Enantio- & chemo-selective preparation of enantiomerically enriched aliphatic nitro alcohols using Candida parapsilosis ATCC 7330

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Enantio- & chemo-selective preparation of enantiomerically enriched aliphatic nitro alcohols using *Candida parapsilosis* ATCC 7330

Sowmyalakshmi Venkataraman* and Anju Chadha*\(^{a,b}\)

Enantiomerically pure β- and γ-nitro alcohols were prepared from their respective nitro ketones by asymmetric reduction mediated by the biocatalyst, *Candida parapsilosis* ATCC 7330 under optimized reaction conditions (ee up to >99%; yields up to 76%). This biocatalyst exhibits high chemoselectivity and reduces the keto group in preference to nitro group along with good enantioselectivity to produce enantiomerically enriched nitro alkanols. The asymmetric reduction of aliphatic nitro ketones was carried out in water with ethanol as cosolvent and glucose as cosubstrate using the whole cells of *Candida parapsilosis* ATCC 7330 in much lesser time (4 h). For the first time, the biocatalytic asymmetric reduction of the following ketones is reported here: 1-nitro-but-2-one, 1-nitro-pentan-2-one, 3-methyl-1-nitro-but-2-one and 1-cyclohexyl-2-nitroethanone to produce (R)-alcohols [ee up to 79%, yield up to 74%] and 1-nitro-hexan-2-one and 1-nitro-heptan-2-one to produce (S)-alcohols [ee up to 81%, yield up to 76%].

**Introduction**

Organic nitro compounds are important for various applications in synthetic chemistry as the electron withdrawing nature of the nitro group make the protons acidic at α-position to generate and stabilise the carbanion, thereby facilitating the formation of a new carbon-carbon bond. The nitro group can also be converted into several other functional groups like amines, carboxyl compounds among others.\(^1\) Enantiomerically pure β- and γ-nitro alcohols are important chiral building blocks for the preparation of various bioactive molecules like ephedrine, chloramphenicol, various β-adrenergic receptor agonists like (S)-propranolol, (S)-pindolol\(^7\) and in pheromone preparations.\(^3\)

Asymmetric Henry reaction is a widely used method for the preparation of enantiomerically pure β-nitro alcohols. Numerous reports are available for the synthesis of these β-nitro alkanols using different chiral chemocatalysts, namely, C\(_2\)-asymmetric bis(oxazoline)-Cu(OAc)\(_2\), H\(_2\)O,\(^5\) 5-bioxazolines from tartaric acid,\(^6\) zinc-amino alcohols system\(^7\) and many others.\(^8\)-\(^12\) In general, however, on an industrial scale, the use of biocatalysts is preferred over chemocatalysts due to ambient operating conditions\(^13\) and the urgent need for 'green' methods has resulted in developing biocatalysed reactions for a host of organic transformations.

Enantiomerically pure β- and γ-nitro alcohols can be prepared by using different biocatalysts. Naemura *et al.* reported the kinetic resolution of racemic 5-nitro-2-pentanol to produce the enantiomerically enriched (S)-5-nitro-2-pentanol using lipase-QL from *Alcaligenes* sp. with 45% conversion and 22% enantiomeric excess (ee),\(^14, 15\) which is the chiral building block for the preparation of spirocyclic pheromone of *Andrena haemorrhoa*.\(^16\) In another instance, goat liver lipase was used in the kinetic resolutions of 4-nitro-2-butanol and 5-nitro-2-pentanol to produce the (S)-enantiomers (up to 72% ee and up to 40% yields).\(^17\) Borah *et al.* studied the kinetic resolution of several racemic aliphatic β-nitro alcohols like 1-nitro-hexan-2-ol, 1-nitro-octan-2-ol etc., using *Pseudomonas fluorescens* lipase to produce the (S)-alcohol with ee up to 94%.\(^18\) The effect of different organic solvents on the resolution of these aliphatic β-nitro alcohols using lipase from *Pseudomonas* sp. to produce their (S)-alcohols in up to 78% ee\(^19\) was also reported. Apart from lipases,\(^20, 21\) several other enzymes were also employed for the preparation of these β-nitro alcohols. Among them, hydroxy nitrile lyase from *Hevea brasiliensis* catalyses the Henry reaction. Using this enzyme, various (S)-aryl and aliphatic nitro alkanols were prepared with high ee (up to 99%) and yields (up to 77%).\(^22, 23\) In another report, (R)-enantiomers of nitro alcohols were preferably formed by a hydroxy nitrile lyase from *Arabidopsis thalliana*.\(^24\) Notably, other biocatalytic methods are also available for the preparation of aliphatic nitro alcohols using different enzymes, namely halohydrin dehalogenase from *Agrobacterium radiobacter* AD1,\(^25\) TGase

