Microbial Reduction of Aromatic Carboxylic Acids

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Several benzoic, cinnamic and phenylacetic acid derivatives were screened with 20 microorganisms, mainly fungi, for the reduction of their carboxylic function. For all organisms several compounds were reduced in fairly good yields up to 80% to the corresponding alcohol. No general rule could be established, concerning the substitution pattern, as to which compounds were transformed to the alcohol. Generally the reactions were accomplished within 48–70 h. Only minor, if any, side products were detected. Dicarboxylic acids, such as phthalic or phenylglutaric acids and similar compounds could not be reduced by the microorganisms tested.

Introduction

During the last decade much literature has accumulated on the reduction of ketones, ketoesters and related substances with bakers yeast ([1], and ref. cited herein), in contrast that for the reduction of carboxylic acids is sparse. During the course of our investigations on aromatic carboxylic acids [2], several microorganisms were selected as they were able to reduce these acids to corresponding alcohols. Based on this observation, further derivatives of several aromatic carboxylic acids varying in side chain and substitution pattern of the phenyl moiety were incubated with a limited number of strains, mainly fungi, to elucidate their substrate specificity and reducing power. The capacity and specificity of several fungi to reduce aromatic carboxylic acids to their respective alcohols is presented here.

Materials and Methods

All microorganisms, from various culture collections (DSM, ATCC, CBS, IFO), were kept as agar slants at 4 °C or frozen in liquid nitrogen. Growth medium was: 10 g glucose, 20 g malt extract, 10 g peptone, 3 g yeast extract in 1 l water, final pH 5.7; for transformation reactions the medium was composed of 5 g glucose, 2 g peptone, 5 g malt extract, 1 g yeast extract in 1 l water, pH 5.7. Precultures and analytical runs were performed in 100 ml Erlenmeyer flasks containing 20 ml medium, preparative scale experiments were performed in 1000 ml Erlenmeyer flasks containing 200 ml medium. The substrate concentrations were 0.5 mg/ml in each case. The reactions were followed by analytical HPTLC developed with dichloromethane/methanol (9/1 v/v). Scaled up fermentations were performed using a stirred fermenter (B 10, Giovanola) with an actual working volume of 10 l medium (100 rpm, 0.1 vvm air). Chemicals were of highest purity available. All operations were exactly performed as described previously [2].

Products were isolated by extracting culture broth and mycelia three times with ethyl acetate (broth/solvent 3:1 v/v). After solvent evaporation the crude extract was fractionated on a Si-60 column (Lobar, Merck) by eluting with a gradient of *n*-hexane/ethyl acetate changing from 9/1 to 1/1. When necessary the pooled fractions were further purified by preparative TLC. Instruments used: NMR: The ^{1}H NMR spectra were recorded at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 spectrometer; $CDCl_3$ was used as solvent and TMS as internal standard. Mass spectra were recorded on an AEI 902S mass spectrometer with 70 eV.

Fermentation of 200 mg of 3-methoxybenzoic acid (**2**) with *Aspergillus niger ATCC 9142* yielded after 24 h 107 mg of 3-methoxybenzyl alcohol [6971-51-3] (**2a**), the same amount of acid was converted after 70 h by *Corynespora melonis IFO 7483* into 105 mg of alcohol.

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200 mg of 4-methoxybenzoic acid (**3**) was transformed by *Corynespora melonis IFO 7483* within 46 h to 95 mg of 4-methoxybenzyl alcohol [105-13-5] (**3a**).

Coriolus hirsutus IFO 4917 needed 70 h to convert 200 mg of 3,4,5-trimethoxybenzoic acid (**4**) to 110 mg of 3,4,5-trimethoxybenzyl alcohol [3840-31-1] (**4a**).

After a 48 h contact of 200 mg of 3-nitrobenzoic acid (**9**) with *Coriolus versicolor IFO 4937* 145 mg of 3-nitrobenzyl alcohol [619-25-0] (**9a**) were obtained.

200 mg of 4-nitrobenzoic acid (**10**) were converted by *C. versicolor IFO 4937* to 49 mg of 4-nitrobenzyl alcohol [619-73-8] (**10a**) after 24 h.

Coriolus versicolor IFO 4941 transformed 200 mg of 3-aminobenzoic acid (**11**) after 50 h to 21 mg of 3-aminobenzyl alcohol [1877-77-6] (**11a**).

