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ORIGINAL PAPER

Didymosphaeria igniaria: a new microorganism useful for the enantioselective reduction of aryl-aliphatic ketones

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Abstract *Didymosphaeria igniaria* is a promising biocatalyst in asymmetric reductions of prochiral aromatic-aliphatic ketones such as acetonaphthones, acetophenones, and acetylpyridines. The organism converted the substrates mainly to (*S*)-alcohols. Excellent results in terms of conversion and enantioselectivity (100% yield, >99% ee) were obtained with acetonaphthones. In case of acetyl pyridines, the optical purity of the product depended on the position of the carbonyl group on the pyridine ring and followed the order 2-acetyl ≫ 4-acetyl > 3-acetyl-pyridine. Transformation of *o*-methoxy-acetophenone gave optically pure (*S*)-(-)-1-(2-methoxyphenyl)-ethanol in 95% yield. The transformation of *para*-methyl ketone gave (*R*)-alcohol (81% ee), whereas *para*-bromo ketone gave (*S*)-alcohol (98% ee). Monitoring of the biotransformation of these substrates over time led to the conclusion that for both substrates, non-selective carbonyl group reduction occurred in the first step, followed by selective oxidation of the (*R*)-isomer of *p*-bromo-phenylethanol and selective oxidation of the (*S*)-isomer of *p*-methyl-phenylethanol. *D. igniaria* exhibited poor enantioselectivity in the reduction of bicyclic aryl-aliphatic ketones such as 1- and 2-tetralones. Only (*S*)-5-methoxy-1-tetralol was obtained in optically pure (>99% ee) form.

Keywords Asymmetric bioreduction · Chiral alcohol · Enantioselectivity · *Didymosphaeria igniaria*

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Introduction

The enantioselective synthesis of chiral alcohols, a highly versatile and attractive group of chiral building blocks for synthesis of molecules with desirable biological activities [1, 2], has been studied extensively. One of the most attractive methods of preparing enantiomerically pure alcohols is catalytic reduction of the corresponding prochiral ketones. Though chiral transition metal complexes have been successfully applied for this purpose, the use of biocatalytic reduction has some advantages, and in some cases it is able to improve reaction stereoselectivity. Moreover, the biological methods are environmentally friendly due to the use of natural resources and complete degradation of biocatalysts after their use. Until now, many examples of the reduction of ketones by whole cells of microorganisms, mainly yeast, have been described [3–12]. Many other biocatalysts, such as fungal cells [13–17], bacterial strains [18, 19], and even plant organs [3, 20–23] or edible mushrooms [24] were also employed for the preparation of enantiomerically pure alcohols. Recently, germinated radish sprouts have been used as a new type of biocatalyst for the asymmetric reduction of ketones [25]. The use of the whole microbial or plant cells is particularly advantageous since they do not require costly recycling of cofactors because they are recycled by the cell. However, in many cases, biocatalysts have disadvantages such as: low chemotolerance to substrates and organic solvents, narrow substrate specificity, or insufficient stereoselectivity due to the coexistence of several reductases with different specific activities. The use of plant cells additionally is problematic due to the reproducibility of experiments, since the activity of these biocatalysts depends on the places they come from and is easily changeable throughout a year. Thus, a special catalyst needs to be searched for each substrate.

Herein, we focused on the study of a new isolate from forest plants, the filamentous fungus *Didymosphaeria igniaria* KCH 6670, which could be easily manipulated and used as a reducing agent for substituted acetophenones, acetylpyridines, acetonaphthones, and tetralones. The arylethanol derivatives, especially the single enantiomers of halogen-containing aromatic alcohols, are one of the most important kinds of chiral building blocks for synthesis of enantiomerically pure pharmaceuticals, such as L-chlorprenaline, *R*-salbutamol, *R*-tomoxetine, and *R*-denopamine [26–28]. (*S*)-1-(1′-Naphthyl)-ethanol is an important intermediate for the synthesis of a mevinic acid analogue—a potential inhibitor of 3-hydroxy methyl glutaryl coenzyme A reductase [29]. Enantiomerically pure 1- and 2-tetralols and their derivatives are useful chirons for the synthesis of bioactive compounds such as the antidepressant sertraline [30, 31], anti-arrythmical MK-0499 [32], or potent hypnotic and antimycotic agents and empomil inhibitors [33].

In our previous report, *D. igniaria* was used for regio- and stereoselective hydroxylation of bicyclic enones [34], but activity of dehydrogenases from this fungi hasn't been reported before.

Materials and methods

Chemicals and microorganism

All the substrates were purchased from Aldrich (Sigma-Aldrich Corporation, St. Louis, MO, USA) (**1**, **14**–**16**, **18**–**20**, **23**–**28**), Fluka (Sigma-Aldrich Corporation, St. Louis, MO, USA) (**2**–**12**, **17**, **21**–**22**) or Alfa-Aesar (Alfa Aesar, Germany) (**13**). The microorganism *D. igniaria* KCH 6670 used in this study was obtained from the collection of the Department of Forest Phytopathology of the University of Agriculture in Kraków, Poland. The strain was maintained on Sabouraud 4% dextrose-agar slopes and freshly subcultured before use in the transformation experiments.

Conditions of cultivation and transformation

In biotransformations reactions, 250-ml Erlenmeyer flasks, each containing 100 ml of a medium consisting of glucose 30 g l^{-1} and peptone 10 g l^{-1}, were inoculated with 5 ml spore suspensions, and incubated for 72 h at 20°C in a rotary shaker. After this growth period, 0.6 mMol of ketone dissolved in 0.5 ml of acetone was added to each of the cultures, and the transformations continued under the same conditions until it gave the best results in terms of conversion and enantiomeric excess of alcohols. The progress of the reaction was periodically monitored by GC and TLC analysis. Each experiment was performed with at least three replicates.

Isolation and identification of products

At regular intervals (24 h), samples (5 ml) of the reaction medium were taken and extracted with an equal volume of diethyl ether. The organic phase was dried over anhydrous magnesium sulfate, solvent was removed under reduced pressure, and the residues were analyzed by TLC and GC. Authentic reference samples of the racemic alcohol were prepared by reducing the ketones with sodium borohydride in methanol. TLC was carried out using Merck Kieselgel 60 F_{254} plates (Merck, Germany) with hexane/acetone (2:1 v/v) as eluent. Visualization of products was performed by spraying the plates with 2% solution of $H_3[P(Mo_3O_{10})_4]$ and 1% solution of $Ce(SO_4)_2$ in 10% sulphuric acid, followed by heating to 120°C. GC analysis was performed using a Hewlett Packard 5890A Series II GC instrument (FID, carrier gas H_2 at flow rate of 2 ml min^{-1}), equipped with a chiral column:

A: ChrompackWCOT Fused Silica (CP Chirasil-DEX CB), 25 m, DF = 0.25 μm × 0.25 mm ID (Varian Inc. Lake Forest, CA, USA)
B: Supelco Beta DEX^{TM}120, Fused Silica, 30 m, DF = 0.25 μm × 0.25 mm ID (Supelco, Bellafonte, PA, USA)
C: Supelco Beta DEX^{TM}225, Fused Silica, 30 m, DF = 0.25 μm × 0.25 mm ID
D: ChrompackWCOT Fused Silica (CPCycloDEX B 236 M), 25 m, DF = 0.25 μm × 0.25 mm ID
E: Chrompack Chirasil Fused Silica (Chirasil-L-Val), 25 m, DF = 0.12 μm × 0.25 mm ID
F: Varian capillary column (CP-Chirasil-Dex CB), 25 m, DF = 0.25 μm × 0.25 mm ID (Varian Inc. Lake Forest, CA, USA)

The injector temperature was 200°C and detector was 250°C. Temperature programs are given below.