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(protein-glutamine-γ-glutamyl transferase EC 2.3.2.13) from *Streptorericillum griseovercillatum* and others.\(^{27}\)

Another strategy for the preparation of these aliphatic nitro alcohols is the asymmetric reduction of the respective nitro ketones. The asymmetric reduction of β- and γ-nitro ketones is reported using different biocatalysts. Nakamura *et al.* studied the asymmetric reduction of these nitro ketones using baker’s yeast to produce the (S)-nitro alcohols with high ee (up to 99%) and yields (up to 66%).\(^{1, 28}\) The enantiomerically pure (S)-4-nitro-2-butanol acts as a chiral precursor for the preparation of various natural products like (+)-brefeldin A, a compound with a wide range of biological applications like antiviral, antifungal, antitumor activities and in the synthesis of (S)-(−)-sulcatol, a pheromone.\(^{29}\) The asymmetric reduction of 4-nitro-2-butanol by baker’s yeast to produce the (S)-alcohol with high ee (up to 99%) and yields (up to 74%) is also reported.\(^{30, 31}\) Fantin *et al.* reported the asymmetric reduction of β- and γ-nitro ketones using different strains of Yarrowia lipolytica, Saccharomyces cerevisiae and Rhizopus sp. among others. Among the strains tested, Yarrowia lipolytica selectively produced the (S)-enantiomer of 5-nitro-pentan-2-ol (ee: 60-90%; yields: up to 100%) and (R)-enantiomer of 5-nitro-pentan-3-ol (ee: 26-84%; yields: up to 8%) while different strains of Saccharomyces cerevisiae produced both enantiomers of 5-nitro-pentan-3-ol (ee: up to 76%; yields: up to 30%) and 5-nitro-pentan-2-ol (ee: up to >99%; yields: up to 97%).\(^{32}\) Unlike β- and γ-nitro ketones, the asymmetric reduction of aliphatic α-nitro ketones is scarcely reported. One such study was the asymmetric reduction of 1-nitro-octan-2-one using the lyophilised cells of *Comamonas testosteroni* to produce the (S)-1-nitro-2-octanol in 48 h (ee: >99%; yield 47%).\(^{33}\)

*Candida parapsilosis* ATCC 7330 is established as a versatile biocatalyst for asymmetric reduction and deracemisation reactions to yield several enantiomerically pure molecules: e.g. aryl α-hydroxy esters,\(^{34}\) β-hydroxy esters,\(^{35}\) alkyl 2-hydroxy-4-arylobut-3-ynoates,\(^{36}\) 1-phenyl ethanols\(^{37}\) etc., are prepared through deracemisation from their racemates and aryl-1,2-diols,\(^{38}\) aryl α-hydroxy amides,\(^{39}\) aryl α-hydroxy esters,\(^{40, 42}\) etc., by asymmetric reduction of the corresponding prochiral ketones in high optical purity and yields. The biocatalyst is chemoselective\(^{36, 40, 42}\) and regioselective.\(^{43}\) The substrate repertoire of the biocatalyst also includes aliphatic substrates. The enantioselective asymmetric reduction of ethyl-4-chloro-3-oxobutanoate using this biocatalyst produces the (S)-hydroxy ester\(^{44}\) (ee >99%; yield 96%). Notably, a change in the carbon source in the growth medium, results in a change in the absolute configuration of the product alcohol.\(^{35}\) Enantiomerically enriched (S)-alkyl 3-hydroxybutanoates (Prelog products) were prepared from the corresponding prochiral ketones using *Candida parapsilosis* ATCC 7330 in water (pH 6.8) with glucose as cosubstrate and acetoneitrile as cosolvent\(^{46}\) with high ee (up to >99%) and yields (up to 71%). Using the same biocatalyst, the deracemisation of different racemic alkyl β-hydroxy esters was carried out in a different reaction medium (pH 8.5 Tris-HCl buffer with acetone as cosubstrate and DMF/DMSO as cosolvent) to produce their (R)-hydroxy esters (anti-Prelog products) predominantly with high ee (up to >99%).\(^{37}\) The present work reports a biocatalytic asymmetric reduction for the preparation of nitro alcohols from nitro ketones thus using the chemoselectivity of the biocatalyst to advantage.

