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Regio- and enantio-selective oxidation of diols by *Candida parapsilosis* ATCC 7330

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Selectivity between primary and secondary alcohols was observed in oxidation using whole cells of *Candida parapsilosis* ATCC 7330, where the secondary alcohol was preferentially oxidized. In racemic *sec* alcohols, the '*R*' enantiomer was selectively oxidized to the corresponding keto alcohol (yield = 18–54%) leaving the '*S*' diol (yield = 31–69% and enantiomeric excess from 14% to >99%). A biphasic system consisting of isooctane–water (48 : 2 v/v) was used as a medium for biotransformation at 25 °C. This is the first report of the regio- and enantio-selective oxidation of diols using *C. parapsilosis* ATCC 7330.

Introduction

Optically pure substituted 1-phenylethane-1,2-diols are useful in the synthesis of pharmaceuticals, liquid crystals, agrochemicals,[1] inhibitors of fatty acid amide hydrolase,[2] antiviral agents,[3] anti-cancer compounds,[4,5] (*R*)-selegiline (which is widely used, together with L-DOPA, in the treatment of Parkinson's disease as well as Alzheimer's disease),[6] and scyphostatin (which is a potent inhibitor of neutral sphingomyelinase).[7] Chemical syntheses of optically pure diols by dihydroxylation of olefins using ligands such as cinchona alkaloid derivatives have been reported but the ligands need to be synthesized.[8] Hydrogenation by a rhodium catalyst involves a multi-step synthesis of the ligand[9] and asymmetric borane reduction by Corey–Bakshi–Shibata (CBS) reagent involves protection and deprotection of primary alcohols.[10] Biocatalytic preparations of optically pure diols have been reported for *Geotrichum candidum* IFO 5767 *via* stereoinversion,[11] asymmetric reduction of the corresponding phenylglyoxals,[12] chemo-enzymatic synthesis using immobilized lipase from *Pseudomonas cepacia*[13] and enantioselective hydrolysis by epoxide hydrolase from the potato *Solanum tuberosum*.[14] Optically pure diols can also be prepared by regio- and enantio-selective oxidation of secondary alcohols in diols.

Regio- and enantio-selective oxidation of secondary alcohols in diols gives keto alcohols together with optically pure diols. In the present study, substituted 2-hydroxy-1-phenylethanones are formed by the regio- and enantio-selective oxidation of secondary alcohols of diols. These ketones are starting materials for the syntheses of inhibitors of Tie-2 and VEGFR-2 receptor tyrosine kinases,[15] antibacterial LpxC inhibitors for the treatment of Gram negative infections,[16] androgen receptor modulators,[17] and isotopically labelled structurally diverse α-amino acids, which are useful for research on amino acid and protein metabolism.[18] Preparation of 2-hydroxy-1-phenylethanones has been reported using chemical oxidizing agents. Chemical oxidation of alcohols is a well-established reaction in organic chemistry, but reagents for selective oxidation are still limited.[19] Several chemical reagents have been used to selectively oxidize secondary alcohols in the presence of primary alcohols, but they are not enantioselective.[20] In the case of biocatalysts, selective oxidation of primary alcohols over secondary alcohols has been reported using *Sphingomonas* sp. HXN-200 for 3-*O*-benzylglycerol, phenyl-1,2-ethanediol, *p*-chlorophenyl-1,2-ethanediol and *p*-methylphenyl-1,2-ethanediol.[21] Selective oxidation of secondary alcohols over primary alcohols for *n*-octane-1,2-diol, hexane-1,5-diol and 2,3-octanediol has also been reported using the alcohol dehydrogenase (ADH-'A') from *Rhodococcus ruber* DSM 44541 overexpressed in *Escherichia coli*,[22] and in 1,*n*-alkane diols (n = 2–6) by *R. ruber* DSM 44541.[23]

Candida parapsilosis ATCC 7330 has been used for deracemization of *sec* alcohols, asymmetric reduction of prochiral ketones and separation of DL-N-protected amino acid esters.[24–28] Mechanistically, the deracemization using *C. parapsilosis* ATCC 7330 proceeds *via* stereo-inversion, *i.e.*, enantioselective oxidation followed by reduction [a keto intermediate was observed using high-performance liquid chromatography (HPLC)].[29] Another mechanistic investigation of such deracemization using a deuterated substrate also showed the formation of an undeuterated product, which is possible when the oxidation of an enantiomer is followed by its reduction.[30] These investigations have led us to carry out the enantioselective oxidation of allylic alcohols and 4-phenylbutan-2-ols was established.[31]

To address the selectivity in the oxidation between primary and *sec* alcohols, 1-phenyl-1,2-ethanediol was selected as a substrate for oxidation using the same biocatalyst in the current

