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ARTICLE TYPE

Regio- and enantioselective 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; secondary alcohol was preferred over primary alcohol. 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 ee 14 to >99%). A biphasic system consisting of isooctane: water (48:2) (v/v) was used as medium for biotransformation at 25 ºC. This is the first report for the regio- and enantioselective oxidation of diols using *Candida parapsilosis* ATCC 7330.

Introduction

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

Regio- and enantioselective oxidation of secondary alcohols in diols gives keto alcohols along with optically pure diols. In the present study, substituted 2-hydroxy-1-phenylethanones are formed by the regio- and enantioselective oxidation of secondary alcohols of diols. These ketones are starting materials for the synthesis of inhibitors of Tie-2 and VEGFR2 receptor tyrosine kinases,[15] antibacterial LpxC inhibitors for the treatment of Gram-negative infections,[16] androgen receptor modulators,[17] 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 is 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 are reported for the selective oxidation of 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 is reported using *Sphingomonas* sp. HXN-200 for 3-O-benzylglycerol, phenyl-1,2-ethanediol, *p*-Cl phenyl-1,2-ethanediol and *p*-Me phenyl-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 is reported using alcohol dehydrogenase ADH-'A from *Rhodococcus ruber* DSM 44541 over-expressed in *E. coli*,[22] and in 1,*n*-alkane diols (*n* = 2-6) by *Rhodococcus ruber* DSM 44541.[23]

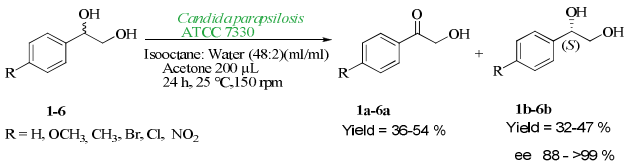
Candida parapsilosis ATCC 7330 is reported for deracemization of *sec.* alcohols, asymmetric reduction of prochiral ketones and resolution of N-protected amino acid esters.[24-28] Mechanistically, deracemization using *C. parapsilosis* ATCC 7330 proceeds *via* stereo-inversion, i.e enantioselective oxidation followed by reduction [keto intermediate was observed in HPLC].[29] Another mechanistic investigation on deracemization using deuterated substrate showed the formation of undeuterated product also, which is possible because of oxidation of one enantiomer followed by its reduction.[30] Based on this clue for oxidation of alcohols, enantioselective oxidation of allylic alcohols and 4-phenylbutan-2-ols was established.[31]

Taking this further and addressing the question of selectivity in oxidation between primary and secondary alcohols, 1-phenyl-1, 2-ethanediol was selected as a substrate for oxidation using the same biocatalyst. Regio- and enantioselective oxidation of the secondary 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 enantioselective 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 selectively one *sec.* alcohol group was oxidised of the two *sec.* alcohol groups present in the molecule. This is the first report for the biocatalytic regio- and enantioselective oxidation of diols [1-11] from the

corresponding racemic alcohols using whole cells of *C. parapsilosis* ATCC 7330. 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 *Saccaromyces 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 chiral copper catalyst.[33] In the present study, the starting material is a mixture of all the four diastereomers which is certainly an advantage as it eliminates a purification step of the starting material and gives the product keto (*S*)- alcohol in high ee and yield except in the case of diol 8. No other chemical methods are reported for the regio- and enantioselective oxidation of racemic diols [1-11].

Results and discussion

1-Phenylethane-1, 2-diol **1** (Scheme 1, Table 1) was the model substrate for regio- and enantioselective oxidation of diols using whole cells of *C. parapsilosis* ATCC 7330. For the reaction, 14 h culture was used based on our previous report.[34] Different parameters like reaction medium, reaction time, cosolvent screening, substrate concentration and acetone (cosolvent) concentration were optimized in order to get maximum conversion to the corresponding keto alcohol. Formation of product was confirmed by HPLC using a reverse phase C18 column.