### Results and discussion

For this study, 1-nitro-2-butane 1a was the model substrate. The asymmetric reduction was carried out using the earlier reported method.\(^{44}\) In this reaction, the substrate 1a in ethanol was incubated with the wet cells of *Candida parapsilosis* ATCC 7330 suspended in water with glucose (50 g/L) as a cosubstrate. The reaction was monitored using thin-layer chromatography and after 4 h, the product formed was identified as (R)-1-nitro-2-butanol 2a with 42% ee and 95% conversion (Scheme 1).

![Scheme 1 Asymmetric reduction of 1-nitro-2-butane 1a using *C. parapsilosis* ATCC 7380](image)

#### Screening of cosolvents

To further improve the ee, the asymmetric reduction of 1a was carried out using different cosolvents. Among the cosolvents tested, chloroform showed hardly any change in ee (43%). All the other solvents, viz. dimethyl sulfoxide (ee: 35%), tetrahydrofuran (ee: 36%), 1,4-dioxane (ee: 31%), ethyl acetate (ee: 39%) and 2-propanol (ee: 35%) showed comparatively lower ee than ethanol (ee: 42%) (Table 1). Ethanol was found to be the most suitable cosolvent for the biotransformations as it also plays an important role in the regeneration of cofactors.

The importance of the cosolvents in enhancing ee of the product alcohols in different biotransformations was reported earlier from our group.\(^{45, 46}\) The effect of different solvents reported for the kinetic resolution of racemic 1-nitro-2-pentanol using Novozym 435, showed that the resolution proceeded favourably in diisopropyl ether with good conversion (54%) and ee (92%).\(^{47}\) In certain instances, a switch in enantioselectivity is also observed in the product when different solvents are used in the reaction. In an earlier report on the asymmetric reduction of ethyl-4-chloro-3-oxobutanoate carried out using baker’s yeast in water as well as in other organic solvents, the (S)-enantiomer (ee 14%, conversion 100%) was produced in water and the (R)-enantiomer was obtained when the reaction was carried out with different organic solvents.\(^{48}\) In the present study, the product nitro alcohols did not show a switch in enantioselectivity with such a change in solvents.
dehydrogenase which in turn gets oxidised to produce acrolein, which acts as a potent inhibitor for the yeast alcohol dehydrogenase enzymes.\textsuperscript{54} Hence, in the present study the increase in the concentration of allyl alcohol (>8.6 mM) was not attempted due to its irreversible inhibitory effect on the yeast alcohol dehydrogenase enzyme.\textsuperscript{55} In addition, the use of allyl alcohol as inhibitor in the reaction had shown only a marginal improvement in ee of (R)-2a' (from 42% to 50%) with a drastic decrease in conversion (from 95% to 74%).

![Scheme 2](image)

Scheme 2 Asymmetric reduction of 1-nitro-2-butane 1a using C. parapsilosis ATCC 7330 using allyl alcohol as inhibitor

Therefore, the reaction conditions for the asymmetric reduction of \( \alpha \), \( \beta \) and \( \gamma \)-nitro ketones using \textit{Candida parapsilosis} ATCC 7330 was optimised to carry out in water (pH 6.8) using ethanol as cosolvent and glucose as cosubstrate in the absence of inhibitor (Scheme 3). The results obtained are tabulated in Table 2.

