We investigated the degradation pathways and kinetics of 2,4-dichlorophenol (DCP) by an endemic soil fungus, Mortierella sp. (Zygomycetes). Mortierella sp. degraded 32% of added DCP (final concentration, 250 μM) within 1 h. We identified four aromatic metabolites and found two DCP degradation pathways (a hydroxylation pathway and a dechlorination pathway). This is the first report of a dechlorination pathway in Zygomycetes.

Key words: dechlorination; 2,4-dichlorophenol; degradation of 2,4-dichlorophenol; Mortierella sp.

Chlorophenols have been used as fungicides, herbicides, insecticides, and precursors in the synthesis of other herbicides.1) Because of their abundance, toxicity, and tendency to persist in the environment, many chlorophenols have been classified as important pollutants by the U.S. Environmental Protection Agency.2) Chlorophenols have been a serious worldwide problem for decades.

Some bacteria and fungi are able to degrade chlorophenols.3–6) The degradation pathways of chlorophenols have been clarified for several bacteria and higher fungi (Basidiomycetes and Deuteromycetes),3–5) but not for the lower fungi, Zygomycetes.6) We used the edaphic soil fungus Mortierella sp. (Zygomycetes) and tested its ability to degrade 2,4-dichlorophenol (DCP) as a model substrate. The DCP degradation pathways and its kinetics in Mortierella sp. were also investigated.

Mortierella sp. (FERM p-17687) was isolated from forest soil in Innoshima City, Hiroshima Prefecture, Western Japan.7) The strain was maintained at room temperature on 3.9% (w/v) potato dextrose agar medium (Becton Dickinson, Franklin Lakes, NJ) by subculturing at 3-month intervals. In the degradation experiments, Mortierella sp. was cultured in 200-ml Erlenmeyer flasks supplemented with 30 ml of 2.4% (w/v) potato dextrose broth medium (Becton Dickinson) on a reciprocal shaker at 120 rpm at 25 °C for 3 d. Then DCP dissolved in acetone was added to cultures to a final concentration of 250 μM. After an additional incubation of 0, 1, 6, 12, 24, or 48 h, the products were isolated as described below. Cultures were acidified with 0.4 ml of 1 N H₂SO₄ to pH 2, and then mycelia were separated from the culture medium by filtration. The mycelia were suspended in 50 ml of water and homogenized in a Waring Blender. The homogenized mycelia and the extracellular medium were individually extracted three times with ethyl acetate (50 ml each time), dehydrated with NaHSO₄, and dried under reduced pressure. The products were dissolved in MeOH (1 ml) and analyzed chromatographically. Gas chromatography–mass spectroscopy (GC–MS) was performed at 70 eV on an HP 5973 mass spectrometer (Hewlett Packard, Palo Alto, CA) linked to an HP 6890 gas chromatograph (Hewlett Packard) with a 30-m fused-silica column (HP-5 MS; Agilent Technologies, Palo Alto, CA). The GC oven temperature was programmed to increase from 50 °C to 320 °C at 10 °C/min. Chlorinated products were also detected by gas chromatography with an electron capture detector (GC–ECD, Varian 3300) (Varian, Palo Alto, CA) with the 30-m fused-silica column (see above). The products were identified by comparing their retention times and their mass spectra with authentic standards. DCP and the products were quantified by high performance liquid chromatography (HPLC) with an ODS column (Wako 5C18; 250 × 4.6-mm ID) (Wako Pure Chemical Industries, Osaka, Japan) using CH₂CN–H₂O–CH₃COOH (40:59:1) as the eluent.

In the non-fungal culture, DCP decreased gradually (reaching a 26% decrease after 48 h of incubation),
possibly as a result of evaporation. On the other hand, in the fungal culture, DCP decreased by more than 30% after 1 h and decreased by 55% after 48 h. In the fungal culture, most of the DCP was in the extracellular fraction. In the intracellular fraction, the DCP concentration increased from 0 to 13% of the initial concentration in the culture, and then decreased. In addition to the more rapid decrease in DCP in the fungal culture, four DCP-like compounds (chlorohydroquinone, CHQ [II]; 3,5-dichlorocatechol, 3,5-DCC [III]; 3,5-dichloroguaiacol, 3,5-DCG [IV]; and 4,6-dichloroguaiacol, 4,6-DCG [V]; Table 1) were identified only in the fungal culture by GC–MS and GC–ECD analyses. The structures of these compounds are shown in Fig. 2. These findings suggest that DCP was not bound to viscous materials secreted by the fungus, but was metabolized, as shown below.