1a: A; 88°C/1 min, 4°C min^{-1} to 107°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 8.3, R_t (*R*) 8.9
2a: B; 85°C/1 min, 0.8°C min^{-1} to 103°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 8.2, R_t (*R*) 8.6
3a: A; 100°C/1 min, 2°C min^{-1} to 110°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 4.8, R_t (*R*) 5.1
4a: A; as **3a**, R_t (*S*) 5.9, R_t (*R*) 6.2
5a: A; as **3a**, R_t (*S*) 3.6, R_t (*R*) 4.0
6a: A; 90°C/1 min, 2°C min^{-1} to 110°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 4.7, R_t (*R*) 5.2
7a: A; 110°C/1 min, 2°C min^{-1} to 120°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 7.1, R_t (*R*) 7.7
8a: C; as **2a**, R_t (*S*) 11.0, R_t (*R*) 12.2

9a: D; 90°C/1 min, 0.8°C min^{-1} to 110°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 11.9, R_t (*R*) 12.8
10a: D; as **9a**, R_t (*S*) 12.2, R_t (*R*) 13.1
11a: E; 110°C/1 min, 0.4°C min^{-1} to 125°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 32.5, R_t (*R*) 33.1
12a: E; as **11a**, R_t (*S*) 33.4, R_t (*R*) 33.9
13a: E; 110°C/1 min, 0.4°C min^{-1} to 127°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 40.2, R_t (*R*) 41.1
14a: A; 120°C/1 min, 1°C min^{-1} to 145°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 23.0, R_t (*R*) 23.5
15a: C; 80°C/1 min, 2°C min^{-1} to 140°C, 30°C min^{-1} to 200°C/5 min, R_t (*R*) 32.5, R_t (*S*) 33.2
17a: C; 120°C/1 min, 0.4°C min^{-1} to 131°C, 30°C min^{-1} to 200°C/5 min, R_t (*R*) 24.0, R_t (*S*) 24.6
18a: E; 120°C/1 min, 1.4°C min^{-1} to 155°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 15.4, R_t (*R*) 16.1
23a: E; 80°C/1 min, 1°C min^{-1} to 141°C, 30°C min^{-1} to 200°C/5 min, R_t (*S*) 26.9, R_t (*R*) 27.3
24a: D; 125°C/1 min, 1°C min^{-1} to 156°C, 30°C min^{-1} to 200°C/5 min, after conversion to the acetate R_t (*S*) 22.8, R_t (*R*) 23.4
25a: D; as **24a**, after conversion to the acetate R_t (*S*) 17.5, R_t (*R*) 17.8
26a: F; as **15a**, R_t (*S*) 34.0, R_t (*R*) 35.1
27a: D; 130°C/1 min, 1.4°C min^{-1} to 143°C, 30°C min^{-1} to 200°C/5 min., after conversion to the acetate R_t (*S*) 6.5, R_t (*R*) 6.8

Enantiomeric excesses were determined by GC analysis using the chiral columns described above. Structures of the biotransformation products were confirmed by ^{1}H NMR. The spectra were recorded on a DRX 300-MHz Bruker spectrometer (Bruker, Billerica, MA, USA) and measured in $CDCl_3$ with TMS as internal standard. Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The absolute configuration of the products was determined by comparison of their optical rotational values with literature data. For alcohols **13a** and **27a** it was established by comparison of the sign of specific rotation provided by the literature and the elution order of the enantiomers in chiral GC for these compounds and their structural analogues.

(*S*)-1-phenylethanol (**1a**): $[\alpha]_D^{20} = -49.0$ (*c* 2.5, $CHCl_3$) {lit. $[\alpha]_D^{25} = -55.1$ (*c* 1.63, $CHCl_3$), >99% ee, *S*, [35]}; ^{1}H NMR: δ (ppm) 1.47 (d, 3H, $J = 6.5$ Hz, CH_3), 2.79 (s, 1H, O*H*), 4.79 (q, 1H, $J = 6.5$ Hz, C*H*OH), 6.86 (d, 1H, $J = 8.3$ Hz, *H*-6), 7.18 (m, 5H, *H*-Ar)

(*S*)-2,2,2-trifluoro-1-phenylethanol (**2a**): $[\alpha]_D^{20} = +8.6$ (*c* 1.8, $CHCl_3$) {lit. $[\alpha]_D^{25} = -5.5$ (*c* 5.26, EtOH), *R*, [36]}; ^{1}H NMR: δ (ppm) 2.05 (s, 1H, O*H*), 5.02 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.39 (m, 3H, *H*-3 and *H*-4 and *H*-5), 7.47 (m, 2H, *H*-2 and *H*-6)

(*S*)-1-(2-methoxyphenyl)-ethanol (**3a**): $[\alpha]_D^{20} = -54.2$ (*c* 1.2, $CHCl_3$) {lit. $[\alpha]_D^{23} = -63.0$ (*c* 1.10, toluene), >99% ee, *S*, [35]}, ^{1}H NMR: δ (ppm) 1.49 (d, 3H, $J = 6.6$ Hz, CH_3), 2.73 (s, 1H, O*H*), 3.84 (s, 3H, OCH_3), 5.08 (q, 1H, $J = 6.6$ Hz, C*H*OH), 6.86 (d, 1H, $J = 8.3$ Hz, *H*-6), 6.95 (t, 1H, $J = 7.5$ Hz, *H*-4), 7.23 (td, 1H, $J = 8.3$ Hz, $J = 1.6$ Hz, *H*-5), 7.33 (dd, 1H, $J = 7.5$ Hz, $J = 1.6$ Hz, *H*-3)

(*R*)-1-(3-methoxyphenyl)-ethanol (**4a**): $[\alpha]_D^{20} = +31.6$ (*c* 0.8, $CHCl_3$) {lit. $[\alpha]_D^{22} = -34.9$ (*c* 0.849, MeOH), >99% ee, *S*, [35]}, ^{1}H NMR: δ (ppm) 1.51 (d, 3H, $J = 6.5$ Hz, CH_3), 2.05 (s, 1H, O*H*), 3.84 (s, 3H, OCH_3), 4.89 (q, 1H, $J = 6.5$ Hz, C*H*OH), 6.84 (dd, 1H, $J = 8.0$ Hz, $J = 2.5$ Hz, *H*-4), 6.97 (s, 1H, *H*-2), 6.99 (dd, 1H, $J = 8.0$ Hz, $J = 2.5$ Hz, *H*-6), 7.29 (t, 1H, $J = 8.0$ Hz, *H*-5)

(*S*)-1-(4-methoxyphenyl)-ethanol (**5a**): $[\alpha]_D^{20} = -23.3$ (*c* 1.3, $CHCl_3$) {lit. $[\alpha]_D^{25} = -30.0$ (*c* 1.75, $CHCl_3$), 94% ee, *S*, [20]}; ^{1}H NMR: δ (ppm) 1.51 (d, 3H, $J = 6.4$ Hz, CH_3), 1.84 (s, 1H, O*H*), 3.84 (s, 3H, OCH_3), 4.89 (q, 1H, $J = 6.4$ Hz, C*H*OH), 6.91 (m, 2H, *H*-3 and *H*-5), 7.33 (m, 2H, *H*-2 and *H*-6)

(*R*)-1-(4-methylphenyl)-ethanol (**6a**): $[\alpha]_D^{20} = +21.8$ (*c* 2.1, $CHCl_3$) {lit. $[\alpha]_D^{25} = -21.0$ (*c* 1.5, $CHCl_3$), 92% ee, *S*, [20]}; ^{1}H NMR: δ (ppm) 1.47 (d, 3H, $J = 6.4$ Hz, CH_3), 1.72 (s, 1H, O*H*), 2.33 (s, 3H, Ar–CH_3), 4.86 (q, 1H, $J = 6.4$ Hz, C*H*OH), 7.14 (m, 2H, *H*-2 and *H*-6); 7.33 (m, 2H, *H*-3 and *H*-5)

