(2) Schwack, W.; Flober-Muller, H. Fungicides and photochemistry. Photodehalogenation of captan. *Chemosphere* **1990**, *21* (7), 905−912.
(3) Gilvydis, D. M.; Walters, S. M. Gas chromatographic determination of captan, folpet, and captafol residues in tomatoes, cucumbers, and apples using a wide-bore capillary column: interlaboratory study *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 830−835.
(4) Elliot, G. Captan: Transition from "B2" to "not likely". How pesticide registrants affected the EPA Cancer Classification Update. *J. Appl. Toxicol.* **2007**, *27* (5), 519−526.
(5) U.S. Environmental Protection Agency. Registration Eligibility Decision (RED): Captan; EPA: Washington, DC, 2009; http://www.epa.gov/oppsrrdl/REDs/0120red.pdf (accessed Dec 15, 2009).
(6) Barbera, C. *Pesticidas agricolas*. Omega. Barcelona. 1989, 259−265.
(7) Anonymous. A carcinogenesis bioassay of captan for possible carcinogenicity. CAS No. 133-06-NCI-CG-TR-15. National Cancer Institute Technical Report. Series 15, NCI, Bethesda, MD, USA, **1977**.
(8) Rawn, D. F. K.; Quade, S. C.; Sun, W. −F.; Fouguet, A.; Belanger, A.; Smith, M. Captan residue reduction in apples as a result of rinsing and peeling. *Food Chem.* **2008**, *109* (4), 790−796.
(9) Spero, J. M.; Devito, B.; Theodore, L. *Regulatory Chemicals Handbook*; Dekker: New York, 2000.
(10) Chen, K.; Mackie, J. C.; Kennedy, E. M.; Dlugogorski, B. Z. Thermal decomposition of captan and formation pathways of toxic air pollutants. *Environ. Sci. Technol.* **2010**, *44*, 4149−4154.
(11) Edwards, I. R.; Ferry, D. G.; Temple, W. A. Fungicides and Related Compounds. In *Handbook of Pesticide Toxicology*; Hayes, W. S., Jr., Laws, E. R., Jr., Eds.; Academic Press: New York, 1991; pp 1410−1415.
(12) Gordon, E. B. Captan and Folpet. In *Handbook of Pesticide Toxicology: Vol. 2, Agents*, 2nd ed.; Kreiger, R., Ed.; Academic Press: San Diego, CA, 2001; pp 1711−1742.
(13) *The Pesticide Manual: A World Compendium*, 12th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Farnham, U.K., 2000; pp 132−133.
(14) Hines, C. J.; Deddens, J. A.; Jaycox, L. B.; Andrews, R. N.; Striley, C. A. F.; Alavanja, M. C. R. Captan exposure and evaluation of a pesticide exposure algorithm among orchard pesticide applicators in the agricultural health study. *Ann. Occup. Hyg.* **2008**, *52* (3), 267−278.
(15) Kada, T.; Moriya, M.; Shirasu, Y. Screening of pesticides for DNA interactions by rec-assay and mutagenesis testing and frameshift mutagens detected. *Mutat. Res.* **1974**, *26*, 243−248.
(16) Roberts, L. Pesticides and kids. *Science* **1989**, *243*, 1280.
(17) Rahden-Staron, I.; Szumilo, M.; Ziemkiewicz, P. The effect of captan and captafol on different bacterial strains and on c-mitosis in V79 Chinese hamster fibroblasts. *Acta Biochim. Pol.* **1994**, *41*, 45−55.
(18) Shirasu, Y.; Moriya, M.; Kato, K.; Furuhashi, A.; Kada, T. Mutagenicity screening of pesticides in the microbial system. *Mutat. Res.* **1976**, *40*, 19−30.
(19) Agnihotri, V. P. Persistance of captan and its effect on microflora, respiration and nitrification of forest nursery soil. *Can. J. Microbiol.* **1971**, *17*, 377−383.
(20) Banerjee, A.; Banerjee, A. K. Influence of captan on some microorganisms and microbial processes related to the nitrogen cycle. *Plant Soil*. **1987**, *102*, 239−245.