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

Optimization studies

Reaction medium

A major challenge in biocatalysed reactions is that they are generally water based [a few isolated enzymes *e.g* lipases are active in organic and other unconventional solvents[35, 36]] which makes high loading of organic substrates difficult in this medium. It is therefore very important to study the effects of solvents on these biocatalysed reactions. Initially different solvents *viz*. water, buffer and hexane: water (48:2) (v/v)[37] were tried for the biotransformation. The solvent, hexane: water (48:2) (v/v) was tried based on our unpublished work which was optimized for oxidation of primary alcohols using the same biocatalyst. The reaction was monitored up to 48 h, with a 4 h time interval. The substrate concentration used was 0.03 mM (4 mg), acetone 500 µl, i.e. 1% of the final volume of 50 ml [(hexane: water) (48:2) (v/v)] [since the substrate is a solid, acetone (cosolvent) was used to dissolve the substrate and added to the reaction medium], 2.6 g of wet cells of *C. parapsilosis* ATCC 7330 were used. The alcohol did not get oxidised in water and buffer in 48 h. In hexane: water (48:2, v/v), 13.22% conversion to the corresponding keto alcohol **1a** in 24 h was observed. Further, different ratios of hexane: water [i.e (48:2), (45:5) and (25:25) (v/v)] were tried 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. In order to improve the conversion to keto alcohol 'isooctane' was tried instead of hexane. Result with isooctane is discussed below [under 'reaction time'].

Reaction time

Reaction time was monitored again for isooctane: water (48:2) (v/v) from 0 to 30 h at a constant substrate concentration of 0.03 mM (4 mg), acetone 500 µl i.e. 1% of the final volume 50 ml [(isooctane: water) (48:2) (v/v)] and 2.6 g of wet cells. For isooctane: water (48:2) (v/v) 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 1,4-dioxane, ACN, DMSO, THF and DMF were tried instead of acetone. Very low conversions 3-15% were observed for the cosolvents other than acetone. From this it is clear that acetone was useful not only for dissolving 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, acetone 500 µl i.e. 1% of the final volume 50 ml [(isooctane: water) (48:2) (v/v)] and 2.6 g of wet cells. Maximum conversion 41% was observed at 0.04 mM (6 mg) substrate concentration.

Acetone concentration

Further, amount of acetone [cosolvent] was optimized using the range of 50-1000 µl in a final volume of 50 ml. Maximum conversion 45% was observed with 200 µl of acetone [i.e. 0.4% of the final reaction volume of 50 ml].

Under the above 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% and 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%]. Optically pure (*S*)-1-phenylethane-1, 2-diols were reported earlier using the same biocatalyst *via* asymmetric reduction of the corresponding phenyl glyoxals,[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.

Benefit of regio- and enantio-selective oxidation over asymmetric reduction of the prochiral ketones is twofold- the corresponding oxidized keto alcohol is also formed in 50% along with the optically pure diol.

It is to be noted that only a few chemical reagents are available for the selective oxidation of secondary 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 $RuCl_3$ and 3-(dichloroiodo) benzoic acid with a yield of 26% in 3 h, but here, benzaldehyde was also isolated in a yield 74% (which is due to over oxidation to keto acid followed by decarboxylation).[39] Another report for oxidation to keto alcohol is using a resin/TEMPO in 24 h with yield 44%.[40] Biocatalytic oxidation of **1** is not reported so far. Biocatalytic oxidative kinetic resolution of **1** is reported using

glycerol dehydrogenase in 64 h (conversion 50%, ee >99%).[41] Another report for the preparation of **1b** was by stereoinversion using *Candida parapsilosis* CCTCC M203011 in 60 h (yield 90%, ee 99.02%).[42] Preparation of **1b** is also reported by biocatalytic asymmetric dihydroxylation of styrene in 43 h (ee 99.9%, yield 76%) using styrene monooxygenase from *Escherichia coli* JM101and 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**.

Table 1 Regio- and enantioselective 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 $[\alpha]_D^{30\,℃}$ **1b-6b**
1*	H	45.35±2.01	38.22±1.90	54.65±2.01	46.07±2.31	97	24	+64.86 (*C* 1, $CHCl_3$)[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 (*C* 0.5, $CHCl_3$)[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 (*C*1.12, $CHCl_3$)[12]
4*	*p*-Br	43.66±0.25	36.3±0.06	56.34±0.25	47.12±0.48	88	24	+38.19[12] (C 1.0, $CHCl_3$)[12]
5*	*p*-Cl	45.95±0.06	38.48±0.96	54.05±0.06	46.69±0.33	98	24	+50.96 (C 1.6, $CHCl_3$)[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.

* The keto alcohol [4.05%-6.34%] is enantioselectively reduced to the (*S*) diol in the case of 1, 4 and 5, that's why increase in ee was observed.