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work. Regio- and enantio-selective oxidation of the *sec* alcohol group in preference to the primary alcohol was observed. In this molecule, only the '*R*' diol is oxidized to the keto alcohol, leaving the '*S*' diol intact. This study also presents the regio- and enantio-selective oxidation of other diols [2–11], where the *sec* alcohol group is oxidized in preference to the primary alcohol group, except for **7** and **8** where one of the two *sec* alcohol groups present in the molecule was selectively oxidised. This is the first report for the biocatalytic regio- and enantio-selective oxidation of diols [1–11] from the corresponding racemic alcohols using whole cells of *C. parapsilosis* ATCC 7330. An earlier kinetic resolution of (1*R*,2*S*)-1-phenyl-1,2-propanediol to obtain the keto (*S*)-alcohol was reported using resting cells of *Saccharomyces cerevisiae*.[32] Similarly, asymmetric oxidation of (1*S*,2*S*)-1,2-diphenylethane-1,2-diol was reported using *N*-bromosuccinimide in the presence of a chiral copper catalyst.[33] In the present study, the starting material is a mixture of all four diastereomers, which is certainly an advantage as it eliminates a step in the purification of the starting material, and gives the product keto (*S*)-alcohol with high enantiomeric excess (ee) and yield, except in the case of diol **8**. For the regio- and enantio-selective oxidation of racemic diols [1–11], chemical catalyst based methods are not reported so far in the literature.

Results and discussion

1-Phenylethane-1,2-diol **1** (Scheme 1, Table 1) was the model substrate for regio- and enantio-selective oxidation of diols using whole cells of *C. parapsilosis* ATCC 7330. For the reaction, a 14 h culture was used based on our previous report.[34] Different parameters such as type of reaction medium, reaction time, cosolvent screening, substrate concentration and acetone (cosolvent) concentration were optimized in order to maximize the conversion to the corresponding keto alcohol. Formation of product was confirmed using HPLC using a reverse-phase C_{18} column.

Optimization studies

Reaction medium. A major challenge in most biocatalysed reactions is that they are generally water-based, and high loading of organic substrates is difficult in this medium. (A few enzymes such as lipases are active in organic and other unconventional solvents.[35,36]) It is therefore very important to study the effects of solvents on these biocatalysed reactions. Initially different solvents, namely, water, buffer, and a 48 : 2 v/v mixture of hexane and water[37] were tested for the biotransformation. The mixture of hexane and water was investigated because this mixture optimized the oxidation of primary alcohols using the same biocatalyst, according to our unpublished data. The reaction was monitored every 4 h for up to 48 h. The substrate concentration used was 0.03 mM (which amounts to 4 mg). A portion of acetone (500 μl, *i.e.*, 1% of the final volume of 50 ml of the hexane–water mixture) and 2.6 g of wet cells of *C. parapsilosis* ATCC 7330 were also used. (Because the substrate is a solid, acetone (cosolvent) was used to dissolve the substrate and was added to the reaction medium.) The alcohol was not oxidised in water and buffer in 48 h. In the mixture of hexane–water, a 13.22% conversion to the corresponding keto alcohol **1a** was observed in 24 h. Furthermore, different ratios of hexane–water (*i.e.*, (48 : 2 v/v), (45 : 5 v/v) and (25 : 25 v/v) were tested and it was found that 48 : 2 (v/v) was optimum. But the conversion was only 13.22% in a reaction time of 24 h.

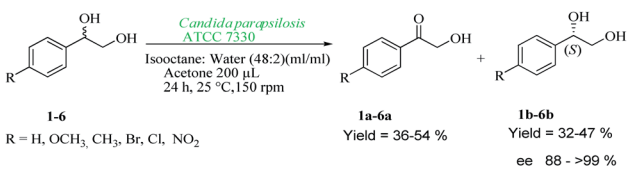
Reaction time. In order to improve the conversion to the keto alcohol, 'isooctane' was used to replace the hexane. The reaction time was monitored for a 48 : 2 (v/v) mixture of isooctane to water from 0 to 30 h at a constant substrate concentration of 0.03 mM (4 mg) with 500 μl of acetone (*i.e.*, 1% of the final volume of 50 ml of the 48 : 2 (v/v) isooctane–water mixture), and with 2.6 g of wet fungal cells. For this mixture, a maximum conversion of 36% was observed at 24 h and the same solvent system was used for further studies.

Cosolvent screening. To increase the conversion to the corresponding keto alcohol, different cosolvents, such as 1,4-dioxane, acetonitrile (ACN), dimethylsulfoxide, tetrahydrofuran and dimethylformamide, were used instead of acetone. Very low conversions of 3–15% were observed for the cosolvents other than acetone. From this it is clear that acetone was useful not only for dissolving the substrate but also for cofactor regeneration.[38] Acetone was used as cosolvent for further reactions.

Substrate concentration. At 24 h, the conversion was monitored at varying substrate concentrations, *i.e.*, from 0.02–0.07 mM, with 500 μl of acetone, (*i.e.*, 1% of the final 50 ml volume of the 48 : 2 (v/v) isooctane–water mixture), and 2.6 g of wet fungal cells. A maximum conversion of 41% was observed at 0.04 mM substrate (which amounts to 6 mg).