(*S*)-1-(4-bromophenyl)-ethanol (**7a**): $[\alpha]_D^{20} = -35.3$ (*c* 1.8, $CHCl_3$) {lit. $[\alpha]_D^{25} = -35.3$ (*c* 1.0, $CHCl_3$), 92% ee, *S*, [9]}; ^{1}H NMR: δ (ppm) 1.49 (d, 3H, $J = 6.5$ Hz, CH_3), 1.94 (s, 1H, O*H*), 4.89 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.27 (m, 2H, *H*-3 and *H*-5), 7.49 (m, 2H, *H*-2 and *H*-6)

(*S*)-1-(2-pyridyl)-ethanol (**8a**): $[\alpha]_D^{20} = -24.7$ (*c* 0.9, $CHCl_3$) {lit. $[\alpha]_D^{25} = -25.0$, (*c* 1.39, $CHCl_3$), >99% ee, *S*, [12]}; ^{1}H NMR: δ (ppm) 1.52 (d, 3H, $J = 6.5$ Hz, CH_3), 1.84 (s, 1H, O*H*), 4.87 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.18 (dd, 1H, $J = 7.7$ Hz, $J = 4.8$ Hz, *H*-4), 7.26 (d, 1H, $J = 7.7$ Hz, *H*-6), 7.67 (t, 1H, $J = 7.7$ Hz, *H*-5), 8.51 (d, 1H, $J = 4.8$ Hz, *H*-3)

(*S*)-1-(3-pyridyl)-ethanol (**9a**): $[\alpha]_D^{20} = -26.0$ (*c* 0.9, $CHCl_3$) {lit. $[\alpha]_D^{25} = -47.0$ (*c* 0.625, MeOH), 99% ee, *S*, [12]}; ^{1}H NMR: δ (ppm) 1.52 (d, 3H, $J = 6.5$ Hz, CH_3), 1.91 (s, 1H, O*H*), 4.94 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.26 (dd, 1H, $J = 7.8$ Hz, $J = 4.8$ Hz, *H*-5), 7.72 (d, 1H, $J = 7.8$ Hz, *H*-6), 8.48 (dd, 1H, $J = 4.8$ Hz, $J = 1.2$ Hz, *H*-4), 8.56 (d, 1H, $J = 1.2$ Hz, *H*-2)

(*S*)-1-(4-pyridyl)-ethanol (**10a**): $[\alpha]_D^{20} = -29.3$ (*c* 0.8, $CHCl_3$) {lit. $[\alpha]_D^{25} = -39.0$, (*c* 0.82, MeOH, 92% ee, *S*, [29]}; ^{1}H NMR: δ (ppm) 1.52 (d, 3H, $J = 6.5$ Hz, CH_3), 1.84 (s, 1H, O*H*), 4.90 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.32 (m, 2H, *H*-2 and *H*-6), 8.54 (m, 2H, *H*-3 and *H*-5)

(*S*)-1-(1′-naphthyl)-ethanol (**11a**): $[\alpha]_D^{20} = -70.0$ (*c* 1.2, $CHCl_3$) {lit. $[\alpha]_D^{25} = -79.4$ (EtOH, >99% ee, *S*, [37]}; ^{1}H NMR: δ (ppm) 1.65 (d, 3H, $J = 6.5$ Hz, CH_3), 1.98 (s, 1H, O*H*), 5.66 (q, 1H, $J = 6.4$ Hz, C*H*OH), 7.54 (m, 3H, *H*-3′ and *H*-6′ and *H*-7′), 7.67 (d, 1H, $J = 7.1$ Hz, *H*-5′), 7.77 (d, 1H, $J = 8.2$ Hz, *H*-2′), 7.86 (d, 1H, $J = 9.3$ Hz, *H*-4′), 8.12 (d, 1H, $J = 6.6$ Hz, *H*-8′)

(*S*)-1-(2′-naphthyl)-ethanol (**12a**): $[\alpha]_D^{20} = -37$ (*c* 2.9, $CHCl_3$) {lit. $[\alpha]_D^{24} = -43.1$, (EtOH, >99% ee, *S*, [37]}; ^{1}H NMR: δ (ppm) 1.55 (d, 3H, $J = 6.5$ Hz, CH_3), 2.40 (s, 1H, O*H*), 5.02 (q, 1H, $J = 6.5$ Hz, C*H*OH), 7.47 (m, 3H), 7.79 (m, 4H)

(*S*)-1-(5,6,7,8-tetrahydro-naphthalen-2-yl)-ethanol (**13a**): $[\alpha]_D^{20} = -48.3$ (*c* 1.8, $CHCl_3$); ^{1}H NMR: δ (ppm) 1.46 (d, 3H, $J = 6.6$ Hz, CH_3), 1.77 (m, 4H, *H*-6 and *H*-7), 1.95 (s, 1H, O*H*), 2.73 (m, 4H, *H*-5 and *H*-8), 4.81 (q, 1H, $J = 6.6$ Hz, C*H*OH), 7.05 (m, 3H, *H*-1 and *H*-3 and *H*-4)

(*S*)-1-tetralol (**14a**): $[\alpha]_D^{20} = +25.4$ (*c* 2.5, $CHCl_3$) {lit. $[\alpha]_D^{25} = -33.5$ (*c* 1.01, $CHCl_3$, 99% ee, *R*, [34]}; ^{1}H NMR: δ ppm: 1.76–2.01 (m, 4H, *H*-2 and *H*-3), 2.73–2.82 (m, 2H, *H*-4), 4.75 (t, 1H, $J = 4.9$ Hz, C*H*OH), 7.03–7.37 (m, 3H, *H*-5 and *H*-7 and *H*-8), 7.45 (1H, m, *H*-6)

(*S*)-5-methoxy-1-tetralol (**15a**): $[\alpha]_D^{23} = +12.5$ (*c* 1.3, $CHCl_3$) {lit. $[\alpha]_D^{23} = -12.3$ (*c* 1.01, $CHCl_3$, 98% ee, *R*, [34]}; ^{1}H NMR: δ ppm 1.81 (m, 1H, *H*-3$_{eq}$), 1.86 (s, 1H, O*H*), 1.91 (m, 1H, *H*-3$_{ax}$), 1.97 (m, 2H, *H*-2), 2.58 (ddd, 1H, $J = 17.5$ Hz, $J = 7.6$ Hz, $J = 6.6$ Hz, *H*-4$_{eq}$), 2.78 (dt, 1H, $J = 17.5$ Hz, $J = 5.4$ Hz, *H*-4$_{ax}$), 3.85 (s, 3H, OCH_3), 4.79 (t, 1H, $J = 4.6$ Hz, C*H*OH), 6.79 (d, 1H, $J = 7.8$ Hz, *H*-6), 7.09 (d, 1H, $J = 7.8$ Hz, *H*-8), 7.23 (t, 1H, $J = 7.8$ Hz, *H*-7)

(*R*)-7-methoxy-1-tetralol (**17a**): $[\alpha]_D^{23} = -14.5$ (*c* 1.5, $CHCl_3$) {lit. $[\alpha]_D^{22} = -38.0$ (*c* 1.02, $CHCl_3$, 99% ee, *R*, [34]}; ^{1}H NMR: δ ppm 1.67 (s, 1H, O*H*), 1.75 (m, 1H, *H*-3$_{eq}$), 1.85 (m, 1H, *H*-3$_{ax}$), 1.93 (m, 1H, *H*-2$_{eq}$), 1.99 (m, 1H, *H*-2$_{ax}$), 2.63 (ddd, 1H, $J = 16.9$ Hz, $J = 7.8$ Hz, $J = 6.7$ Hz, *H*-4$_{eq}$), 2.78 (dt, 1H, $J = 16.9$ Hz, $J = 5.6$ Hz, *H*-4$_{ax}$), 3.78 (s, 3H, OCH_3), 4.79 (t, 1H, $J = 4.8$ Hz, C*H*OH), 6.76 (dd, 1H, $J = 8.4$ Hz, $J = 2.4$ Hz, *H*-5), 6.97 (d, 1H, $J = 2.4$ Hz, *H*-8), 7.00 (d, 1H, $J = 8.4$ Hz, *H*-6)