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 under the above optimized conditions. In all the cases regio- and enantioselective oxidation was seen. Electron donating groups viz *p*-OMe and *p*-Me on the aromatic ring increased the conversion to the corresponding keto alcohol. Presence of *p*-OMe group in the case of 1-(4-methoxyphenyl)ethane-1,2-diol **2**, increased the conversion to 59.06% to the corresponding 2-hydroxy-1-(4-methoxyphenyl)ethanone **2a** with yield of 51.12% (36.36 mg). The unreacted corresponding optically pure (*S*)-diol **2b** was isolated in 32.38% yield (23.31 mg). Similarly, *p*-Me group in the case of 1-*p*-tolylethane-1,2-diol **3**, gave a conversion of 61.71% to the corresponding 2-hydroxy-1-*p*-tolylethanone **3a**, with yield 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 *p*-Br group in the case of 1-(4-bromophenyl)ethane-1,2-diol **4** decreased the conversion to 43.61% to the corresponding 1-(4-bromophenyl)-2-hydroxyethanone **4a**, 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 45.96%, 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 an 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 oxidized corresponding keto alcohol **6a** even after 72 h.

Preparation of **2a**, **3a** and **5a** was reported from the corresponding diols by oxidation using silica-encapsulated $H_3PW_{12}O_{40}$ as a recyclable heterogeneous photo catalyst in 1 to 1.5 h with yields of 84-90%. The catalyst needs to be synthesised and metal was unavoidable which is not environmentally benign.[44] Another report for the oxidation of **2** and **5** to the corresponding hydroxy ketones **2a**, **5a** uses tetrapropyl-ammonium perruthenate (TPAP), which involves three steps. In addition, here the primary alcohol had to be protected for the selective oxidation of the secondary alcohol.[45] **3a** was also reported using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 5 h with yield 67%, but here the corresponding keto aldehyde was also formed in a 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 in 13 h with yield 82%, in which synthesis of DIDA involves multiple steps.[47] Hence the present method is better, as it is relatively 'green' for the preparation of hydroxy ketones from the corresponding diols where synthesis of catalyst, protection and deprotection are not needed and there is no over oxidation to keto aldehyde/ketoacid.

(*S*)-1-(4-Methoxyphenyl)ethane-1,2-diol **2b** was reported *via* asymmetric reduction using *Yamadazyma farinosa* IFO 10896 in 48 h (yield 95%, ee >99%)[45]. Chemically, lithiated N-boc-thiazolidine (yield 55%, ee 66%) which involves multiple steps[48] has also been used for the synthesis of **2b**. Synthesis of **3b** (yield 96%, ee >99%) was reported by asymmetric reduction of the corresponding keto alcohol using a N-phenylamine–borane complex and it involves protection and deprotection of the primary alcohol.[10] (*S*)-1-*p*-Tolylethane-1,2-diol **3b** was also prepared using lipase in 12 h (yield 45%, ee 77%) in three steps.[49] (*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 *Pseudomonas cepacia* lipase in 10 h.[49] Synthesis of **4b** (yield 62%, ee 94%) and **5b** (yield 67%, ee 98%) was reported using 25 mol% of a polymer-supported chiral sulfonamide, $NaBH_4/Me_3SiCl$, in 1.5 h under reflux conditions.[50]

Significantly, the current study presents a simple method for the preparation of optically pure diols in good ee and yields, along with the corresponding keto alcohols under mild reaction conditions at room temperature.

Substrate scope

To expand the substrate scope, different substrates **7**-**11** (Chart 1, Table 3) were subjected to the biotransformation. In the case of 1-phenylpropane-1,2-diol **7** {*erythro* [(*R*, *S*) and (*S*, *R*)]: *threo* [(*R*,*R*) and (*S*, *S*)] were in 78:22 ratio based on 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 corresponding unreacted diol **7b [(***erythro: threo*): (87: 13) ratio based on NMR] [yield 41.59%, 29.95 mg, ee 90.26% (*S*, *R*-major)] was the other product. Here increase in *erythro* and decrease in *threo* in the case of **7b** compared to the starting diol **7** indicates that one of the *threo* form is oxidised. This was confirmed by NMR and HPLC[51] and it was the (*S*, *S*) isomer which was oxidised completely to the (*S*)- keto alcohol. Overall formation of (*S*)-keto alcohol **7a** is due to the oxidation of *erythro* (*R*, *S*) and *threo* (*S*, *S*) (confirmed by HPLC). Unreacted diol **7b** is mixture of *erythro* [(*S*, *R*)-major (ee 90.26%), (*R*,*S*)-minor] and *threo* (*R*, *R*). **7a** Was also reported by *in situ* generated dioxirane from the fructose-derived ketone in 2 h from *threo* 1-phenylpropane-1,2-diol with ee 69%, from *erythro* with ee 23%, where the conversion as calculated by ^{1}H NMR was 20 and 34% respectively (the corresponding 1-hydroxy-1-phenyl 2-propanone also formed in 84:16 ratio for *threo* and 89:11 for *erythro*).[52] Another report for the preparation of **7a** used (1*R*, 2*S*)-1-phenyl-1,2-propanediol and resting cells of *Saccaromyces 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** is formed in 72 h in good ee [99%], 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 24.41% to the corresponding keto alcohol **8a**, [yield 18.06% (12.88 mg), ee 14% (*R*)]. The unreacted diol [(*meso*:±):(78:22) ratio from NMR], **8b** 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 staring diol **8** indicates that the *meso* gets oxidised to the corresponding (*R*)-keto alcohol **8a**. Reduced yields are also reported for **8** as compared to **7** in the case of catalytic asymmetric dihydroxylation of olefins,[53] possibly due to steric hindrance.