Acetone concentration. Furthermore, the amount of acetone (cosolvent) was optimized by testing volumes between 50 μl and 1000 μl in a final volume of 50 ml. The maximum conversion of 45% was observed with 200 μl of acetone (*i.e.*, 0.4% of the final reaction volume of 50 ml).

Under the previously mentioned optimum reaction conditions, yield experiments were carried out for 0.52 mM (72 mg) substrate. Conversion of 1-phenylethane-1,2-diol **1** to the corresponding 2-hydroxy-1-phenylethanone **1a** was 45.35% with an isolated yield of 38.22% (27.12 mg). The unreacted optically pure (*S*)-1-phenylethane-1,2-diol **1b** was also isolated (yield 46.07% *i.e.*, 33.17 mg, ee 97%). The synthesis of optically pure (*S*)-1-phenylethane-1,2-diols were reported previously using the same biocatalyst *via* asymmetric reduction of the corresponding phenylglyoxals,[12] indicating the presence of multiple oxidoreductases in *C. parapsilosis* ATCC 7330 and highlighting the fact that different oxidoreductases act on different substrates under different reaction conditions.



Scheme 1 Regio- and enantio-selective oxidation of *p*-substituted 1-phenylethane-1,2-diols **1–6** by *C. parapsilosis* ATCC 7330.

Table 1 Regio- and enantio-selective oxidation of *p*-substituted 1-phenylethane-1, 2-diols **1–6** by *C. parapsilosis* ATCC 7330

Entry	R	Conversion to keto alcohol[a] (%) **1a–6a**	Isolated yield of keto alcohol[b] (%) **1a–6a**	Unreacted diol[a] (%) (from HPLC) **1b–6b**	Isolated yield of diol[b] (%) **1b–6b**	ee (%) **1b–6b**	Reaction time (h)	Specific rotation $[\alpha]_D^{30\ °C}$ **1b–6b**
1[c]	H	45.35 ± 2.01	38.22 ± 1.90	54.65 ± 2.01	46.07 ± 2.31	97	24	+64.86 (C1, $CHCl_3$)[12]
2	*p*-OCH_3	59.06 ± 0.07	51.12 ± 0.16	40.94 ± 0.07	32.38 ± 0.23	>99	24	+60.17 (C0.5, $CHCl_3$)[12]
3	*p*-CH_3	61.70 ± 0.22	54.11 ± 0.32	38.30 ± 0.22	31.90 ± 0.01	>99	24	+68.45 (C1.12, $CHCl_3$)[12]
4[c]	*p*-Br	43.66 ± 0.25	36.3 ± 0.06	56.34 ± 0.25	47.12 ± 0.48	88	24	+38.19 (ref. 12) (C1.0, $CHCl_3$)[12]
5[c]	*p*-Cl	45.95 ± 0.06	38.48 ± 0.96	54.05 ± 0.06	46.69 ± 0.33	98	24	+50.96 (C1.6, $CHCl_3$)[12]
6	*p*-NO_2	—	—	100	—	—	72	—

[a] Conversion was checked by HPLC. [b] Isolated yields were calculated for 0.52 mM (72 mg) substrate as starting material. [c] The keto alcohol [4.05–6.34%] was enantio-selectively reduced to the (*S*) diol in the case of 1, 4 and 5, and this is why an increase in ee is observed.

The benefit of regio- and enantio-selective oxidation over asymmetric reduction of the prochiral ketones is two-fold—the corresponding oxidized keto alcohol is also formed with a yield of 50% along with the optically pure diol.

Note that only a few chemical reagents are available for the selective oxidation of *sec* alcohols in the presence of primary alcohols, *i.e.*, in a diol. Selective oxidation of the secondary alcohol in 1-phenylethane-1,2-diol **1** was reported using SiO_2-supported ruthenium chloride and 3-(dichloroiodo)benzoic acid with a yield of 26% in 3 h, but here benzaldehyde was also isolated with a yield of 74% (which is because of over-oxidation to a keto acid followed by decarboxylation).[39] In another report, oxidation to a keto alcohol used a resin/2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) for 24 h with a yield of 44%.[40] Biocatalytic oxidation of **1** has not been reported so far. Biocatalytic oxidative kinetic resolution of **1** was reported using glycerol dehydrogenase for 64 h (conversion 50%, ee >99%).[41] Another report for the preparation of **1b** was by stereoinversion using *C. parapsilosis* CCTCC M203011 for 60 h (yield 90%, ee 99.02%).[42] Compound **1b** was also prepared by biocatalytic asymmetric dihydroxylation of styrene for 43 h (yield 76%, ee 99.9%) using styrene monooxygenase from *E. coli* JM101 and the epoxide hydrolase from *Sphingomonas* sp. HXN-200.[43] Thus, it can be seen that the method reported here is better in terms of yield for the production of keto alcohol **1a** and in terms of the reaction time for the production of optically pure (*S*)-diol **1b**.