200 mg of 2-chlorobenzoic acid (**12**) were converted by *Coriolus versicolor IFO 4937* after 167 h to 90 mg of 2-chlorobenzyl alcohol [17849-38-6] (**12a**) and 28 mg of (**12b**). **12b:** ^{1}H NMR: 3.30 (1 H, dd, $J = 12$, 9 Hz, 12-H), 3.42 (1 H, dd, $J = 7$, 6 Hz, 9-H), 3.45 (1 H, t, $J = 7$ Hz, 10-H), 3.63 (1 H, ddd, $J = 9$, 7, 5 Hz, 11-H), 4.00 (1 H, dd, $J = 12$, 5 Hz, 12′-H), 4.44 (1 H, d, $J = 6$ Hz, 8-H), 4.75 (1 H, d, $J = 12$ Hz, 7-H), 4.96 (1 H, d, $J = 12$ Hz, 7′-H), 7.25 (2 H, m, 4-, 5-H), 7.36 (1 H, m, 6-H), 7.54 (1 H, m, 3-H). ^{13}C NMR: 65.0 (t, C-7), 68.1 (t, C-12), 69.4 (d, C-11), 72.6 (d, C-9), 75.3 (d, C-10), 102.5 (d, C-8), 126.8 (d, C-5), 129.0 (d, C-4), 129.2 (d, C-3), 129.5 (d, C-6), 133.0 (s, C-2), 134.8 (s, C-1).

Corynespora melonis IFO 7483 reduced 200 mg of cinnamic acid (**15**) within 48 h to 7 mg of cinnamic alcohol [104-54-1] (**15a**).

200 mg of 3,4-dimethoxycinnamic acid (**18**) were reduced to 100 mg of 3,4-dimethoxycinnamic alcohol [40918-90-9] (**18a**) by *Coriolus hirsutus IFO 4917* after 140 h.

Aspergillus niger ATCC 4192 transformed 200 mg of 3,5-dimethoxycinnamic acid (**19**) within 43 h to 50 mg of 3,5-dimethoxycinnamic alcohol [29548-66-5] (**19a**).

Fermentation of 200 mg of ferulic acid (**24**) with *Mycobacterium phlei DSM 43286* resulted in 13 mg of coniferylalcohol [458-35-5] (**24a**), 24 mg coniferylaldehyde and 13 mg 3-(4-hydroxy-3-methoxy)phenylpropan-1-ol.

5 g of ferulic acid (**24**) gave, after incubation with *Psilocybe zapotekorum DSM 1891* for 96 h, 2.87 g of coniferylalcohol [458-35-5] (**24a**).

Corynespora melonis CBS 12925 converted 200 mg of 2-hydroxycinnamic acid (**20**) after 45 h to 63 mg 2-hydroxycinnamic alcohol [13523-27-8] (**20a**).

200 mg of 4-chlorocinnamic acid (**25**) were converted after 69 h to 20 mg of 4-chloro-(3-phenyl)-propanol by *Coriolus versicolor IFO 4941*.

Fermentation of 200 mg of 2-methoxycinnamic acid (**16**) with *Aspergillus flavus DSM 1959* gave 3 mg 2-methoxycinnamic alcohol [1504-61-6] (**16a**) after 47 h.

200 mg of 2-methoxyphenylacetic acid (**29**) were reduced to 46 mg 2-methoxy-(2-phenyl)-ethanol [7417-18-7] (**29a**) by *Coriolus hirsutus IFO 4817* after 192 h.

Coriolus versicolor IFO 4941 transformed 200 mg of 2-nitrophenylacetic acid (**33**) to 12 mg of 2-nitro-(2-phenyl)-ethanol [15121-84-3] (**33a**) after 141 h incubation.

200 mg 3-nitrophenylacetic acid (**34**) were converted to 2 mg of 3-nitro-(2-phenyl)-ethanol [52022-77-2] (**34a**) after 69 h with *Coriolus versicolor IFO 4941*.

Results

Substituted benzoic acids

Incubation of 3-methoxybenzoic acid (**2**) with *Aspergillus niger ATCC 9142* for 24 h yielded 58.9% of the alcohol (**2a**) and 8 mg of a demethylation product. The same compound could be transformed to (**2a**) with *Corynespora melonis IFO 7483* within 46 h and with *A. flavus DSM 1959* within 70 h in yields of 57.8% and 32.5%, respectively. Minor amounts of an *o*-demethylation product of (**2**) occurred in this reaction too.