The four metabolic products of DCP found in the fungal culture ([II], [III], [IV], and [V]) increased with time (Fig. 1). CHQ (II) in the extracellular fraction was detected at levels of about 15 µM after 6 h, increasing gradually up to 22 µM at 48 h, but was hardly detected in the intracellular fraction during cultivation (Fig. III). CHQ (II) was found to be metabolized to hydroquinone (HQ, [VI]) in a bacterium 3) and a higher fungus, 5) but HQ (VI) was not detected in the DCP-added fungal culture, possibly because the CHQ (II) concentration was too low. In a separate experiment, a higher concentration of CHQ (II) was added to a new fungal culture, and was found to decrease with an accompanying increase in HQ (VI) (data not shown), indicating that Mortierella sp. also metabolizes CHQ (II) to HQ (VI). Both extracellular and intracellular 3,5-DCC (III) increased up to about 35 µM and 1.5 µM until 12 h and slightly decreased with time (Fig. III). A small amount of 3,5-DCC (III) was also detected in the non-fungal culture, suggesting that it was present in the potato dextrose broth medium as an impurity. 3,5-DCG (IV) and 4,6-DCG (V) showed similar kinetics with gradual increases until 48 h (Fig. IV and V). Like DCP, all four metabolites were detected mainly in the extracellular fraction.

The composition of metabolites and their appearance during DCP degradation by Mortierella sp. suggest that the fungus uses two different degradation pathways (Fig. 2). In the first pathway, ortho-oxidation of DCP results in the formation of 3,5-DCC (III), which is then converted into two different dichloroguaiacols, 3,5-DCG (IV) and 4,6-DCG (V), depending on which of the two hydroxyl groups of 3,5-DCC (III) is methylated. This pathway is similar to that of Penicillium frequentans.4) The second pathway removes two chloride ions, forming CHQ (II) and HQ (VI). Here, DCP is oxidatively dechlorinated, resulting in CHQ (II), and then the remaining chloride ion is removed. As mentioned above, this pathway has been found in other microorganisms.3,5)

Our results indicate that an endemic fungus, Mortierella sp., can detoxify DCP in natural soil environments. This is the first report of DCP dechlorination by Zygomycetes.

Acknowledgments

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<tr>
<th>Metabolite</th>
<th>Mass spectrum m/z (relative intensity)</th>
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<tr>
<td>2,4-Dichlorophenol (I)</td>
<td>166(10), 164(63), 162(100), 126(10), 98(26), 73(8), 63(31)</td>
</tr>
<tr>
<td>Chlorohydroquinone (II)</td>
<td>146(33), 144(100), 80(31), 52(37)</td>
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<tr>
<td>3,5-Dichlorocatechol (III)</td>
<td>182(14), 180(63), 178(100), 160(1), 142(5), 114(34), 97(14), 79(19), 51(15)</td>
</tr>
<tr>
<td>3,5-Dichloroguaiacol (IV)</td>
<td>196(5), 194(39), 192(56), 181(11), 179(63), 177(100), 151(28), 149(41), 113(9), 85(12)</td>
</tr>
<tr>
<td>4,6-Dichloroguaiacol (V)</td>
<td>196(10), 194(39), 192(60), 181(13), 179(65), 177(100), 149(42), 113(12), 85(14)</td>
</tr>
<tr>
<td>Hydroquinone (VI)</td>
<td>110(100), 81(25), 53(16)</td>
</tr>
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Table 1. Mass Spectra of DCP and Five Metabolites Produced by Mortierella sp.
References


Fig. 2. Proposed DCP-Degradation Pathways of the Mortierella sp., Zygomyccete Fungus.