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 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)]. Since unsaturated keto alcohol **9a** and saturated keto alcohol were inseparable, 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** is not reported so far in literature. Earlier **9b** was reported by the same catalyst *via* deracemization in 24 h (yield 86%, ee >99%).[54] **9b** (yield 60%, ee 98%) Was also reported by CBS-oxazaborolidine by a multi-step synthesis.[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 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 *Rhodococcus ruber* DSM 44541.[56] In the case of **11**, [this 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 2-phenylethanol dehydrogenase (PEDH), phenyl acetaldehyde dehydrogenase (PADH) and NADH oxidase (NOX), a three enzyme system.[57]

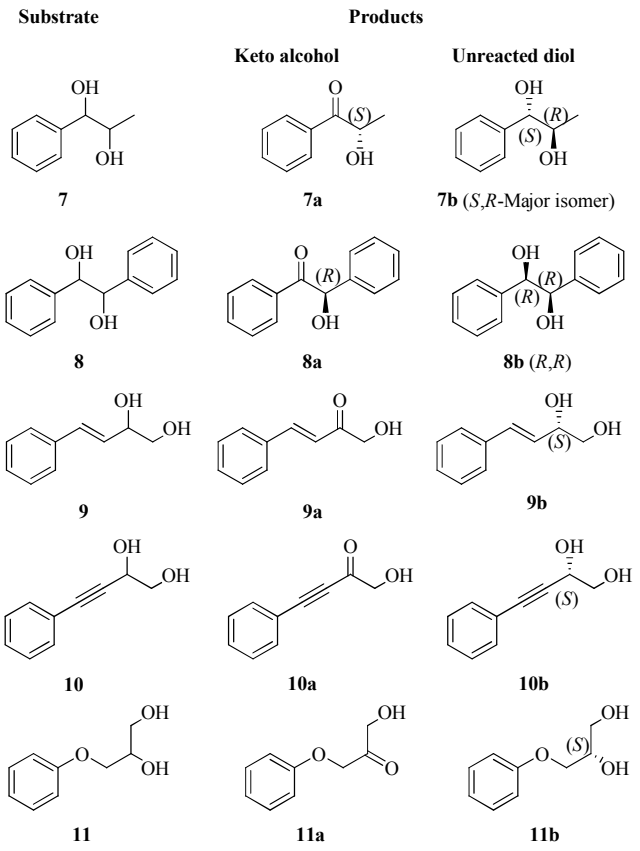


Chart.1 Regio- and enantioselective oxidation of diols **7**-**11** by *C. parapsilosis* ATCC 7330

Cell viability

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

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.

presented (**Table 2**).

Immobilization studies

Preliminary studies using immobilized cells of *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. 1st and 2nd cycles showed 100 % activity while decrease in activity to 87 % was observed in the 3rd cycle, which further reduced drastically to 2 % in the 4th cycle.

Experimental

All substituted benzaldehydes and selenium, Pd/C were purchased from Spectrochem. Sodiumborohydride and Calcium chloride dihydrate were purchased from Merck. Sodium algenate was baught from SRL. Yeast malt agar and Yeast malt broath components (Glucose, Soya peptone, Yeast extract powder and Malt extract powder). HPLC analysis of diols was carried out on a Jasco PU-1580 liquid chromatography with a PDA detector. Conversion was checked using reverse phase C_{18} column, with acetonitrile: water (60:40) (v/v) as mobile phase. Resolution was done using Daicel OJ-H, OB-H, OD-H and AD-H chiral columns. Hexane: isopropanol mixture was used as the mobile phase. The proportion of solvents varied for different diols. Optical rotations were recorded on a Rudolph, Autopol IV digital polarimeter. The characterization of racemic and enantiomerically pure diols was carried out by ^{1}H and ^{13}C NMR; spectra were recorded in $CDCl_3$ on Bruker AVANCE III 500 MHz spectrometer operating at 500 and 125 MHz.