(*R*)-5,7-dimethyl-1-tetralol (**18a**): $[\alpha]_D^{23} = -16.5$ (*c* 1.5, $CHCl_3$) {lit. $[\alpha]_D^{22} = -31.6$ (*c* 1.05, $CHCl_3$, 95% ee, *R*, [34]}; ^{1}H NMR: δ ppm 1.83 (m, 1H, *H*-3$_{eq}$), 1.87–1.99 (m, 2H, *H*-2$_{eq}$ and *H*-3$_{ax}$), 2.05 (m, 1H, *H*-2$_{ax}$), 2.23 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.54 (ddd, 1H, $J = 17.1$ Hz, $J = 7.9$ Hz, $J = 6.6$ Hz, *H*-4$_{eq}$), 2.68 (dt, 1H, $J = 17.1$ Hz, $J = 5.6$ Hz, *H*-4$_{ax}$), 4.75 (t, 1H, $J = 4.9$ Hz, *H*-1$_{ax}$), 6.96 (s, 1H, *H*-8), 7.15 (s, 1H, *H*-6)

(*S*)-2-tetralol (**23a**): $[\alpha]_D^{23} = -2.2$ (*c* 1.1, MeOH) {lit. $[\alpha]_D^{25} = -54.4$ (*c* 0.7, $CHCl_3$, 81% ee, *S*, [38]}; ^{1}H NMR: δ (ppm) 1.82 (dtd, 1H, $J = 12.7$ Hz, $J = 9.1$ Hz, $J = 5.9$ Hz, *H*-3$_{eq}$), 2.05 (m, 1H, *H*-3$_{ax}$), 2.76 (dd, 1H, $J = 16.2$ Hz, $J = 7.5$ Hz, *H*-1$_{ax}$), 2.86 (m, 1H, *H*-4$_{ax}$), 2.96 (dt, 1H, $J = 17.0$ Hz, $J = 5.8$ Hz, *H*-4$_{eq}$), 3.09 (dd, 1H, $J = 16.2$ Hz, $J = 4.9$ Hz, *H*-1$_{eq}$), 4.16 (m, 1H, C*H*OH), 7.10 (m, 4H, *H*-Ar)

(*S*)-6-methoxy-2-tetralol (**24a**): $[\alpha]_D^{23} = -17.4$ (*c* 0.33, MeOH) {lit. $[\alpha]_D^{25} = -16.1$ (*c* 0.22 MeOH, 34% ee, *S*, [4]}; ^{1}H NMR: δ (ppm) 1.82 (dtd, 1H, $J = 12.7$ Hz, $J = 9.1$ Hz, $J = 5.9$ Hz, *H*-3$_{eq}$), 2.05 (m, 1H, *H*-3$_{ax}$), 2.76 (dd, 1H, $J = 16.2$ Hz, $J = 7.5$ Hz, *H*-1$_{ax}$), 2.86 (m, 1H, *H*-4$_{ax}$), 2.96 (dt, 1H, $J = 17.0$ Hz, $J = 5.8$ Hz, *H*-4$_{eq}$), 3.09 (dd, 1H, $J = 16.2$ Hz, $J = 4.9$ Hz, *H*-1$_{eq}$), 3.76 (s, 3H, OCH_3), 4.16 (m, 1H, C*H*OH), 6.62 (d, 1H, $J = 2.4$ Hz, *H*-5), 6.70 (dd, 1H, $J = 8.4$ Hz, $J = 2.4$ Hz, *H*-7), 6.98 (dd, 1H, $J = 8.4$ Hz, *H*-8)

(*S*)-7-methoxy-2-tetralol (**25a**): $[\alpha]_D^{23} = -23.6$ (*c* 0.24, MeOH) {lit. $[\alpha]_D^{25} = +16.3$ (*c* 0.23 MeOH, 38% ee, *R*, [4]}; ^{1}H NMR: δ ppm 1.58 (s, 1H, O*H*), 1.81 (dtd, 1H, $J = 13.2$ Hz, $J = 9.0$ Hz, $J = 5.9$ Hz, *H*-3$_{ax}$), 1.92 (m, 1H, *H*-3$_{eq}$), 2.72 (dd, 1H, $J = 16.2$ Hz, $J = 7.8$ Hz, *H*-1$_{ax}$), 2.81 (ddd, 1H, $J = 16.8$ Hz, $J = 9.0$ Hz, $J = 6.0$ Hz, *H*-4$_{ax}$), 2.88 (dt, 1H, $J = 16.8$ Hz, $J = 6.0$ Hz, *H*-4$_{eq}$), 3.05 (dd, 1H, $J = 16.2$ Hz, $J = 4.9$ Hz, *H*-1$_{eq}$), 3.75 (3H, s, OCH_3), 4.13 (m, 1H, C*H*OH), 6.60 (d, 1H, $J = 2.4$ Hz, *H*-8), 6.69 (dd, 1H, $J = 8.4$ Hz, $J = 2.4$ Hz, *H*-6), 6.98 (d, 1H, $J = 8.4$ Hz, *H*-5)

(*S*)-8-methoxy-2-tetralol (**26a**): $[\alpha]_D^{20} = -29.2$ (*c* 1.0, EtOH {lit. $[\alpha]_D = -50.5$ (*c* 0.87 $CHCl_3$, *S*, [39]}; ^{1}H NMR: δ (ppm) 1.82 (dtd, 1H, $J = 12.6$ Hz, $J = 9.0$ Hz, $J = 6.0$ Hz, *H*-3$_{eq}$), 2.00 (s, 1H, O*H*), 2.05 (m, 1H, *H*-3$_{ax}$), 2.60 (dd, 1H, $J = 16.8$ Hz, $J = 7.2$ Hz, *H*-1$_{ax}$), 2.86 (ddd, 1H, $J = 16.8$ Hz, $J = 9.0$ Hz, $J = 6.0$ Hz, *H*-4$_{ax}$), 2.96 (dt, 1H, $J = 16.8$ Hz, $J = 6.0$ Hz, *H*-4$_{eq}$), 3.06 (dd, 1H, $J = 16.8$ Hz, $J = 5.4$ Hz, *H*-1$_{eq}$), 3.84 (s, 3H, OCH_3), 4.18 (m, 1H, C*H*OH), 6.71 (d, 1H, $J = 8.1$ Hz, *H*-5), 6.77 (d, 1H, $J = 7.9$ Hz, *H*-7), 7.12 (t, 1H, $J = 8.0$ Hz, *H*-6)

(*S*)-6-chloro-2-tetralol (**27a**): $[\alpha]_D^{15} = -20.8$ (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ (ppm) 1.84 (dtd, 1H, $J = 13.2$ Hz, $J = 9.0$ Hz, $J = 5.9$ Hz, *H*-3$_{eq}$), 1.93 (s, 1H, OH), 2.05 (m, 1H, *H*-3$_a$), 2.72 (dd, 1H, $J = 16.2$ Hz, $J = 7.8$ Hz, *H*-1$_{ax}$), 2.82 (ddd, 1H, $J = 16.8$ Hz, $J = 9.0$ Hz, $J = 6.0$ Hz, *H*-4$_a$), 2.96 (dt, 1H, $J = 16.8$ Hz, $J = 6.0$ Hz, *H*-4$_{eq}$), 3.06 (dd, 1H, $J = 16.2$ Hz, $J = 4.9$ Hz, *H*-1$_{eq}$), 4.18 (m, 1H, *H*-2$_{ax}$), 7.02 (d, 1H, $J = 7.8$ Hz, *H*-8), 7.10 (dd, 1H, $J = 7.8$, $J = 1.8$ Hz, *H*-7), 7.12 (d, 1H, $J = 1.8$ Hz, *H*-5)

