Biodegradation and Transformation of 4,4’- and 2,4-
Dihalodiphenyl Ethers by Sphingomonas sp. Strain SS33

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The bacterium Sphingomonas sp. strain SS33, obtained from parent diphenyl ether-mineralizing strain SS33
(S. Schmidt, R.-M. Wittich, D. Erdmann, H. Wilkes, W. Francke, and P. Fortnagel, Appl. Environ. Microbiol. 58:2744–2750, 1992) after several weeks of adaptation on 4,4’-difluorodiphenyl ether as the new
target compound, also utilized 4,4’-dichlorodiphenyl ether for growth. Intermediary halocatechols were also
mineralized via the ortho pathway by type I enzymes. 4,4’-Dibromodiphenyl ether was not used as a carbon
source although transformation by resting cells yielded mononuclear haloaromatic compounds, such as
4-bromophenol and 4-bromocatechol. The same was true for the conversion of 2,4-dichlorodiphenyl ether,
which yielded the respective (halo-) phenols and (halo-) catechols.

In recent years there has been interest in using polyhalo-
genated diphenyl ethers as chemicals to replace the polyhalo-
genated biphenyls formerly utilized as flame retardants. 4,4’-Dibromodiphenyl ether (44DFDE) is the lowest haloge-
nated commercial congener belonging to this class of com-
pounds of environmental concern. Hydroxy and/or nitro
derivatives of diphenyl ethers have also been produced in
bulk and have been used as pesticides in agriculture. During
the last few decades these compounds have been detected in
environmental samples and have entered the food chain (4,
8). They are subject to microbial and mammalian catabolism
depending on their structure (9, 10). In this paper we
describe the extension of the catabolic potential of Sphin-
gomonas sp. strain SS33 to dihalogenated diphenyl ethers.

The growth conditions used, the methods used for prepara-
tion of resting cells and extracts, oxygen uptake measure-
ments, enzyme assays, and analytical procedures, and the
chemicals used have been described previously (5, 7). 2,4-
Dichloro-4’-nitrodiphenyl ether (NITROFEN) was obtained
from Wako Chemicals GmbH, Neuss, Germany. 4,4’-Difu-
orodiphenyl ether (44DFDE) was obtained from Alfa Prod-
ucts and was supplied by Johnson Matthey, Karlsruhe,
Germany. 44DBDE was obtained from Aldrich Chemie,
Steinheim, Germany. A crude preparation of 2,4-dichlo-
rodiphenyl ether was kindly supplied by K. Figge, Natesc
Institute, Hamburg, Germany, and was purified by prepara-
tive high-performance liquid chromatography (HPLC). 4,4’-
Dichlorodiphenyl ether (44DCDE) was prepared from 4,4’-
diaminodiphenyl ether (Fluka GmbH, Neu-Ulm, Germany)
by chlorinating its diazonium salt, using the Sandmeyer reaction (2).

We were not successful in our attempts to screen directly
for potent bacterial strains capable of utilizing dihalogenated
diphenyl ethers for growth. However, we succeeded in
adapting Sphingomonas sp. strain SS3, an organism that
mineralizes diphenyl ether and several 4-halodiphenyl ethers,
to the utilization or transformation of some dihalogenated
diphenyl ethers; Sphingomonas sp. strain SS3 utilized diphe-
nyl ether, 4-fluorodiphenyl ether, and (to a considerably
lesser extent) 4-chlorodiphenyl ether for growth (7) but did
not grow at the expense of dihalogenated congeners. An
adaptation period of several weeks was needed to achieve
growth with 44DFDE; the doubling times of our adapted
strain, designated SS33, were about 19 h (Fig. 1) in the
systems that we used for growing the organisms, as described
previously (5, 7). During growth with 44DCDE the doubling
times were considerably greater (more than 40 h), and
44DBDE was not utilized at all. The levels of halide ions
released from 44DFDE and 44DCDE when endpoint determi-
nations were made were 91 and 93%, respectively; no bro-
mide ions were detected after conversion of 44DBDE. The
pesticides NITROFEN (2,4-dichloro-4’-nitrodiphenyl ether)
and TRICLOSAN (2,4,4’-trichloro-2-hydroxydiphenyl ether)
were not transformed by resting cells.