Synthesis of substrates

All substituted 1-phenyl-1, 2-ethanediols **1-8**[12] [**4**, **7** and **8** (racemic keto alcohol for HPLC analysis) were given by Pula Mahajabeen], **9**,[54] **10**[54, 60] [**9**, **10** synthesized by Thangavel Saravanan] and **11**[61] were synthesized according to the reported procedure and characterized by ^{1}H and ^{13}C NMR.

Table 3 Regio- and enantioselective 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 (*C* 1, $CHCl_3$)[62] **7a** (*S*) +27.49 (*C* 3.2, $CHCl_3$)[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 (*C*1, CH_3COCH_3)[64] **8a** (*R*) +12.77 (*C* 1, 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 (*C* 1, $CHCl_3$)[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**.

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 biotransformation.[66]

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

Isooctane: water (48:1) (v/v) was sonicated for 5 min using a Vibra-Cell sonicator (pulse 5 sec on, 5 sec 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 water were added to the isooctane: water (48: 1) (v/v) 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 volume 50 ml] was added to the above cell suspension, incubated at 25 ºC, 150 rpm for 24 h. For yield experiment 0.52 mM (72 mg) substrate 1 was used (parallely in 12 different flasks i.e 6 mg × 12 flasks). After 24 h reaction mass from all flasks was combined and extracted with ethyl acetate 3 × 50 ml, dried over anhydrous sodium sulphate and concentrated using rotary evaporator. Conversion to the corresponding keto alcohol **1a** was checked using HPLC on a C_{18} column (Table1). Enantiomeric excess for the unreacted diol **1b** was determined by HPLC using OB-H column. Isolated yields for the products were determined by column chromatography using hexane: ethyl acetate (98: 2) (ml/ml) 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 enantioselective 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 **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 $CaCl_2$ aqueous solution (100 ml, 2% w/v) were autoclaved prior to the biotransformation. Wet cells (2.6 g) of *C. parapsilosis* ATCC 7330 suspended in 2.8 ml of distilled water were added to the 13 ml of sodium alginate and stirred for 1 h to make it homogeneous. This homogeneous cell suspension was added drop wise to the pre-chilled $CaCl_2$ solution (2% w/v), that resulted in the formation of beads. The beads were kept in $CaCl_2$ solution for 12 h, then washed with distilled water [3x 100 mL], stored at 8˚C before biotransformation.

Immobilized cells were added to the isooctane: water (48:1) (v/v) (sonicated as mentioned above) using 1 ml 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 above immobilized cells suspended in isooctane: water and incubated at 25 ˚C, 150 rpm for 24 h. After 24 h immobilized cells were removed by filtration and products were extracted with ethyl acetate (3x10 ml), and analyzed by HPLC. Beads were washed with distilled water (3x30 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 coincidence with the literature reported values.

For all compounds HPLC resolution details are given below.

2-hydroxy-1-phenylpropan-1-one ***7a***: AD-H column [hexanes/2-propanol = 95:05, 0.5 ml/min; retention times 15.37 min (*R*-minor), 17.51 min (*S*-major)].

2-hydroxy-1,2-diphenylethanone ***8a***: AD-H column [hexanes/2-propanol = 90:10, 1.0 ml/min; retention times 9.73 min (*S*-minor), 13.55 min (*R*-major)].

1-phenylethane-1,2-diol ***1b***: OB-H column [hexanes/2-propanol = 90:10, 0.5 ml/min; retention times 14.01 min (*R*-minor), 17.81 min (*S*-major)].

1-(4-methoxyphenyl)ethane-1,2-diol ***2b***: OD-H column [hexanes/2-propanol = 98:02, 0.8 ml/min; retention times 68.29 min (*R*-minor), 73.03 min (*S*-major)].

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

1-(4-bromophenyl)ethane-1,2-diol ***4b***: OD-H column [hexanes/2-propanol = 98:02, 0.8 ml/min; retention times 67.80 min (*R*-minor), 74.19 min (*S*-major)].

1-(4-chlorophenyl)ethane-1,2-diol ***5b***: OD-H column [hexanes/2-propanol = 98:02, 0.8 ml/min; retention times 53.36 min (*R*-minor), 59.41 min (*S*-major)].

1-phenylpropane-1,2-diol ***7b***: AD-H column [hexanes/2-propanol = 98:02, 1.0 ml/min; retention times 40.85 min (*S*, *R*-major), 43.79 min (*R*, *S*)-minor)].

1,2-diphenylethane-1,2-diol ***8b***: O-JH column [hexanes/2-propanol = 90:10, 1.0 ml/min; 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 [hexanes/2-propanol = 90:10, 1.0 ml/min; retention times 13. 67 min (*S*-major),15.55 min(*R*-minor)].

Conclusion

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

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Notes and references

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