Effect of substitution on aromatic ring. To study the effect of substitution on the aromatic ring, different *para*-substituted 1-phenylethane-1,2-diols **2–6** (Scheme 1, Table 1) were subjected to oxidation using the previously optimized conditions. In all the cases regio- and enantio-selective oxidation was seen. Electron donating groups, namely, *p*-OMe and *p*-Me on the aromatic ring increased the conversion to the corresponding keto alcohol. The presence of the *p*-OMe group in the case of 1-(4-methoxyphenyl)ethane-1,2-diol **2** increased the conversion to the corresponding 2-hydroxy-1-(4-methoxyphenyl)ethanone **2a** to 59.06% with a yield of 51.12% (36.36 mg). The corresponding unreacted optically pure (*S*)-diol **2b** was isolated with a 32.38% yield (23.31 mg). Similarly, a *p*-Me group in the case of 1-*p*-tolylethane-1,2-diol **3** gave a 61.70% conversion to the corresponding 2-hydroxy-1-*p*-tolylethanone **3a**, with a yield of 54.11% (38.44 mg) while the unreacted optically pure (*S*)-diol **3b** gave a yield of 31.9% (22.97 mg). The presence of the *p*-Br group in the case of 1-(4-bromophenyl)ethane-1,2-diol **4** decreased the conversion to the corresponding 1-(4-bromophenyl)-2-hydroxyethanone **4a** to 43.66%, giving a yield of 36.3% (25.89 mg). The unreacted optically pure (*S*)-diol **4b** gave a yield of 47.12% (33.93 mg). Similarly, in the case of the *p*-Cl substituent 1-(4-chlorophenyl) ethane-1,2-diol **5**, the conversion to the corresponding 1-(4-chlorophenyl)-2-hydroxyethanone **5a** was 45.95%, and the yield was 38.48% (27.38 mg). The unreacted optically pure (*S*)-diol **5b** gave a yield of 46.69% (33.62 mg). The presence of the electron withdrawing group *p*-NO_2 in the case of 1-(4-nitrophenyl)ethane-1,2-diol **6** did not result in the formation of the corresponding oxidized keto alcohol **6a** even after 72 h.

Preparation of **2a**, **3a** and **5a** from the corresponding diols by oxidation using silica-encapsulated phosphotungstic acid ($H_3PW_{12}O_{40}$) as a recyclable heterogeneous photocatalyst was reported to occur in 1 to 1.5 h with yields of 84–90%. The catalyst needed to be synthesised, and the use of a metal, which is not environmentally benign, was unavoidable.[44] In another report, the oxidation of **2** and **5** to the corresponding hydroxy ketones **2a** and **5a** uses tetrapropylammonium perruthenate (TPAP), and involves three steps and in this case the primary alcohol had to be protected for the selective oxidation of the secondary alcohol.[45] The synthesis of **3a** was also reported using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone for 5 h with a yield of 67%, but here the corresponding keto aldehyde was also formed in an 8 : 1 ratio.[46] Oxidation of **4** to the corresponding hydroxy ketone **4a** was reported using 3,3-diiodo-2,2,6,6-tetramethoxy-4,4-biphenyldicarboxylic acid (DIDA)/Oxone® for 13 h, which gave a yield of 82%; note that the synthesis of DIDA involves multiple steps.[47] Thus, the present method is better than the others: it is relatively 'green' for the preparation of hydroxy ketones from the corresponding diols, requires neither synthesis of a catalyst nor protection and deprotection, and there is no over-oxidation to keto aldehyde/ketoacid.

(*S*)-1-(4-Methoxyphenyl)ethane-1,2-diol **2b** was shown to be synthesized *via* an asymmetric reduction using *Yamadazyma*

farinosa IFO 10896 for 48 h (yield 95%, ee >99%).[45] Lithiated *N*-boc thiazolidine which is obtained by multi step synthesis, has also been reported for the synthesis of **2b** [yield 55%, ee 66%].[48] Synthesis of **3b** (yield 96%, ee >99%) by asymmetric reduction of the corresponding keto alcohol using an *N*-phenylamine–borane complex was reported, and it involved protection and deprotection of the primary alcohol.[10] (*S*)-1-*p*-Tolylethane-1,2-diol **3b** was also prepared within 12 h using lipase (yield 45%, ee 77%) in three steps.[49] Ten hour syntheses of (*S*)-1-(4-bromophenyl)ethane-1,2-diol **4b** (yield 39%, ee 91%) and (*S*)-1-(4-chlorophenyl)ethane-1,2-diol **5b** (yield 47%, ee 72%) were reported using *Ps. cepacia* lipase.[49] Syntheses of **4b** (yield 62%, ee 94%) and **5b** (yield 67%, ee 98%) were reported using 25 mol% of a polymer supported chiral sulfonamide, $NaBH_4$/Me_3SiCl for 1.5 h under reflux conditions.[50] Significantly, the current study presents a simple method for the preparation of optically pure diols with good ee and yields, together with the corresponding keto alcohols under mild reaction conditions at room temperature.

