Degradation of dioxin like compounds by the yeast Trichosporon beigelii

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Polychlorinated and polybrominated dioxins, dibenzofurans and diphenyl ethers are environmental pollutants of high toxicity[1].
The halogens and the ether linkage cause the persistence of those compounds and the resistance to microbial attack. In most cases, model compounds are used to search for microorganisms with the ability to break down this class of pollutants and to understand the mechanisms, which are involved in the degradation process. Information about the role of bacteria is available for degradation of dibenzofuran[2,3]. But there are only a few reports on the hydroxylation of diaryl compounds by yeasts or filamentous fungi[4].
The yeast Trichosporon beigelii SBUG 752 is able to oxidize several aromatic and diaryl compounds. The metabolism of diphenyl ether was investigated in detail[5].

Materials and methods

The yeast isolated from soil (Greifswald, Germany) polluted with car exhaust was incubated with the substrates after growth on a glucose-medium.
Manometric and polarographic methods we used for oxidation measurements. After optimization of the substrate concentration for the added compounds the specific oxygen uptake rates were calculated as differences to endogenous respiration.
For identification of metabolites, the data from HPLC, GC-MS, UV and NMR spectroscopy were evaluated.

Results

To test the ability of the yeast Trichosporon beigelii to oxidize different aromatic and diaryl compounds, the oxygen uptake by resting cells was measured (see fig. 1).
The accumulation of metabolites during incubation of yeast cells with diphenyl ether was observed by HPLC (see fig. 2).

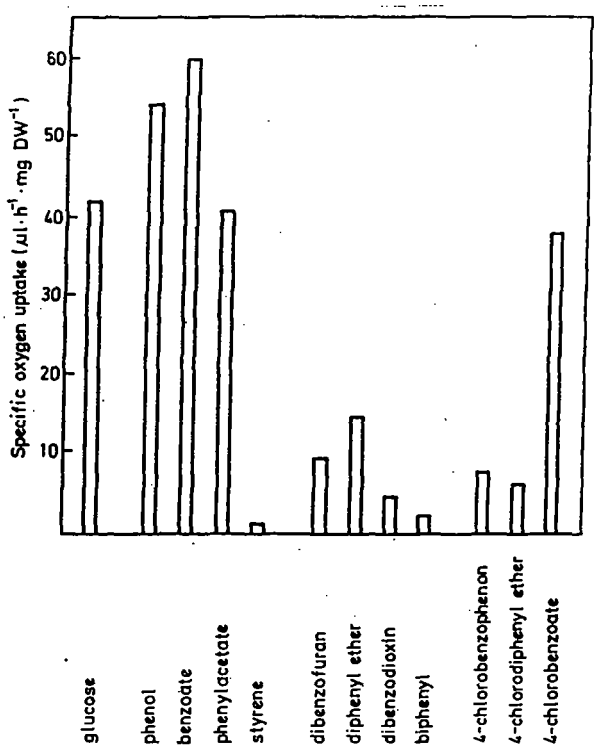

Fig. 1: Specific oxygen uptake (µl O_2 . h^{-1} . mg^{-1} dry weight) of resting cells of *Trichosporon beigelii* pregrown on glucose during incubation with different substrates (average values of a 3 h incubation period).

Besides the characteristic retention time, information about the compounds was given by in situ UV spectra (for the acid product see fig. 3). Further data were available after extraction at pH 5,5 and pH 2 of the incubation medium and methylation of the extracts. After comparison of UV-, HPLC-, and GC-data with available standards, 2-, 3- and 4-hydroxydiphenyl ether (OH-DPE) have been identified in the neutral extracts. The peak in the HPLC chromatogram at t_R = 4,0 min (see fig. 2) was identified as 3,4-dihydroxydiphenyl ether by comparison with the synthesized authentic compound.
The main compound in the acid extract showed a mass spectrum (see fig. 3) with a molecular ion peak at m/z 246 and diagnostically important ion peaks at m/z 218 (M-CO), 187 (M-$COOCH_3$), 159 ($HCOOCH_3$-CO), 93 (C_6H_5O), 77 (C_6H_5) and 59 ($COOCH_3$). Preparative HPLC was necessary for separation of the compound to get a NMR spectrum of this polar product. According to all data, 6-carboxy-4-phenoxy-2-pyrone (the lactone of 2-hydroxy-4-phenoxymuconic acid) has been identified.