Results and discussion

Reduction of acetophenones (1–7)

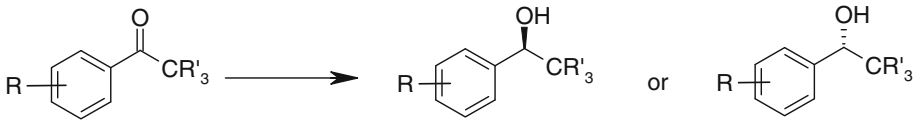
The result of transformation of acetophenone (**1**) indicated that it could be reduced by *D. igniaria* to chiral 1-phenylethanol with good enantioselectivity and chemical yield. In view of these good results, we decided to test the

efficiency and stereoselectivity of bioreduction of its trifluoro analogue as well as other acetophenone derivatives with different substituents (methoxy and methyl groups, bromine atom) in the benzene ring (Table 1). As expected, all the substrates were converted to arylethanols with moderate to high ee's (77–>99%). Configurationally different alcohols were obtained by subjecting acetophenone (**1**) and its trifluorinated analogue **2** (*S*-**1a** and *S*-**2a** are opposite according to definition), but the stereoselectivities were high for both substrates (91 and 82% ee, respectively). Moreover, **2** was more efficiently reduced than **1**. The similar difference was described for reduction of fluorinated and nonfluorinated substrates by two enzymes with opposite stereochemical preferences contained in the acetone powder of yeast of *Geotrichum candidum* [40].

Ortho-, *meta*-, and *para*-substituted methoxy acetophenones (**3**–**5**) were reduced to the corresponding alcohols according to the Prelog's or anti-Prelog's rule, depending on the structure of the substrate. The best results were obtained for *ortho*-acetophenone **3**. The reaction gave the enantiomerically pure (>99% ee) (*S*)-1-(2-methoxyphenyl)-ethanol with good yield (95%). The yield of reduction of *meta*-substituted acetophenone **4** was slightly lower, and decreased for *para*-substituted acetophenone **5**. It is worthy of note that *ortho*- and *para*-methoxy substituted acetophenones were reduced more slowly than *meta*-methoxy-acetophenone (**4**). Unexpectedly, as a result of reduction of **4** with high ee (90%), (*R*)-enantiomer of the corresponding alcohol was obtained. A plausible explanation for this effect is that it is determined by the equilibrium between the reduction and oxidation of resulting alcohols. It is known that phenylethanol and its derivatives undergo stereoinversion [13, 41, 42]. For this reason, we studied the time-course of transformation of all substrates (data not presented). These experiments evidently indicated that the ee's of resulting alcohols were not changing with prolonged reaction times for **1a–5a**. Thus, (*R*)-1-(3-methoxyphenyl)-ethanol (**4a**) was a result of preference of *R*-reduction of *meta*-methoxy-acetophenone (**4**) by the investigated microorganism.

The different behavior of *D. igniaria* was observed against *para*-methyl- and *para*-bromo-substituted acetophenones **6** and **7**. The transformation of *para*-methyl ketone **6** gave (*R*)-alcohol, whereas *para*-bromo ketone **7** gave (*S*)-alcohol. Initially, both substrates were reduced with low selectivity or yield to *S*-alcohols (compound **6**: 70% ee, 51% yield, and compound **7**: 7% ee, 94% yield). The amount of (*R*)-1-(4-methylphenyl)-ethanol (**6a**) and the amount of (*S*)-1-(4-bromophenyl)-ethanol (**7a**) increased with prolonged transformation time of respective substrates (Figs. 1 and 2). An inversion of configuration from the (*S*)-**6a** to (*R*)-**6a** was detected after 4 days of transformation of **6**. Time course of reaction suggested that ketone **6** was converted into a mixture of (*S*)-**6a** and (*R*)-**6a**, but only (*S*)-**6a** could be oxidized under the reaction conditions. Thus, possible mechanism of the stereoinversion is that oxidoreduction between (*S*)-**6a**

Table 1 Reduction of acethophenones by *D. igniaria*



1-7 **1a-7a**

Substrate	R	R′	Time (days)[a]	Yield[b,c] (%)	Ee[b] (%)	Config.[d]
1	H	H	3	90 (84)	91	*S*
2	H	F	1	100 (87)	82	*S*
3	2-OCH_3	H	3	95 (90)	>99	*S*
4	3-OCH_3	H	1	92 (90)	90	*R*
5	4-OCH_3	H	6	78 (71)	77	*S*
6	4-CH_3	H	6	70 (63)	81	*R*
7	4-Br	H	6	98 (95)	98	*S*

[a] Optimal time for highest yield and enantioselectivity

[b] Determined by chiral GC analyses

[c] Isolated yield in parentheses

[d] Assigned by comparison of the specific rotations with the literature values

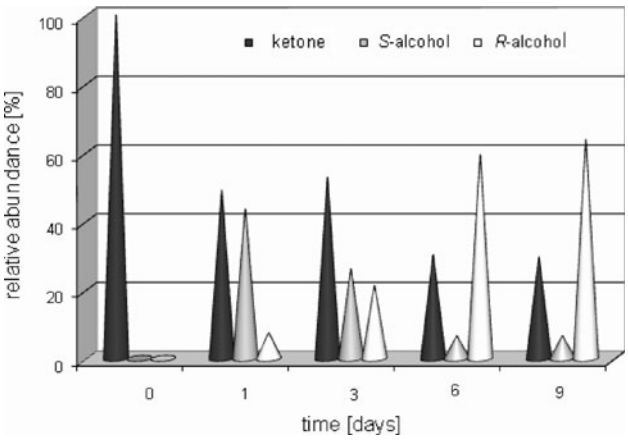


Fig. 1 Time course of biotransformation of *para*-methyl-acetophenone (**6**)

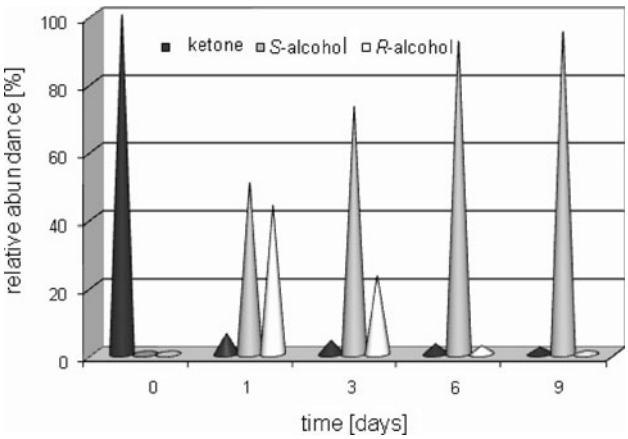
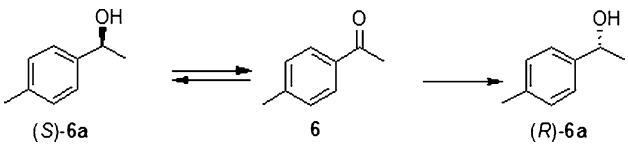
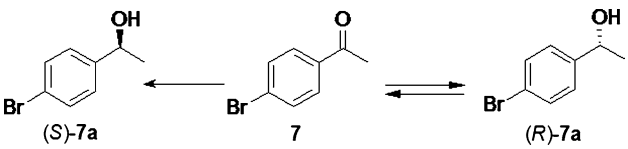


Fig. 2 Time course of biotransformation of *para*-bromo-acetophenone (**7**)



Scheme 1 Mechanistic pathway proposed for the biotransformation of *para*-methyl-acetophenone (**6**)



Scheme 2 Mechanistic pathway proposed for the biotransformation of *para*-bromo-acetophenone (**7**)

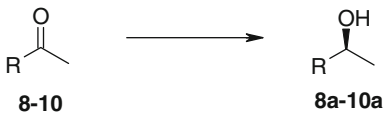
and **6** is reversible and the reduction of **6** to the (*R*)-**6a** is irreversible (Scheme 1). On the contrary, the oxidoreduction from ketone **7** to the corresponding (*R*)-alcohol **7a** is reversible and the reduction from ketone **7** to the (*S*)-alcohol **7a** is irreversible (Scheme 2).