Fermentation of 4-methoxybenzoic acid (**3**) with *Corynespora melonis IFO 7483* gave after 46 h 52.3% of the alcohol (**3a**) without byproducts. The two organisms *A. flavus DSM 62065* and *Coriolus hirsutus IFO 4917* yielded only 1–2% of (**3a**) after 70 h. When 3,4,5-trimethoxybenzoic acid (**4**) was subjected to transformation with *Coriolus hirsutus IFO 4917* for 70 h exclusively the alcohol (**4a**) was obtained in 58.9% yield.

3-Nitrobenzoic acid (**9**) could be reduced to the alcohol (**9a**) by *Coriolus versicolor IFO 4937* after

50 h in a yield of 79% without further reaction products.

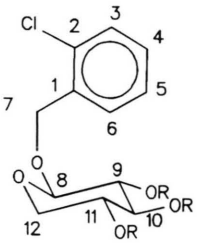
Fermentation of 5 g of (**9**) in 10 l medium with *C. versicolor IFO 4937* under the same conditions reduce the yield of the alcohol (**9a**) to 20.4% as was to be expected.

In contrast, 4-nitrobenzoic acid (**10**) yielded with the same organism after 24 h only 26.8% of (**10a**). No other products were formed. An incubation of (**10**) with *Curvularia affinis DSM 63274* for 46 h did not attack the carboxyl function, but instead reduced the nitro group to yield 16.5% of 4-aminobenzoic acid as the only product.

An incubation of 4-nitrobenzoic acid (**10**) with a mixed culture of *C. affinis DSM 63274* and *C. versicolor IFO 4937* to synthesize 4-aminobenzyl alcohol was not successful, only traces of reaction product were obtained, leaving the substrate unchanged.

3-Aminobenzoic acid (**11**) could be reduced within 50 h by *Coriolus versicolor IFO 4941* to the alcohol (**11a**) in 11.7% yield. No other products were detected.

Incubation of 2-chlorobenzoic acid (**12**) with *Coriolus versicolor IFO 4937* resulted after 167 h in 49.4% of alcohol (**12a**). Beside (**12a**) 28 mg of (**12b**) were formed (structure see Fig. 1).



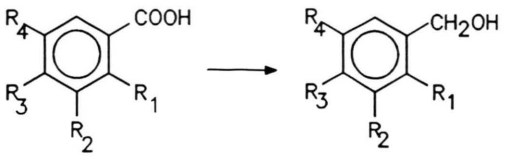
12b

Fig. 1. Structure of the β-D-xylopyranoside **12b.**

Benzoic acid (**14**) its derivatives (**1**), (**5**), (**6**), (**7**), (**8**) and (**13**) could not be reduced with the microorganisms utilized in the screen (Fig. 2).

Cinnamic acid derivatives

Cinnamic acid itself (**15**) could only be transformed to the alcohol (**15a**) in a yield of 3.9% by incubation with *Corynespora melonis IFO 7483* for



1–14 **1a–14a**

	R_1	R_2	R_3	R_4
1	OCH_3	H	H	H
2	H	OCH_3	H	H
3	H	H	OCH_3	H
4	H	OCH_3	OCH_3	OCH_3
5	H	OCH_3	OH	H
6	OH	H	H	H
7	H	OH	H	H
8	H	H	OH	H
9	H	NO_2	H	H
10	H	H	NO_2	H
11	H	NH_2	H	H
12	Cl	H	H	H
13	H	H	Cl	H
14	H	H	H	H

Fig. 2. Benzoic acid derivatives which were tested for reduction.

48 h. In this reaction nearly 50% of the substrate remained unchanged, even at longer incubation times.

Coriolus hirsutus IFO 4917 reduced 3,4-dimethoxycinnamic acid (**18**) during 140 h to (**18a**) in a yield of 53.6%. As a second product 26.5% of 3-(3,4-dimethoxy)-propanol was obtained.

Aspergillus niger ATCC 9142 produced after incubation with 3,5-dimethoxycinnamic acid (**19**) for 43 h 28.6% of alcohol (**19a**) and 11 mg of 3,5-dimethoxybenzoic acid as a result of β-oxidation of the side chain of (**19**).