![Scheme 3](image)

Scheme 3 Asymmetric reduction of nitro ketones 1a-i using \textit{C. parapsilosis} ATCC 7330

Scope of substrates

The ee of 2a'-2e' (Table 2) alters with increase in chain lengths of alkyl groups attached to the carbonyl carbon along with the switch in enantioselectivity. As observed with products 2a' and 2b', there is a decrease in ee for 2b' compared to 2a' due to the increase in the enantioselectivity for the (S)-enantiomer.\textsuperscript{59} The products 2a', 2b' and 2c' were obtained as (R)-enantiomers (ee: 8.2-79.5%) which could be due to the presence of alkyl groups like ethyl, propyl, isopropyl groups on the carbonyl carbon, where the enzyme prefers to direct the hydride attack from the \( S \)-face resulting in the formation of (R)-alcohols.\textsuperscript{56} The increase in ee as well as enantio-reversal were observed with products 2d' and 2e'. Zhou et al. and Keinan et al. reported the switch in enantioselectivity and improvement in ee upon increasing the length of the alkyl chain on the carbonyl carbon.\textsuperscript{57} Further increase in the alkyl chain to up to 6 carbons showed a substantial decrease in the ee (59%) of 2g' (Table 2) which may be attributed to steric factors as reported by us earlier.\textsuperscript{46} In the case of 2g' (Table 2) with bulky cyclohexyl group, the ee observed was considerably low (11%) with an opposite

### Table 1: Asymmetric reduction of 1-nitro-2-butane 1a using \textit{C. parapsilosis} ATCC 7330 using different cosolvents

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cosolvents used</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>Dimethyl sulphoxide</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>Tetrahydrofuran</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>1,4-Dioxane</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl acetate</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>2-Propanol</td>
<td>35</td>
</tr>
</tbody>
</table>

### Effect of inhibitors

The low ee in whole cell mediated biotransformations could be due to the presence of multiple oxidoreductases in the biocatalyst with opposite stereochemical preferences.\textsuperscript{57} This can be averted by using selective inhibitors to improve the ee of the product. Inhibitors like allyl alcohol or allyl bromide are known to inhibit the \( R \)-face yeast enzymes and \( S \)-face yeast enzymes respectively.\textsuperscript{56} Hence in the present study, these inhibitors were used to improve the ee of the product. The concentration of allyl alcohol used was as per our earlier report.\textsuperscript{51} Allyl alcohol (8.6 mM) or allyl bromide (4.13 mM) was added to the cell suspension (separately) and incubated for 1h. The product 2a' obtained after allyl alcohol treatment showed a marginal increase in ee of up to 50%, giving the (R)-alcohol with a marked decrease in conversion (74%) unlike the untreated cells which produced (R)-alcohol with 42% ee and 95% conversion (Scheme 2). The marginal increase in ee of (R)-2a' could be attributed to the selective inhibition of (S)-specific enzymes present in the biocatalyst. The cells treated with allyl bromide produced (R)-2a' with a low ee (<12%). The drastic decrease in ee could be due to the selective inhibition of (R)-specific enzymes\textsuperscript{55} present in the cells. A series of experiments were also carried out earlier with the model substrate using lower concentrations of allyl alcohol (1.7 - 8.6 mM). Under the conditions tested, there is a marginal increase in the ee from 42% to 50% (maximum ee with 8.6 mM allyl alcohol treatment) with a gradual decrease in conversion (up to 74%) was observed. Nevertheless, increasing the period of pretreatment of the biocatalyst with allyl alcohol (up to 2 h 30 min) did not show any improvement in the ee. In our earlier study on the asymmetric reduction of ethyl 4,4,4-trifluoro-3-oxobutanoate, the biocatalyst was pretreated with different concentrations (1.7-8.6 mM) of allyl alcohol up to 2 h 30 min. The formation of (S)-alcohol with a gradual increase in ee from 63% to 84% was observed while increase in allyl alcohol concentration to 10.3 mM showed a marked decrease in ee (79%) and conversion (52%).\textsuperscript{51} In the asymmetric reduction of ethyl-4-chloro-3-oxobutanoate using allyl alcohol treated baker's yeast, showed initial inhibition of the \( R \)-face specific enzymes at lower concentration, which facilitates the increased availability of cofactors to the \( S \)-face specific enzymes to produce (S)-alcohol [change in \( R \), \( S \) configuration due to substituent priority as per CIP rules] and later the increase in allyl alcohol concentration also inhibits the \( S \)-face enzymes thus resulting in the low conversion of the (S)-product.\textsuperscript{55} It is known that allyl alcohol is a good substrate for alcohol dehydrogenase which in turn gets oxidised to produce acrolein, which acts as a potent inhibitor for the yeast alcohol dehydrogenase enzymes.\textsuperscript{54} Hence, in the present study the increase in the concentration of allyl alcohol (>8.6 mM) was not attempted due to its irreversible inhibitory effect on the yeast alcohol dehydrogenase enzyme.\textsuperscript{55} In addition, the use of allyl alcohol as inhibitor in the reaction had shown only a marginal improvement in ee of (R)-2a' (from 42% to 50%) with a drastic decrease in conversion (from 95% to 74%).