Substrate scope. To expand the substrate scope, different substrates, in particular **7–11** (Chart 1, Table 3), were subjected to the biotransformation. In the case of 1-phenylpropane-1,2-diol **7** {for which the ratio of *erythro* [(*R*,*S*) and (*S*,*R*)] : *threo* [(*R*,*R*) and (*S*,*S*)] was 78 : 22 based on nuclear magnetic resonance (NMR)}, the reaction time was increased significantly from 24 h to 72 h. The conversion to (*S*)-2-hydroxy-1-phenylpropan-1-one **7a** was 49.67% (yield 42.34%, 30.08 mg, ee 99%). The other product was the corresponding unreacted diol **7b** (with an 87 : 13 ratio of *erythro* : *threo* based on NMR) [yield 41.59%, 29.95 mg, ee 90.26% (*S*,*R*-major)]. Here the increase in *erythro* and decrease in *threo* in the case of **7b** compared to the starting diol **7** indicates that one of the *threo* forms is oxidised. This oxidation was confirmed using NMR and HPLC,[51] and it was the (*S*,*S*) isomer that was oxidised completely to the (*S*)-keto alcohol. The overall formation of the (*S*)-keto alcohol **7a** is because of the oxidation of *erythro* (*R*,*S*) and *threo* (*S*,*S*) (confirmed by HPLC). Unreacted diol **7b** is a mixture of *erythro* [(*S*,*R*)-major (ee 90.26%), (*R*,*S*)-minor] and *threo* (*R*,*R*). An *in situ* generated dioxirane (from the fructose derived ketone) was used previously to carry out a two hour synthesis of **7a** using *threo* 1-phenylpropane-1,2-diol with an ee of 69%, and from *erythro* with an ee of 23%, where the conversion as calculated from the results of ^{1}H-NMR was 20% and 34% respectively. The corresponding 2-hydroxy-1-phenyl-1-propanone and 1-hydroxy-1-phenyl-2-propanone are formed in an 84 : 16 ratio for *threo* and 89 : 11 ratio for *ethryo*.[52] Another preparation of **7a** used (1*R*,2*S*)-1-phenyl-1,2-propanediol and resting cells of *S. cerevisiae* at 30 °C: the product was recovered in 7 days (yield 64%, ee 93%), and the corresponding diketone was also formed.[32] In the present method, (*S*)-2-hydroxy-1-phenylpropan-1-one **7a** was formed in 72 h with a good ee [99%], and without any side product. In the same reaction time of 72 h, the presence of a phenyl group instead of a methyl group as in the case of 1,2-diphenylethane-1,2-diol **8** [(*meso* : ±) : (88 : 12) ratio based on NMR], reduced the conversion to the corresponding keto alcohol **8a** to 24.41% [yield 18.06% (12.88 mg), ee 14% (*R*)]. The unreacted diol **8b** [(*meso* : ±) : (78 : 22) ratio from NMR] gave a yield of 68.92% [49.62 mg, ee 14% (*R*,*R*)]. This decrease in *meso* [(*S*,*R*) or (*R*,*S*)], and corresponding increase in racemic [(*R*,*R*) and (*S*,*S*)] in the case of **8b**, compared to the starting diol **8** indicates that the *meso* form gets oxidised to the corresponding (*R*)-keto alcohol **8a**. Reduced yields for **8** as compared to **7** were also reported in the case of catalytic asymmetric dihydroxylation of olefins,[53] possibly because of steric hindrance.