The fermentation of ferulic acid (**24**) with *Mycobacterium phlei DSM 43286* for 46 h resulted in three main products, 7% of coniferylalcohol (**24a**), 13.1% coniferylaldehyde, and 6.9% of 3-(4-hydroxy-3-methoxy)propan-1-ol and traces of 3-(4-hydroxy-3-methoxy)propionic acid. After incubation of (**24**) with *Psilocybe zapotekorum DSM 1891* for 118 h in a minimal medium under nitrogen limitation (10 g glucose, 1 g KCl, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \times 7\ H_2O$, 0.2 g $CaCl_2 \times 2\ H_2O$, 0.1 g NH_4Cl, 1 l, pH 6.5) 35.6% of (**24a**) were obtained as the only reaction product.

Scaling up the reaction by incubating 5 g of (**24**) in a 10 l medium containing stirred fermenter with *P. zapotekorum DSM 1891* under analogous conditions, coniferylalcohol (**24a**) was obtained in a surprisingly high yield of 61.9%.

Corynespora melonis CBS 12925 converted 2-hydroxycinnamic acid (**20**, *o*-coumaric acid) within 45 h to the alcohol (**20a**) in a yield of 34.5%. The two other isomers, *m*- and *p*-coumaric acid (**21, 22**) could not be transformed to the respectives alcohols with any of the organisms used.

During incubation of 4-chlorocinnamic acid (**25**) with *Coriolus versicolor IFO 4941* concomitant with the reduction a hydrogenation of the side chain occurred resulting in 10.7% of 3-(4-chlorophenyl)propan-1-ol after 69 h. An incubation of (**25**) with *Curvularia fallax DSM 63169* ends up after 65 h with 36 mg of 4-chlorobenzoic acid, a β-oxidation product of (**25**).

During fermentation of the compounds such as (**17**), (**23**), and 2,6-dichlorocinnamic acid no transformation to the respective alcohols could be obtained (Fig. 3). 2-Methoxycinnamic alcohol (**16a**) was obtained only in 1.6% yield after incubating the corresponding acid (**16**) for 47 h with *Aspergillus flavus DSM 1959*.

Phenylacetic acid derivatives

Substrates subjected to transformation reactions are listed in Fig. 4.

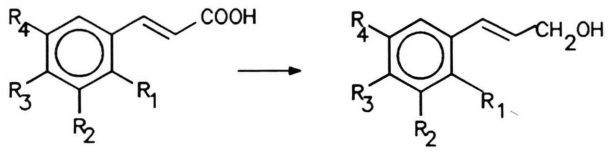
Incubation of 2-methoxyphenylacetic acid (**29**) with *Coriolus hirsutus IFO 4917* gave after 192 h 25% of the alcohol (**29a**).

Contact of 2-nitrophenylacetic acid (**33**) with *Coriolus versicolor IFO 4941* resulted in 6.5% of alcohol (**33a**) after 141 h.

3-Nitrophenylacetic acid (**34**) could be reduced to (**34a**) only in a yield of 1.1% with *C. versicolor IFO 4941* within 69 h.

Phenylacetic acid and the other derivatives tested with the microorganisms (Fig. 4) were not converted to the respective alcohols.

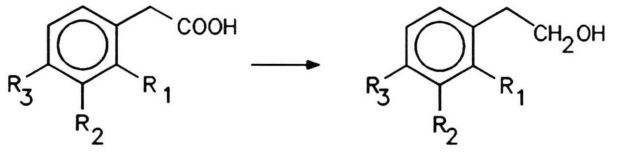
Several further aromatic carboxylic acids with different aromatic moieties, including nicotinic, picolinic, indolpropionic, indolbutyric, phenylmalonic, 3-phenylglutaric, and phthalic acid and derivatives thereof, were subjected to biotransformation. But none of these could be reduced to the respective alcohol with the microorganisms used in our investigations. In most cases the substrates remained unchanged or some were metabolized by the organism.



15–25 **15a–25a**

	R_1	R_2	R_3	R_4
15	H	H	H	H
16	OCH_3	H	H	H
17	OCH_3	H	H	OCH_3
18	H	OCH_3	OCH_3	H
19	H	OCH_3	H	OCH_3
20	OH	H	H	H
21	H	OH	H	H
22	H	H	OH	H
23	H	OH	OH	H
24	H	OCH_3	OH	H
25	H	H	Cl	H

Fig. 3. Cinnamic acid derivatives incubated with the various microorganisms.