Further, the influence of the nature of *para*-substituents in the aromatic moiety on the substrate reduction rate was also studied. We have observed that reduction of substrate **7** containing a bromine atom proceeds eight times faster than for the methyl-substituted ketone **6** (compare Figs. 1 and 2). Thus, the electron-withdrawing substituent accelerated the reduction, which is in accordance with the results reported by Yang et al. [12], Mączka and Mironowicz [21], Yang et al. [23], Nakamura and Matsuda [35], and Mandal et al. [43].

Reduction of acetylpyridines (**8–10**)

2-, 3-, and 4-Acetylpyridines **8–10** were reduced efficiently to the corresponding *S*-alcohols by *D. igniaria* cells. The time for complete conversion depended on the position of the carbonyl group on the pyridine ring and followed the order **10** ≪ **9** < **8** (Table 2). This might be due to lack (for **10**) of the steric hindrance exerted by hydrated (in the aqueous solution) basic nitrogen on the carbonyl group [6]. The enantioselectivity of these ketone reductions varies, and enantiomeric excess for (*S*)-pyridyl-ethanoles (**8a–10a**) ranges from 57% to >99% ee. It was extremely high (>99% ee) when the acyl group was in the 2-position of the pyridine moiety and it was lowest for the reduction of the 3-acyl pyridine. A similar enantioselectivity was reported for the reduction of the same compounds by yeast *Rhodotorula* sp. AS2.2241 [12], and quite different for the reduction by a mutant strain of bacterium *Pseudomonas putida* UV4 [18].

Table 2 Reduction of acetylpyridines by *D. igniaria*



Substrate	R	Time (days)[a]	Yield[b,c] (%)	Ee[b] (%)	Config.[d]
8	2-pyridyl	5	100 (95)	>99	*S*
9	3-pyridyl	3	100 (92)	57	*S*
10	4-pyridyl	1	100 (95)	82	*S*

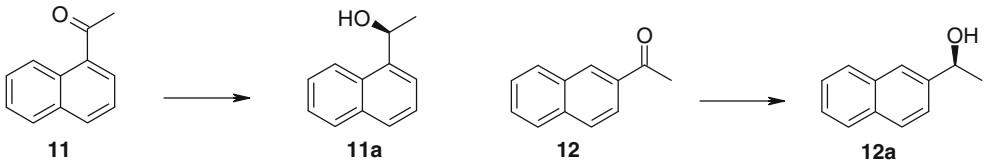
[a] Optimal time for highest yield and enantioselectivity

[b] Determined by chiral GC analyses

[c] Isolated yield in parentheses

[d] Assigned by comparison of the specific rotations with the literature values

Table 3 Reduction of acetonaphthones by *D. igniaria*



Substrate	Time (days)[a]	Yield[b,c] (%)	Ee[b]	Config.[d]
11	3	100 (96)	>99	*S*
	1[e]	100	>99	*S*
12	3	54 (49)	>99	*S*
	1[e]	94	>99	*S*

[a] Optimal time for highest yield and enantioselectivity

[b] Determined by chiral GC analyses

[c] Isolated yield in parentheses

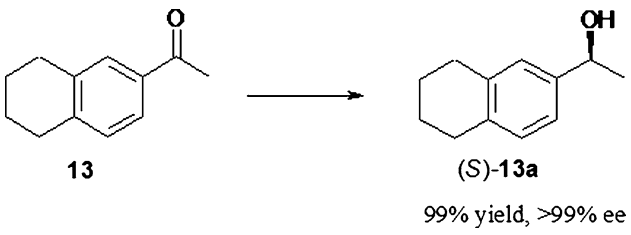
[d] Assigned by comparison of the specific rotations with the literature values

[e] Substrate-induced cultures. Induction conditions: substrate (0.006 mMol) dissolved in acetone (0.02 ml) was added to culture 1 h before transformation

Reduction of acetonaphthones (**11**, **12**)

As shown in Table 3, the incubations of ketones **11** and **12** with *D. igniaria* cells gave the corresponding alcohols with excellent enantioselectivity. Independently of the position of the carbonyl group in the substrate, the enzyme delivered the hydride exclusively from the *re* face of carbonyl group to gave the (*S*)-alcohols. 2-Acetonaphthone (**12**) was a much poorer substrate for reduction by *D. igniaria*. Yield of (*S*)-1-(2′-naphthyl)-ethanol (**12a**) was greatly increased after induction of dehydrogenase by substrate (from 54 to 96%). The both ketones were reduced considerably faster by substrate-induced cells of the microorganism.

In this group of ketones we carried out the reduction of saturated analogue of **12**—1-(5,6,7,8-tetrahydro-naphthalen-2-yl)-ethanone (**13**). The transformation of this substrate during 24 h was complete and gave (*S*)-alcohol with ee above 99% (Scheme 3).

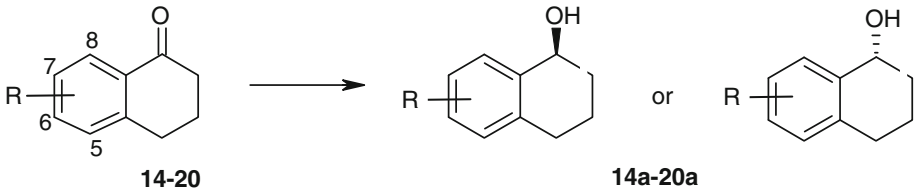


Scheme 3 Reduction of 1-(5,6,7,8-tetrahydro-naphthalen-2-yl)-ethanone (**13**)

Reduction of 1- and 2-tetralones (**14**–**28**)

Whole cells of the fungus *D. igniaria* showed mainly (*S*)-enantiopreference for reduction of 1- and 2-tetralones and their derivatives. Even though, as can be seen in Tables 4 and 5, the yields of these reductions were between 70 and 100%, the enantioselectivities were low. Enantiomerically pure (*S*)-alcohol (>99% ee) could be obtained only from 5-methoxy-1-tetralone (**15**). It clearly appears that providing of hydrogen from the *re*-face of the carbonyl group at C-1 (giving rise to (*S*)-alcohol) is reinforced in the presence of methoxy group at C-5. Surprisingly, this stereoselectivity was reversed when position 7 was occupied. As result of reduction of 7-methoxy-1-tetralone (**17**) the (*R*)-enantiomer of alcohol was obtained. A plausible explanation of this effect could be that it is determined by the equilibrium between the reduction and oxidation of resulting alcohol products. It is known that reduction of 1-tetralones may be accompanied by oxidation of the resulting alcohols [44]. Therefore we studied the time-course of transformation of this substrate. These experiments evidently indicated (Fig. 3) that this product was the result of preference of *R*-reduction of **17** by investigated microorganism, and that alcohol products do not appear to be reoxidized to ketone. A similar result was encountered in the reduction of 5,7-dimethyl-1-tetralone (**18**). *D. igniaria* exhibited no reduction towards 6-methoxy—(**16**), 6,7-dimethoxy—(**19**), and 5,8-dimethoxy—(**20**) 1-tetralones. This result can be caused by the electron-donating effect of the *para*-methoxy group in the aromatic ring of the ketone, or by steric hindrance of bulky substituents, respectively. To confirm this hypothesis, two additional substrates: 2,3-dihydro-1*H*-phenanthren-4-one (**21**)

Table 4 Reduction of 1-tetralones by *D. igniaria*



14-20 **14a-20a**

Substrate	R	Time (days)[a]	Yield[b,c] (%)	Ee[b] (%)	Config.[d]
14	H	3	71 (65)	78	*S*
15	5-OCH_3	3	97 (90)	>99	*S*
16	6-OCH_3	4	n.d.	n.d.	n.d.
17	7-OCH_3	3	92 (86)	41	*R*
18	5,7-$(CH_3)_2$	3	70 (60)	52	*R*
19	6,7-$(OCH_3)_2$	4	n.d.	n.d.	n.d.
20	5,8-$(OCH_3)_2$	4	n.d.	n.d.	n.d.