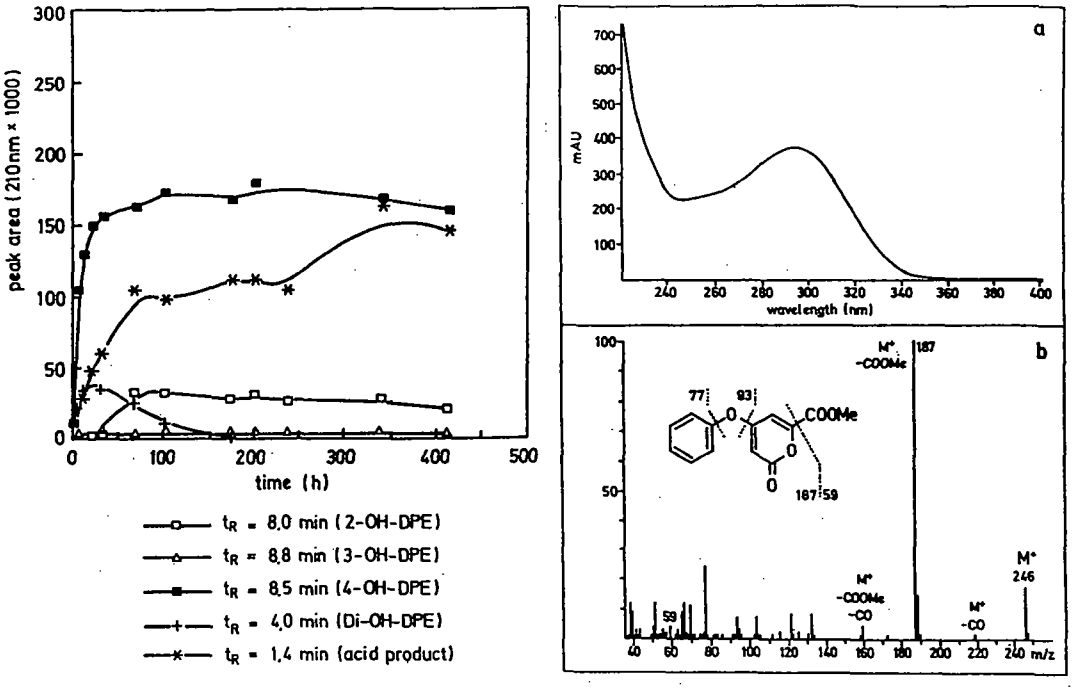

Fig. 2: (left) Formation of metabolites during incubation of Trichosporon beigelii SBUG 752 with diphenyl ether. Glucose grown cells were incubated in 50 ml mineral salts solution (OD_{600} = 2,0) with 3 µl diphenyl ether (DPE) as the sole carbon source.

Fig. 3: (right). In situ UV-spectrum (a) and mass spectrum after methylation (b) of the metabolite at t_R = 1,4 min in HPLC, 6-carboxy-4-phenoxy-2-pyrone

Conclusions

In accordance with the identification of several metabolites, we propose the following degradation pathway (see fig. 4).

The hydroxylation occurs at all three positions. After a second hydroxylation, ring cleavage occurs to yield 6-carboxy-4-phenoxy-2-pyrone.

This is to our knowledge the first report on ring cleavage of a biaryl ether compound by a yeast. Because an m-cleavage is not reported for yeasts, the pathway via the trihydroxylated intermediate seems more likely.

TRANS

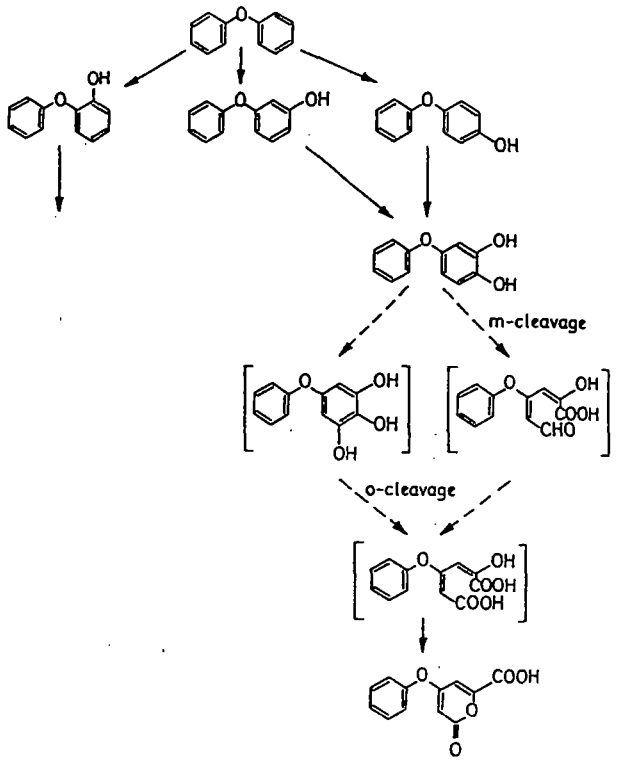

Fig. 4: Proposed pathway for the oxidation of diphenyl ether by the yeast Trichosporon beigelii SBUG 752

References

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