(21) Curley, R. L.; Burton, J. C. Compatibility of *Rhizobium japonicum* with chemical seed protectants. *Agron. J.* **1975**, *67*, 807−808.
(22) Gallori, E.; Bazzicalupo, M. Effect of nitrogen compounds on nitrogenase activity in *Azospirillum brasilense*. *FEMS Microbiol. Lett.* **1985**, *28*, 35−38.
(23) Martinez-Toledo, M. V.; Salmeron, B.; Rodelas, C.; Pozo, J. Gonzalez-Lopez. Effects of fungicide captan on some functional groups of soil microflora. *Appl. Soil Ecol.* **1998**, *7*, 245−255.

(24) Food and Agriculture Organization of the United Nations. Feeding the world: sustainable management of natural resources. Environmental Assessment and Management Unit (NRCE), 2008, Rome.
(25) Buyanovsky, G. A.; Pieczonka, G. J.; Wagner, G. H.; Fairchild, M. A. Degradation of captan under laboratory conditions. *Bull. Environ. Contam. Toxicol.* **1988**, *40*, 689−695.
(26) Megadi, V. B.; Tallur, P. N.; Hoskeri, R. S.; Mulla, S. I.; Ninnekar, H. Z. Biodegradation of pendimethalin by *Bacillus circulans*. *Indian J. Biotechnol.* **2010**, *9*, 173−177.
(27) Arnow, L. E. Colorimetric determination of the components of 3,4-dihydroxyphenyl alaninetyrosine mixtures. *J. Biol. Chem.* **1937**, *118*, 531−537.
(28) Bergmann, J. G.; Sanik, J. Determination of trace amounts of chloride in naphtha. *Anal. Chem.* **1957**, *29*, 241−243.
(29) Svehla, G. *Vogel's qualitative inorganic analysis*, 7th ed.; 1996. Longman group limited.
(30) Bhattacharjee, M.; Gupta, V. K. A new reagent for the detection of captan (N-trichloromethylthiotetrahydropthalimide) pesticide *J. Indian Chem. Soc.* **1995**, *72*, 47−148.
(31) Crawford, R. L. Novel pathway for the degradation of protocatechuic acid in *Bacillus* species. *J. Bacteriol.* **1975**, *121*, 531−536.
(32) Fujisawa, H.; Hayaishi, O. Protocatechuate-3,4-dioxygenase, I. Crystalization and characterization. *J. Biol. Chem.* **1968**, *243*, 2673−2681.
(33) Dagley, S.; Geary, P. T.; Wood, J. M. The metabolism of protocatechuate by *Pseudomonas testoteroni*. *Biochem. J.* **1968**, *109*, 559−568.
(34) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(35) Duncan, D. M. Multiple range & multiple tests. *Biometrics* **1955**, *42*, 1−42.
(36) Doddamani, H. P.; Ninnekar, H. Z. Biodegradation of phenanthrene by a *Bacillus* species. *Curr. Microbiol.* **2000**, *41* (1), 11−14.
(37) Engelhardt, G.; Wallnofer, P. R.; Rast, H. G. Metabolism of o-phthalic acid by different gram-negative and gram-positive soil bacteria. *Arch. Microbiol.* **1976**, *109*, 109−114.
(38) Gosh, D. K.; Mishra, A. K. Oxidation of phenanthrene by a strain of *Micrococcus*: evidence of protocatechuate pathway. *Curr. Microbiol.* **1983**, *9*, 219−224.
(39) Herwijnen, R. V.; Springael, D.; Slot, P.; Govers, H. A. J.; Parsons, J. R. Degradation of anthracene by *Mycobacterium* sp. strain LB 501T proceeds via a novel pathway through *o*-phthalic acid. *Appl. Environ. Microbiol.* **2003**, *69*, 186−190.
(40) Wolfe, N. L.; Zepp, R. G.; Doster, J. C.; Hollis, R. C. Captan hydrolysis. *J. Agric. Food Chem.* **1976**, *24* (5), 1041−1045.

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