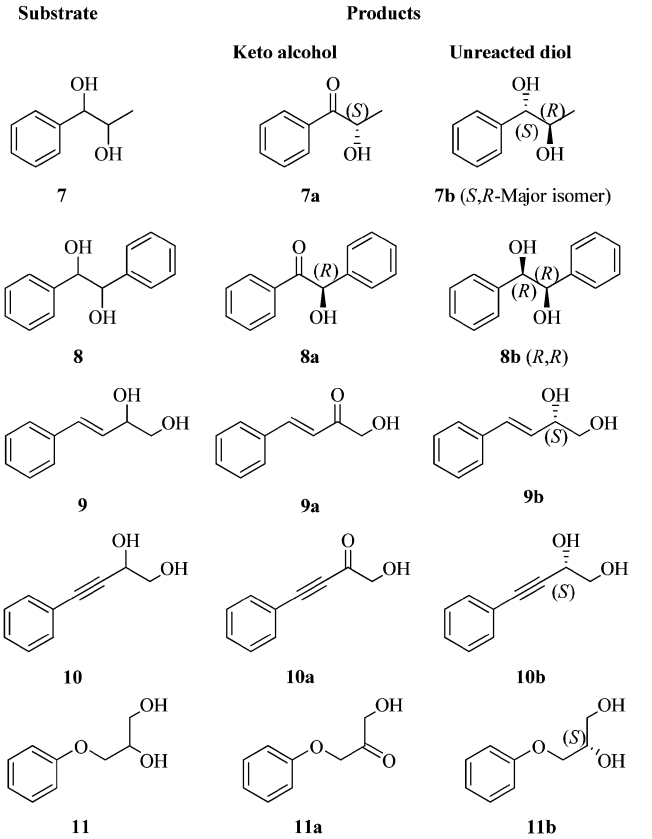


Chart 1 Regio- and enantio-selective oxidation of diols **7–11** by *C. parapsilosis* ATCC 7330.

Introduction of a double bond between the phenyl ring and diol in the case of **9** (Chart 1, Table 3) reduced the reaction time from 24 h to 7 h, as was also reported for allylic alcohols.[31] Conversion to an unsaturated keto alcohol **9a** [yield 22.02% (15.66 mg)] and the corresponding saturated keto alcohol 1-hydroxy-4-phenyl-2-butanone was 63.14% [yield 30.45% (21.92 mg)]. Because the unsaturated keto alcohol **9a** and saturated keto alcohol could not be separated, yields were calculated based on NMR [ratio of unsaturated and saturated keto alcohols was 1 : 1.4]. The unreacted optically pure diol **9b**, [yield 30.74% (22.13 mg), ee >99%] was also formed.

Oxidation of **9** to **9a** has not been reported so far in the literature. Earlier, a synthesis of **9b** that took 24 h was reported using the same catalyst *via* deracemization (yield 86%, ee >99%).[54] A multi-step synthesis of **9b** (yield 60%, ee 98%) was also reported using CBS-oxazaborolidine.[55]

Introduction of a triple bond between the phenyl ring and the diol in the case of **10** showed very low conversion (5.62%) after 24 h. On extending the reaction time to 72 h, no oxidized

Table 2 Cell viability

Reaction time (h)	Cell viability[a] (%)
24	77
48	39
66	21
80	8

[a] Experiments were performed in triplicate and values given are average.

product was detected. A similar observation was reported for the oxidative kinetic resolution of *rac*-4-pentyn-2-ol, and *rac*-1-octyn-3-ol using *R. ruber* DSM 44541.[56] In the case of **11**, which has an ether linkage in addition to the absence of conjugation, the oxidized product was not seen even after 72 h. The presence of an ether linkage reduced the rate of oxidation in the case of oxidation of phenoxymethanol using the three enzyme system that includes 2-phenylethanol dehydrogenase, phenyl acetaldehyde dehydrogenase and NADH oxidase.[57]

Cell viability. The viability of *C. parapsilosis* ATCC 7330 cells was checked using the reaction medium isooctane–water (48 : 2 v/v) for up to 80 h using the conventional agar plate method.[58] The results are presented in Table 2.

Immobilization studies. Preliminary studies using immobilized *C. parapsilosis* ATCC 7330 cells were carried out. Immobilized cells were prepared using sodium alginate according to the reported procedure[59] and used for the biotransformation under the experimental conditions that were optimized for the present study. Each of the first two cycles showed 100% activity, but the activity decreased to 87% in the 3rd cycle, and decreased drastically to 2% in the 4th cycle.

Experimental

All substituted benzaldehydes and selenium, palladium on carbon were purchased from Spectrochem. Sodium borohydride and calcium chloride dihydrate were purchased from Merck. Sodium alginate was purchased from SRL. Yeast malt agar and yeast malt broth components (glucose, soya peptone, yeast extract powder and malt extract powder) were purchased from Himedia. HPLC analysis of diols was carried out on a Jasco PU-1580 liquid chromatograph with a photodiode array detector. Conversion was checked using a reverse-phase C_{18} column, with ACN–water (60 : 40 v/v) as the mobile phase. Resolution was carried out using Daicel OJ-H, OB-H, OD-H and AD-H chiral columns. A hexane–isopropanol mixture was used as the mobile phase. The proportion of solvents varied for different diols. Optical rotations were recorded on a Rudolph Research Autopol IV digital polarimeter. The characterization of racemic and enantiomerically pure diols was carried out by ^{1}H-NMR and ^{13}C-NMR and spectra were recorded in deuterated chloroform on a Bruker Avance III 500 MHz spectrometer operating at 500 MHz and 125 MHz.

Synthesis of substrates

All substituted 1-phenyl-1,2-ethane diols **1–8**,[12] **11**[61] were prepared using reported methods. Substrates **4**, **7** and **8** (racemic keto alcohol for HPLC analysis) were provided by Pula Mahajabeen. Substrates **9**,[54] and **10**[54,60] were synthesized by Thangavel Saravanan. All substrates were characterized using ^{1}H-NMR and ^{13}C-NMR.