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configuration. This observation is in consensus with a recent report from our lab which also highlighted the effect of steric factors on the ee of product alcohol under the conditions studied using this biocatalyst. \(^{58}\)

### Table 2. Asymmetric reduction of nitroketones 1a-i using C. parapsilosis ATCC 7330

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>ee (%)(^a)(^b) Configuration</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>2a'</td>
<td>(\text{CH}_3\text{NO}_2)</td>
<td>42, (R)</td>
<td>74</td>
</tr>
<tr>
<td>2b'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>8, (R)</td>
<td>69</td>
</tr>
<tr>
<td>2c'</td>
<td>(\text{CH}_3\text{NO}_2)</td>
<td>79.5, (R)</td>
<td>74</td>
</tr>
<tr>
<td>2d'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>62, (S)</td>
<td>76</td>
</tr>
<tr>
<td>2e'</td>
<td>(\text{CH}_3\text{NO}_2)</td>
<td>81, (S)</td>
<td>69</td>
</tr>
<tr>
<td>2f'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>59, (S)</td>
<td>76</td>
</tr>
<tr>
<td>2g'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>11, (R)</td>
<td>54</td>
</tr>
<tr>
<td>2h'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>89, (S)</td>
<td>72</td>
</tr>
<tr>
<td>2i'</td>
<td>(\text{CH}_3\text{O}\text{NO}_2)</td>
<td>&gt;99, (S)</td>
<td>74</td>
</tr>
</tbody>
</table>

\(^a\)Enantiomeric excess was determined using chiral GC column

In the asymmetric reduction of \(\beta\)- and \(\gamma\)-nitro ketones \(\text{viz.}, 1\text{h and }1\text{i}\), the products formed were the (S)-enantiomers of \(2\text{h}'\) and \(2\text{i}'\) (Table 2) respectively, with high enantioselectivity (ee: up to >99%) in much lesser time (4 h) unlike the earlier reported methods (2-4 days). \(^{1,32}\)

### Experimental

The racemic \(\beta\)-nitro alcohols (2a-g) were synthesised from the corresponding aldehyde and nitromethane using Henry reaction. \(^{59}\) The \(\alpha\)-nitro ketones (1a-g) were synthesised by the oxidation of \(\beta\)-nitro alcohols using chromium trioxide and sulphuric acid. \(^{60}\) The \(\beta\)- and \(\gamma\)-nitro ketones (1h, 1i) were prepared using the reported methods. \(^{61,62}\) \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra were recorded in CDCl\(_3\) solution on a Bruker AVANCE III 500 MHz spectrometer operating at 500 MHz and 125 MHz respectively using TMS as an internal standard. Infrared spectra were recorded on a JASCO FT/IR - 4200 instrument. The enantiomeric excess (ee) was determined by gas chromatography using Perkin Elmer CLARUS 600 gas chromatograph fitted with FID detector using VARIAN Chirasil Dex CB chiral column (0.25 µm x 25 mm x 25 m). Helium was used as the carrier gas with a flow rate of 2mL/min with a split factor of 10:1. For few compounds ee was determined by HPLC using Chiralpak AD-H (Daicel) column. Hexane: 2-propanol mixture was used as mobile phase. Mobile phase ratio and flow rate were varied for different products. Optical rotations were determined on Rudolph, Autopol IV digital polarimeter. TLC was carried out using Kieselgel 60 F254 aluminium sheets (Merck 1.05554).

### Growth conditions of Candida parapsilosis ATCC 7330

*Candida parapsilosis* ATCC 7330 was purchased from ATCC, Manassas, VA 20108, USA and maintained at 4 °C in yeast malt agar medium that contained 5 g/L peptic digest of animal tissue, 3 g/L malt extract, 3 g/L yeast extract, 10 g/L dextrose and 20 g/L agar. All chemicals used for media preparation were purchased from Himedia.