26–36 **26a–36a**

	R_1	R_2	R_3
26	H	H	OH
27	OH	H	H
28	H	OH	H
29	OCH_3	H	H
30	H	H	OCH_3
31	H	OCH_3	H
32	H	H	NH_2
33	NO_2	H	H
34	H	NO_2	H
35	H	H	NO_2
36	H	H	H

Fig. 4. Phenylacetic acid derivatives used in the reduction screen.

Discussion

The incubation of aromatic carboxylic acids such as benzoic, phenylacetic, and cinnamic acid and several derivatives thereof with microorganisms resulted in the respective aromatic alcohols in fairly good yields, depending on the substrate.

Most of the reactions were examined after a relatively short time and gave only a single product. Benzoic acid itself, as well as the three hydroxyl derivatives, could not be reduced to their respective alcohols by our set of microorganisms. In a screen of benzoic acid with about 300 microorganisms [3] a *Nocardia* was found to reduce the substrate in 60% yield and also several molds could effect the same reaction. In an earlier paper [4] *Polystictus versicolor* was shown to reduce some benzoic and phenylacetic acid derivatives to the respective aldehydes or alcohols. Transformation of the hydroxy derivatives resulted only in a breakdown of the aromatic structure. In our investigation, we did not find any aldehyde with these two acids and derivatives.

In contrast the *o*-hydroxycinnamic acid could be reduced to the respective alcohol in 35% yield, the two other isomers however remained unchanged by the organisms similar to their benzoic acid counterparts.

The microorganisms were also unable to convert caffeic acid (3,4-dihydroxycinnamic acid) to its alcohol. No further dihydroxy compounds were tested.

The *m*- and *p*-methoxybenzoic acids were reduced in yields of 59 or 52% respectively, whereas the *o*-derivative could not be reduced. The *o*-methoxycinnamic acid, the only methoxy compound tested, gave only traces of alcohol, whereas the phenylacetic acid analogue was transformed to its alcohol in 25% yield, the two others however withstood transformation.

Among the three dimethoxycinnamic acid compounds tested, two, namely the 3,4-dimethoxy and 3,5-dimethoxy derivatives could be reduced to their alcohol in 54% and 29%, respectively. The 2,5-dimethoxy compound was resistant to transformation. A yield of only 21.9% of 3,4-dimethoxycinnamyl alcohol was obtained from the respective acid compound with the fungus *Phanerochaete chrysosporium* as reported previously [5]. A 3,4,5-trimethoxybenzoic acid delivered the respective alcohol in 58% yield after contact with the organism.

Although the microorganisms were not able to reduce 4-hydroxy-3-methoxy benzoic acid to the alcohol, ferulic acid, the cinnamic acid counterpart could be transformed yielding up to 62% coniferyl alcohol.

Depending on the microorganism chosen, 4-nitrobenzoic acid could be reduced either to 4-nitrobenzylic alcohol in 27% yield or to 4-aminobenzoic acid in 16% yield. An effort to synthesize 4-aminobenzylic alcohol by using a mixed culture of both organisms remained unsuccessful. In contrast to the low yield of amino compound obtained in our system, the reduction of aromatic nitro compounds carrying no free carboxylic group by baker's yeast, yielded between 4 and 88% of the aromatic amines [6]. The same organism transformed 3-nitrobenzoic acid in a high yield of up to 80% to its alcohol. Contact of 4-nitrophenylacetic acid with the microorganisms did not give any reduction product, although the 2-nitro and the 3-nitro derivative could be reduced in low yields, 6.5% or 1.2% respectively.

Incubation of 3-aminobenzoic acid yielded 12% of its alcohol derivative, whereas the 4-nitrophenylacetic acid withstood any biotransformation reaction.

Two chloro derivatives tested, namely 2-chlorobenzoic acid and 4-chlorocinnamic acid, could be converted to their alcohols in 49% and 11% yield respectively. The 4-chloro-benzoic acid remained unchanged after microbial contact.

Aromatic carboxylic acids which differed in the aromatic nucleus from those described above, and aromatic dicarboxylic acids were also subjected to biotransformation with our set of organisms. None of it could be reduced to the respective alcohol, and most remained unchanged or were metabolized. Reduction of non aromatic acids such as fatty acids as described in the literature [7–10], was not studied with our organisms.

The results obtained, demonstrated, that the microbial reduction of benzoic, cinnamic and phenylacetic acids and derivatives opened up a second avenue to aromatic alcohols under mild conditions and in good yields. They offer a viable alternative to chemical synthesis, which normaly uses toxic solvents or catalysts.

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