The addition of an excess of substrate to cell suspensions
maintained in Erlenmeyer flasks equipped with baffle plates
led to the accumulation of metabolites in the medium.
4-Fluorophenol and 4-fluorocatechol were produced from
44DFDE, whereas the conversion of 44DCDE yielded the
respective chloro derivatives, which were identified by
HPLC analysis by using authentic reference samples as
described previously (7). Although 44DBDE was not utilized
for growth, it was accessible to dioxygenolytic attack by
the first enzyme, as shown by the oxygen uptake rates of
resting cells pregrown with 44DFDE (Table 1) and the detection
and identification of catabolites; the respective bromo deriva-
tives were found as described above. Similar observations
were made during the conversion of 2,4-dichlorodiphenyl
erether. In this case phenol, 2,4-dichlorophenol, catechol,
and 3,5-dichlorocatechol were identified. A scheme for the
turnover of dihalogenated and possibly higher halogenated
diphenyl ethers is proposed in Fig. 2.

The results described above, together with those obtained
from oxygen uptake experiments (Table 1), clearly indicate
that the conversion of dihalogenated diphenyl ethers
strongly depends on steric effects, such as the bulkiness of
the halo substituents, rather than the degree of substitution
by halogens. Further conversion seemed to be restricted by

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FIG. 1. Growth of *Sphingomonas* sp. strain SS33 with 44DFDE as the carbon source. Experiments were performed in parallel sets of closed Erlenmeyer flasks containing 44DFDE dispersed in mineral salts medium. The flasks were inoculated from a just outgrown preculture ( inoculum, about 1%). Batches were worked up after the times indicated. The concentrations of 44DFDE were determined by HPLC. Growth of the culture (△) and consumption of 44DFDE (○) are plotted against time together with the results for controls grown in the absence of 44DFDE (●) and for experiments performed with heat-inactivated inocula (■).

The limited substrate range of phenol hydroxylase and catechol 1,2-dioxygenase, which in this study exhibited substantially the same activities as they did in parent strain SS3 (7) and therefore are not described here, and probably by the subsequent enzymes. Some explanations for the restricted biodegradation of halocatechols have been given previously by Dorn and Knackmuss (3).

Work is in progress to extend the substrate range (i.e., the specificity of the initial dioxygenase having an apparently rather low regio-selectivity [11] and representing the first bottleneck in the pathway for the turnover and utilization of the polyhalogenated substrates) by adaptation experiments under selective pressure in the presence of our target compounds and to achieve short generation times of the organism. Furthermore, we will produce hybrid strains that harbor the genes for production of the type II enzymes which are responsible for the turnover of halogenated substrates, such as a halophenol hydroxylase and the chlorocatechol 1,2-dioxygenase, and the subsequent enzymes contained in halophenol- and halocatechol-degrading strains (6) or in the 3,4,6-trichlorocatechol-mineralizing organism *Pseudomonas* sp. strain PS12/14 (5) in order to circumvent this bottleneck in the lower diphenyl ether pathway. Hybrid strains should offer a nice opportunity to mineralize higher halogenated diphenyl ethers without formation of critical intermediates and therefore should help avoid those problems that occur during the nonproductive co-oxidation of the structurally analogous polyhalogenated biphenyls (1).

We thank Dirk Erdmann (Institute of Organic Chemistry, University of Hamburg) for the purification of 44DCDE by preparative column chromatography and for supplying some noncommercial halocatechols, K. Scheibli (Ciba-Geigy AG, Basel, Switzerland) for a sample of 2,4,4′-trichloro-2′-hydroxydiphenyl ether (TRICLOSAN), and C. Adami for the preparation of photo prints.

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### Table 1. Relative oxygen uptake rates with halogenated diphenyl ethers by resting cells of *Sphingomonas* sp. strain SS33 pregrown with 44DFDE

<table>
<thead>
<tr>
<th>Assay substrate</th>
<th>Relative activity (%)</th>
</tr>
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<tbody>
<tr>
<td>Diphenyl ether</td>
<td>82</td>
</tr>
<tr>
<td>4-Fluorodiphenyl ether</td>
<td>123</td>
</tr>
<tr>
<td>4-Chlorodiphenyl ether</td>
<td>58</td>
</tr>
<tr>
<td>4-Bromodiphenyl ether</td>
<td>41</td>
</tr>
<tr>
<td>44DFDE</td>
<td>100</td>
</tr>
<tr>
<td>44DCDE</td>
<td>35</td>
</tr>
<tr>
<td>44DBDE</td>
<td>15</td>
</tr>
<tr>
<td>2,4-Dichlorodiphenyl ether</td>
<td>18</td>
</tr>
</tbody>
</table>

*Results are the means of at least three independently performed experiments.* The specific rate for the oxidation of 44DFDE was 127 nmol of O₂ per min per mg of protein.

### References


5. Sander, P., R.-M. Wittich, P. Fortnagel, H. Wilkes, and W. Francke. 1991. Degradation of 1,2,4-trichloro- and 1,2,4,5-tetra-