Microorganism maintenance

C. parapsilosis ATCC 7330 was bought from American Type Culture Collection, Manassas, VA 20108, USA and maintained at 4 °C in yeast malt agar (HiMedia). *C. parapsilosis* ATCC 7330 was grown and harvested as per the earlier reported procedure and used for the biotransformation procedures.[66]

General procedure for regio- and enantio-selective oxidation of diols by *C. parapsilosis* ATCC 7330

Isooctane–water (48 : 1 v/v) was mixed ultrasonically for 5 min using a Vibra-Cell ultrasonic processor (pulse 5 s on, 5 s off, amplitude 38) prior to the reaction, to make the reaction medium homogeneous. Wet cells (2.6 g) of *C. parapsilosis* ATCC 7330 suspended in 1 ml of water were added to the isooctane–water mixture to give a total volume of 50 ml. 1-Phenylethane-1,2-diol **1** (0.04 mM, 6 mg) dissolved in 200 μl acetone (*i.e.*, 0.4% of the final 50 ml volume) was added to the above cell suspension, incubated at 25 °C, and mixed at 150 rpm for 24 h. For yield experiments, 0.52 mM (72 mg) of substrate 1 was used (in parallel, in 12 different flasks, *i.e.*, 6 mg × 12 flasks). After reacting for 24 h, the contents of all the flasks were combined and extracted with ethyl acetate (3 × 50 ml), then dried over anhydrous sodium sulfate and concentrated using a rotary evaporator. Conversion to the corresponding keto alcohol **1a** was checked using HPLC with a C_{18} column (Table 1). Enantiomeric excess for the unreacted diol **1b** was determined by using HPLC with an OB-H column. Isolated yields for the products were determined by column chromatography using hexane–ethyl acetate (98 : 2, ml ml^{-1}) as eluent.

The same procedure was followed for the other alcohols **2–6** (Table 1, Scheme 1) and **7–11** (Table 3, Chart 1) with subsequent change in the reaction time for the regio- and enantio-selective oxidation using *C. parapsilosis* ATCC 7330. Reactions were done in triplicate for consistent results and control experiments were carried out in parallel without whole cells and also using heat killed cells under identical conditions. The absolute configuration for all the optically pure alcohols was determined to be (*S*), except for **8**, which gave (*R*,*R*).

General procedure for the biotransformation using immobilized *C. parapsilosis* ATCC 7330 cells

Sodium alginate (100 ml, 2% w/v) and calcium chloride ($CaCl_2$) aqueous solution (100 ml, 2% w/v) were autoclaved prior to the biotransformation.

Wet *C. parapsilosis* ATCC 7330 cells (2.6 g) suspended in 2.8 ml of distilled water were added to 13 ml of the sodium alginate and stirred for 1 h to make a homogeneous mixture. This homogeneous cell suspension was added dropwise to the pre-chilled $CaCl_2$ solution (2% w/v), which resulted in the formation of beads. The beads were kept in the $CaCl_2$ solution for 12

Table 3 Regio- and enantio-selective oxidation of diols **7–11** by *C. parapsilosis* ATCC 7330

Entry	Conversion to keto alcohol[a] (%) **7a–11a**	Isolated yield[b] (%) **7a–11a**	Unreacted diol (%) (from HPLC) **7b–11b**	Isolated yield[b] (%) **7b–11b**	ee (%) **7b–11b**	Reaction time (h)	Specific rotation $[\alpha]_D^{25}$ °C
7	49.67 ± 1.81	42.34 ± 1.62	50.33 ± 1.81	41.59 ± 1.75	>99[d], 90	72	−90.69 (C1, $CHCl_3$)[62] **7a** (*S*) +27.49 (C3.2, $CHCl_3$)[63] **7b** (S,R)
8	24.41 ± 3.08	18.06 ± 2.64	75.59 ± 3.08	68.92 ± 1.68	14[e], 14	72	−10.86 (C1, CH_3COCH_3)[64] **8a** (*R*) +12.77 (C1, EtOH)[65] **8b** (*R*,*R*)
9	63.14 ± 2.93[c]	52.47 ± 0.57[c]	36.86 ± 2.93	30.74 ± 0.59	>99	7	+28.65 (C1, $CHCl_3$)[54] **9b**
10	5.62 ± 2.35	—	94.38 ± 2.35	—	—	24	—
11	—	—	100	—	—	24	—

[a] Conversion was checked by HPLC. [b] Isolated yields were calculated for 72 mg substrate as starting material. [c] Ratio of unsaturated keto alcohol **9a** and the corresponding saturated keto alcohol was 1 : 1.4, based on NMR. [d] ee of **7a**. [e] ee of **8a**.

h, and then washed with distilled water [3 × 100 ml] and stored at 8 °C before biotransformation.