*Candida parapsilosis* ATCC 7330 was precultured for 12 h at 25 °C with shaking at 200 rpm in yeast malt broth medium (contains 5 g/L peptic digest of animal tissue, 3 g/L malt extract, 3 g/L yeast extract, 10 g/L dextrose). The precultured broth, 2 mL (4% v/v) was transferred to a 250 mL Erlenmeyer flask that contained 48 mL of yeast malt broth. The culture was grown on Orbitek shaker at 25 ºC and 200 rpm for 14 h. \(^{64}\) The cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C and subsequently washed thrice with distilled water and the wet cells were used for biotransformation.

### Synthesis of \(\alpha\)-nitro ketones from racemic \(\beta\)-nitro alcohols

The \(\alpha\)-nitro ketones (1a-g) were synthesised from racemic \(\beta\)-nitro alcohols (2a-g) using modified protocol of Elmaaty *et al.*\(^{60}\) About 2 g (20 mmol) of chromium trioxide was added to a three-necked flask containing 9 mL of water and 1 mL of acetone fitted with a mechanical stirrer maintained in ice-bath under nitrogen atmosphere. To this, 5 mmol of the corresponding \(\beta\)-nitro alcohol was added slowly to the cooled solution and stirring was continued for another 10 minutes, followed by the dropwise addition of sulphuric acid (0.8 mL) in ice-cold condition. The reaction was allowed to stir for another 3 h followed by addition of 50 mL of water. The mixture was extracted with dichloromethane followed by subsequent washing with water (200 mL) and 5% sodium carbonate solution (200 mL). The separated organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo to get the crude products, which were purified by silica gel column chromatography using hexane: ethyl acetate (97:3) to obtain the desired \(\alpha\)-nitro ketones 1a-g in quantitative yields. The purified products obtained were characterised using spectroscopic techniques and compared with the literature reported values and were used for the biotransformation.
spectroscopic characterisations of 1-nitroheptan-2-one using $^1$H, $^{13}$C NMR and HRMS analysis are given as follows:

**1-Nitro-heptan-2-one 1e**

Light yellow colour solid; m.p.: 44 °C (uncorrected), IR (cm$^{-1}$): 3542 (enolic OH), 2971, 2937, 1730, 1552, 1383, 1097; $^1$H NMR (CDCl$_3$; 500 MHz; $\delta$ in ppm): 0.88 (t, 3H, $J = 7$ Hz), 1.61-1.67 (quint, 2H, $J = 7.5$ Hz), 2.53 (t, 3H, $J = 7.5$ Hz), 5.26 (s, 2H); $^{13}$C NMR (CDCl$_3$; 125 MHz; $\delta$ in ppm): 13.75, 22.24, 22.79, 30.94, 40.39, 83.18, 196.18; keto:enol tautomerism: consistent with the literature reported values. The absolute configuration was found to be 74% ($\text{Scheme 3}$; $\text{Table 2}$).

The same procedure was followed for other aliphatic $\alpha$, $\beta$-and $\gamma$-nitro ketones $1b$-$i$ ($\text{Scheme 3}$) for the asymmetric reduction using *Candida parapsilosis* ATCC 7330. The reactions were repeated in triplicate for consistent results and control experiments were done in parallel without the whole cells and also using heat-killed cells under identical conditions.

**Spectroscopic data of products**

The purified nitro alcohols were characterised using spectroscopic (IR, $^1$H and $^{13}$C NMR) techniques and were consistent with the literature reported values. The absolute configuration for all the products were assigned by comparing their specific rotation values with literature reported values (given in parentheses). The ee of the products was found to be 42% as determined by the GC and the isolated yield was found to be 74% ($\text{Scheme 3}$; $\text{Table 2}$).

**Typical procedure for the asymmetric reduction of 1-nitro-2-butane 1a using *C. parapsilosis* ATCC 7330 with ethanol or other cosolvents**

In a 150 mL Erlenmeyer flask, 2.4 g of wet cells of *Candida parapsilosis* ATCC 7330 was suspended in 10 mL of sterile distilled water containing 500 mg of glucose. After 10 min, the substrate, 1-nitro-butane-2-one 1a (5 mg, 0.04 mmol) dissolved in 200 µL of ethanol or other cosolvent under study was added and the reaction was continued up to 4 h. After the reaction time, the product was extracted thrice with ethyl acetate and the ee of the product alcohol was determined with a chiral solvent was removed by evaporation using rotary evaporator and the ee of the product alcohol was found with a chiral column. The details are given below.