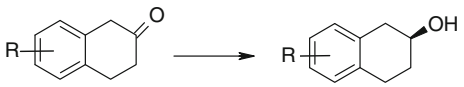
[a] Optimal time for highest yield and enantioselectivity

[b] Determined by chiral GC analyses

[c] Isolated yield in parentheses

[d] Assigned by comparison of the specific rotations with the literature values

Table 5 Reduction of 2-tetralones by *D. igniaria*



23-28 **23a-28a**

Substrate	R	Time (days)[a]	Yield[b,c] (%)	Ee[b] (%)	Config.[d]
23	H	1	100 (95)	3	*S*
24	6-OCH_3	1	96 (90)	40	*S*
25	7-OCH_3	1	92 (90)	52	*S*
26	8-OCH_3	1	98 (96)	53	*S*
27	6-Cl	3	100 (96)	43	*S*
28	6-Br	3	n.d.	n.d.	n.d.

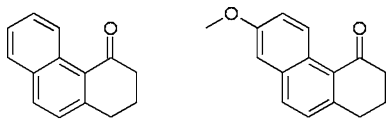
[a] Optimal time for highest yield and enantioselectivity

[b] Determined by chiral GC analyses

[c] Isolated yield in parentheses

[d] Assigned by comparison of the specific rotations with the literature values

and its 7-methoxy derivative (**22**) were tested and it turns out, in accordance with our anticipations, that they undergo almost completely negligible conversion.



21 **22**

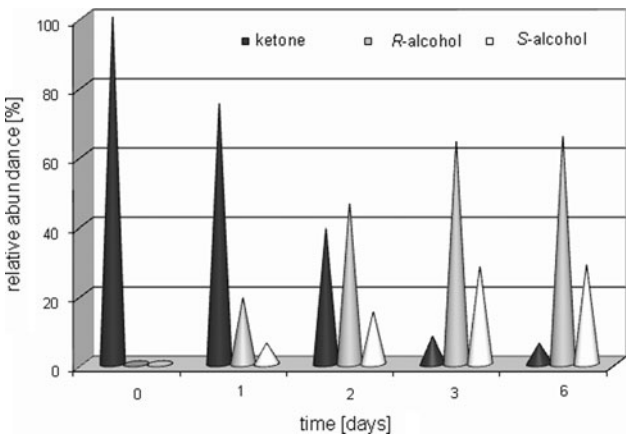


Fig. 3 Time course of transformation of 7-methoxy-1-tetralone (**17**) by *D. igniaria*

2-Tetralones were reduced significantly faster than their 1-isomers. Unfortunately, the enantioselectivity of this reaction was moderate to low (extremely low for 2-tetralone (**23**) which led to the formation of practically racemic mixture of alcohols). The substituted 2-tetralones (**24**–**27**) independently of nature and position of substituent in the benzene ring gave alcohols with higher enantiomeric excess (40–53% ee). The similar results was reported on the reduction by *Fusarium culmorum* [44]. However, 6-bromo-2-tetralone (**28**) cannot be reduced by *D. igniaria*.

Conclusions

It has been shown that *D. igniaria* acts as a new biocatalyst for enantioselective reduction (without oxidase activity against the majority alcohol produced) of variety of aryl-aliphatic ketones. In some cases, the reaction occurred in high yield and enantiomeric excess. Enantiomerically pure (*S*)-alcohols were obtained after reduction of 2-methoxy-acetophenone (**3**), 2-acetylpiridyne (**8**), 1- and 2-acetonaphthones (**11** and **12**), 1-(5,6,7,8-tetrahydro-naphthalen-2-yl)-ethanone (**13**) and 5-methoxy-1-tetralone (**15**). Both high ee and conversion yields allowed us to propose this method as a practical asymmetric synthesis route leading to those alcohols. The interesting reaction pathways were observed in transformations of *para*-substituted derivatives of acetophenone. The transformation of *para*-methyl ketone gave (*R*)-alcohol, whereas *para*-bromo ketone gave (*S*)-alcohol. At first, reduction of those ketones occurred with low selectivity. Reduction of those substrates was accompanied by oxidation of the resulting alcohols. Selective oxidation was observed for (*S*)-1-(4-methylphenyl)-ethanol (**6a**) and (*R*)-1-(4-bromophenyl)-ethanol (**7a**). Re-oxidation was not observed in transformation of *para*-methoxy-acetophenone (**5**). This behavior is different from the one described in literature [13, 35, 41, 42].