Immobilized cells were added to the isooctane–water (48 : 1 v/v) (ultrasonically mixed as described previously) using 1 ml of distilled water. 1-Phenylethane-1,2-diol **1** (0.04 mM, 6 mg) dissolved in acetone (200 μl, 0.4% of the final volume) was added to the immobilized cells suspended in isooctane–water and incubated at 25 °C, 150 rpm for 24 h. After the 24 h, the immobilized cells were removed by filtration and the products were extracted with ethyl acetate (3 × 10 ml), and analyzed by HPLC. Beads were washed with distilled water (3 × 30 ml) and used for the next cycle.

Spectral data

Spectral data for the products **1a**,[67] **2a**,[67] **3a**,[67] **4a**,[67] **5a**,[67] **7a**,[68] **8a**,[64] **9a**,[69] **1b**,[12] **2b**,[12] **3b**,[12] **4b**,[12] **5b**,[12] **7b**,[63] **8b** [53] and **9b** [54] are in agreement with the values reported in the literature.

HPLC resolution details for all compounds are given next.

2-Hydroxy-1-phenylpropan-1-one 7a. AD-H column [hexane–2-propanol = 95 : 05, 0.5 ml min^{-1}; retention times 15.37 min (*R*-minor), 17.51 min (*S*-major)].

2-Hydroxy-1,2-diphenylethanone 8a. AD-H column [hexane–2-propanol = 90 : 10, 1.0 ml min^{-1}; retention times 9.73 min (*S*-minor), 13.55 min (*R*-major)].

1-Phenylethane-1,2-diol 1b. OB-H column [hexane–2-propanol = 90 : 10, 0.5 ml min^{-1}; retention times 14.01 min (*R*-minor), 17.81 min (*S*-major)].

1-(4-Methoxyphenyl)ethane-1,2-diol 2b. OD-H column [hexane–2-propanol = 98 : 02, 0.8 ml min^{-1}; retention times 68.29 min (*R*-minor), 73.03 min (*S*-major)].

1-(4-Methylphenyl)-1,2-ethanediol 3b. OB-H column [hexane–2-propanol = 90 : 10, 0.5 ml min^{-1}; retention times 22.08 min (*R*-minor), 25.72 min (*S*-major)].

1-(4-Bromophenyl)ethane-1,2-diol 4b. OD-H column [hexane–2-propanol = 98 : 02, 0.8 ml min^{-1}; retention times 67.80 min (*R*-minor), 74.19 min (*S*-major)].

1-(4-Chlorophenyl)ethane-1,2-diol 5b. OD-H column [hexane–2-propanol = 98 : 02, 0.8 ml min^{-1}; retention times 53.36 min (*R*-minor), 59.41 min (*S*-major)].

1-Phenylpropane-1,2-diol 7b. AD-H column [hexane–2-propanol = 98 : 02, 1.0 ml min^{-1}; retention times 40.85 min (*S*,*R*-major), 43.79 min (*R*,*S*)-minor)].

1,2-Diphenylethane-1,2-diol 8b. O-JH column [hexane–2-propanol = 90 : 10, 1.0 ml min^{-1}; retention times 10.53 min (*S*,*S*-minor), 11.84 min (*R*,*R*-major)].

(*E*)-4-Phenylbut-3-ene-1,2-diol 9b. OD-H column [hexane–2-propanol = 90 : 10, 1.0 ml min^{-1}; retention times 13.67 min (*S*-major), 15.55 min (*R*-minor)].

Conclusion

Various 1,2-diols (**1–11**) were regio- and enantio-selectively oxidized using whole cells of *C. parapsilosis* ATCC 7330. Regio- and enantio-selective oxidation of diols **1–9** gave corresponding keto alcohols (**1a–9a**) and optically pure (*S*) diols **1b–9b**, except for **8**, which gave (*R*)-keto alcohol **8a** and (*R*,*R*)-diol **8b**. In the case of **7**, the benzylic hydroxy group was preferentially oxidized over the methyl attached hydroxy group. Enantio-selective oxidation of *sec* alcohols in diols (**2–5**, **9**) is reported for the first time in this study. Excellent enantio-selectivities were observed for the optically pure alcohols (up to >99%) except for **6** and **11**, which did not show any oxidized product even after 72 h. Those compounds whose two alcohol groups are both *sec* alcohols, *i.e.*, **7** and **8**, took a longer time to react compared to the compounds having one primary and one secondary alcohol. Introduction of a double bond in (*E*)-4-phenylbut-3-ene-1, 2-diol **9** reduced the reaction time significantly to 7 h. Introduction of a triple bond, in 4-phenylbut-3-yne-1,2-diol (**10**), showed very low conversion. Compounds **2**, **3** and **9** showed conversions of more than 50%, which may be because of the reaction of more than one enzyme (since the biocatalyst is a whole cell). The present study can also be scaled up to 1 g and above using a reactor. For reactor scale-up, separate optimization of the previous parameters such as substrate concentration is needed for free cells. Immobilized cells also can be used for reactor scale-up as they are reusable.

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