References

1. Patel RN (2002) Microbial/enzymatic synthesis of chiral intermediates for pharmaceuticals. Enzyme Microb Technol 31:804–826
2. Panke S, Wubbolts M (2005) Advances in biocatalytic synthesis of pharmaceutical intermediates. Curr Opin Chem Biol 9:188–194
3. Yadav JS, Reddy GSKK, Sabitha G, Krishna AD, Prasad AR, Hafeez-U-R-Rahaman, Vishwaswar Rao K, Bhaskar Rao A (2007) *Daucus carota* and Baker's yeast mediated bio-reduction of prochiral ketones. Tetrahedron Asymm 18:717–723
4. Manitto P, Speranza G, Monti D, Fontana G, Panosetti E (1995) Baker's yeast mediated reduction of aromatic ring substituted 2-tetralones. Tetrahedron 51:11531–11546
5. Li Y-N, Shi X-A, Zong M-H, Meng C, Dong Y-Q, Guo Y-H (2007) Asymmetric reduction of 2-octanone in water/organic solvent biphasic system with Baker's yeast FD-12. Enzyme Microb Technol 40:1305–1311
6. Soni P, Kaur G, Chakraborti AK, Banerjee UC (2005) *Candida viswanathii* as a novel biocatalyst for stereoselective reduction of heteroaryl methyl ketones: a highly efficient enantioselective synthesis of (*S*)-α-(3-pyridyl)ethanol. Tetrahedron Asymm 16:2425–2428
7. Kamble AL, Soni P, Banerjee UC (2005) Biocatalytic synthesis of *S*(-)-1-(1′-naphthyl) ethanol by a novel isolate of *Candida viswanathii*. J Mol Catal B Enzym 35:1–6
8. Nakamura K, Inoune Y, Matsuda T, Misawa I (1999) Stereoselective oxidation and reduction by immobilized *Geotrichum candidum* in organic solvent. J Chem Soc Perkin Trans 1:2397–2402
9. Nakamura K, Takenaka K, Fujii M, Ida Y (2002) Asymmetric synthesis of both enantiomers of secondary alcohols by reduction with a single microbe. Tetrahedron Lett 43:3629–3631
10. Ni Y, Xu J-H (2002) Asymmetric reduction of aryl ketones with a new isolate *Rhodotorula* sp. AS2.2241. J Mol Catal B Enzym 18:233–241
11. Żymańczyk-Duda E, Brzezińska-Rodak M, Lejczak B (2004) Stereochemical control of asymmetric hydrogen transfer employing five different kinds of fungi in anhydrous hexane. Enzyme Microb Technol 34:578–582
12. Yang W, Xu J-H, Xie Y, Xu Y, Zhao G, Lin G-Q (2006) Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2.2241 and synthesis of the *β*-blocker (*R*)-nifenalol. Tetrahedron Asymm 17:1769–1774
13. Comasseto JV, Andrade LH, Omori ÁT, Assis LF, Porto ALM (2004) Deracemization of aryl ethanols and reduction of acetophenones by whole fungal cells of *Aspergillus terreus* CCT 4083, *A. terreus* CCT 3320 and *Rhizopus oryzae* CCT 4964. J Mol Catal B Enzym 29:55–61
14. Carballeira JD, Álvarez E, Compillo M, Pardo L, Sinisterra JV (2004) *Diplogelasinospora grovesi* IMI 171018, a new whole cell biocatalyst for the stereoselective reduction of ketones. Tetrahedron Asymm 15:951–962
15. Comasseto JV, Assis LF, Andrade LH, Schoenlein-Crusius IH, Porto ALM (2006) Biotransformation of *ortho*-, *meta*- and *para*-aromatic nitrocompounds by strain of *Aspergillus terreus*: reduction of ketones and deracemization of alcohols. J Mol Catal B Enzym 39:24–30
16. Kurbanoglu EB, Zilbeyaz K, Kurbanoglu NI, Kilic H (2007) Enantioselective reduction of substituted acetophenones by *Aspergillus niger*. Tetrahedron Asymm 18:1159–1162
17. Kurbanoglu EB, Zilbeyaz K, Kurbanoglu NI, Taskin M (2007) Highly enantioselective reduction of acetophenone by locally isolated *Alternaria alternate* using ram horn peptone. Tetrahedron Asymm 18:1529–1532
18. Garrett MD, Scott R, Sheldrake GN (2002) Enantiocomplementary preparation of (*S*)-and (*R*)-1-pyridylalkanols via ketone reduction and alkane hydroxylation using whole cells of *Pseudomonas putida* UV4. Tetrahedron Asymm 13:2201–2204
19. Andrade LH, Comasseto JV, Rodrigues DF, Pellizari VH, Porto ALM (2005) Enantioselective reduction of *ortho*-substituted acetophenones by bacterial strains isolated from medium enriched with biphenyl or diesel fuel. J Mol Catal B Enzym 33:73–79
20. Yadav JS, Nanda S, Thirupathi Reddy P, Bhaskar Rao A (2002) Efficient enantioselective reduction of ketones with *Daucus carota* root. J Org Chem 67:3900–3903
21. Mączka WK, Mironowicz A (2004) Enantioselective reduction of bromo—and methoxy-acetophenone derivatives using carrot and celeriac enzymatic system. Tetrahedron Asymm 15:1965–1967
22. Orden AA, Bisogno FR, Giordano OS, Sanz MK (2008) Comparative study in the asymmetric bioreduction of ketones by plant organs and undifferentiated cells. J Mol Catal B Enzym 51:49–55
23. Yang Z-H, Zeng R, Yang G, Wang Y, Li L-Z, Lv Z-S, Yao M, Lai B (2008) Asymmetric reduction of prochiral ketones to chiral alcohols catalyzed by plants tissue. J Ind Microbiol Biotechnol 35:1047–1051
24. Barros-Filho BA, de Oliveira MCF, Lemos TLG, de Mattos MC, de Gonzalo G, Gotor-Fernández V, Gotor V (2009) *Lentinus strigellus*: a new versatile stereoselective biocatalyst for the bioreduction of prochiral ketones. Tetrahedron Asymm 20:1057–1061
25. Matsuo K, Kawabe S, Tokuda Y, Eguchi T, Yamanaka R, Nakamura K (2008) Asymmetric reduction of ketones with a germinated plant. Tetrahedron Asymm 19:157–159
26. Barbieri C, Caruso E, D'Arrigo P, Pedrocchi-Fantoni G, Servi S (1999) Chemoenzymatic synthesis of (R)- and (S)-3,4-dichlorophenylbutanolide intermediate in the synthesis of sertraline. Tetrahedron Asymm 10:3931–3937

27. Srebnik M, Ramachandran PV, Brown HC (1988) Chiral synthesis via organoboranes. XVIII: selective reduction. XLIII: Diisopinocampheylchloroborane as an excellent chiral reducing reagent for the synthesis of halo alcohols of high enantiomeric purity. A highly enantioselective synthesis of both optical isomers of tomoxetine, fluoxetine, and nisoxetine. J Org Chem 53:2916–2920
28. Okano K, Murata K, Ikariya T (2000) Stereoselective synthesis of optically active pyridyl alcohols via asymmetric transfer hydrogenation of pyridyl ketones. Tetrahedron Lett 41:9277–9280
29. Salvi NA, Chattopadhyay S (2001) Studies on *Rhizopus arrhizus* mediated enantioselective reduction of arylalkanones. Tetrahedron 57:2833–2839
30. Barbieri C, Bossi L, D'Arrigo D, Fantoni GP, Servi S (2001) Bioreduction of aromatic ketones: preparation of chiral benzyl alcohols in both enantiomeric forms. J Mol Catal B Enzym 11:415–421
31. Wang G, Zheng C, Zao G (2006) Asymmetric reduction of substituted indanones and tetralones catalysed by chiral dendimer and its application to the synthesis of (+)-sertraline. Tetrahedron Asymm 17:2074–2081
32. Reddy J, Tschaen D, Shi Y-J, Pecore V, KZatz L, Greasham R, Chartrain M (1996) Asymmetric bioreduction of a β-tetralone to its corresponding (*S*)-alcohol by the yeast *Trichosporon capitatum* MY 1890. J Ferment Bioeng 81:304–309
33. Okhuma T, Hattori T, Ooka H, Inoue T, Noyori R (2004) BINAP/1, 4-diamine-ruthenium(II) complexes for efficient asymmetric hydrogenation of 1-tetralones and analogues. Org Lett 6:2681–2683
34. Janeczko T, Dmochowska-Gładysz J, Białońska A, Ciunik Z (2006) Microbial hydroxylation of chiral bicyclic enones by *Chaetomium sp*. 1 and *Didymosphaeria igniaria* cultures. Biocatal Biotransform 24:458–463
35. Nakamura K, Matsuda T (1998) Asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum*. J Org Chem 63:8957–8964
36. Peters HM, Feigl DM, Mosher HS (1968) Absolute configuration of substituted trifluoromethylcarbinols. J Org Chem 33:4245–4250
37. Homann MJ, Vail RB, Previte E, Tamarez M, Morgan B, Dodds DR, Zaks A (2004) Rapid identification of enantioselective ketone reductions using targeted microbial libraries. Tetrahedron 60:789–797
38. Palmer MJ, Kenny JA, Walsgrove T, Kawamoto AM, Wills M (2002) Asymmetric transfer hydrogenation of ketones using amino alcohol and monotosylated diamine derivatives of indane. J Chem Soc Perkin Trans I 416:427
39. Orsini F, Sello G, Travaini E, Di Gennaro P (2002) A chemoenzymatic synthesis of (2*R*)-8-substituted-2-aminotetralins. Tetrahedron Asymm 13:253–259
40. Matsuda T, Harada T, Nakajima N, Itoch T, Nakamura K (2000) Two classes of enzymes of opposite stereochemistry in an organism: one for fluorinated and another for nonfluorinated substrates. J Org Chem 65:157–163
41. Utsukihara T, Misumi O, Nakajima K, Koshimura M, Kuniyoshi M, Kuroiwa T, Horiuchi CA (2008) Stereoinversion of 1-arylethanols by *Cyanidioschyzon merolae* NEIS-1332. J Mol Catal B Enzym 35:1–6
42. Nakamura K, Fujii M, Ida Y (2001) Stereoinversion of arylethanols by *Geotrichum candidum*. Tetrahedron Asymm 12:3147–3153
43. Mandal D, Ahmad A, Khan MI, Kumar R (2004) Enantioselective bioreduction of acetophenone and its analogues by the fungus *Trichothecium* sp. J Mol Catal B Enzym 27:61–63
44. Świzdor A, Kołek T (2009) Asymmetric reduction of tetralones and their methoxy derivatives by *Fusarium culmorum*. Biocatal Biotransform 27:179–185