(2R)-1-Nitro-butane-2-ol (2a')

Light yellow oil, Specific rotation: $[\alpha]_D^{24}$ -14.5 (c 0.5, CHCl$_3$), $[\alpha]_D^{25}$ -24.7 (c 1, CHCl$_3$; ee: 85% (R)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 70 °C min, 15 °C/min, 130 °C 5 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 10.30 ($\delta$, minor); 10.37 ($R$, major).

(2R)-1-Nitro-pentan-2-ol (2b')

Light yellow oil, Specific rotation: $[\alpha]_D^{24}$ -1.4 (c 0.5, CHCl$_3$), $[\alpha]_D^{25}$ +12.2 (c 0.2, CHCl$_3$; ee: 77% ($S$)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 2 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 13.57 ($\delta$, minor); 13.80 ($R$, major).

(2R)-3-Methyl-1-nitro-butane-2-ol (2c')

Light yellow oil, Specific rotation: $[\alpha]_D^{24}$ -13.1 (c 1, CHCl$_3$), $[\alpha]_D^{25}$ -24.0 (c 1.05, CHCl$_3$; ee: 86% (R)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 2 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 12.89 ($\delta$, minor); 13.14 ($R$, major).

(2S)-1-Nitro-hexan-2-ol (2d')

Light yellow oil, Specific rotation: $[\alpha]_D^{24}$ +5.0 (c 0.5, CHCl$_3$), $[\alpha]_D^{25}$ -9.0 (c 1.0, CHCl$_3$; ee: 90% (R)). The peaks are resolved...
by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 5 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 15.94 (S, major); 16.12 (R, minor).

(2S)-1-Nitro-heptan-2-ol (2a') 18,66

Yellow oil, Specific rotation: [α]D -24 +12.4 (c 1, CHCl3), [α]D -25 +18.7 (c 1, CHCl3, ee: 88% (R)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 5 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 17.79 (S, major); 18.00 (R, minor).

(2S)-1-Nitro-octan-2-ol (2f') 7,41

Light yellow oil, Specific rotation: [α]D -24 +6.5 (c 0.7, CHCl3), [α]D -25 +6.2 (c 1.6, CHCl3, ee: 62% (S)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 5 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 19.59 (S, major); 19.79 (R, minor).

(2R)-1-Cyclohexyl-2-nitroethanol (2g') 66,67

Light yellow oil, Specific rotation: [α]D -24 -1.6 (c 0.7, CHCl3), [α]D -25 -14.7 (c 1.03, CHCl3, ee: 73% (R)). The peaks are resolved by HPLC using CHIRALPAK AD-H column at 0.8 mL/min flow rate using hexane: 2-propanol (97:3) as mobile phase with retention times (min): 21.21 (S, minor); 22.89 (R, major).

(2S)-4-Nitrobutan-2-ol (2h') 29,68

Light yellow oil, Specific rotation: [α]D -24 +30.1 (c 1.7, CHCl3), [α]D -25 +40.6 (c 1.15, CHCl3, ee: 99% (S)). The peaks are resolved by HPLC using CHIRALPAK AD-H column at 1 mL/min flow rate using hexane: 2-propanol (95:5) as mobile phase with retention times (min): 18.29 (R, minor); 21.41 (S, major).

(2S)-5-Nitropentan-2-ol (2i') 17,30

Light yellow oil, Specific rotation: [α]D -24 +22.1 (c 2, CHCl3), [α]D -25 +11.1 (c 1.6, CHCl3, ee >99% (S)). The peaks are resolved by GC using the chiral column (injector and detector temperature: 220 °C, oven temperature: 90 °C 5 min, 5 °C/min, 170 °C 5 min; split: 1:10, flow rate: 2.0 mL/min) with retention times (min): 10.97 (S, major); 11.83 (R, minor).

Conclusion

The asymmetric reduction of aliphatic α-, β- and γ-nitro ketones were efficiently carried out using Candida parapsilosis ATCC 7330 to produce their corresponding enantiomerically enriched nitro alcohols (ee up to >99%; yields up to 76%) in much lesser time (4 h) as compared to earlier reports. Several optimisation studies carried out with the 1-nitro-butan-2-one (model substrate) using different cosolvents and inhibitors enhanced the ee of the product alcohol [α]-1-nitro-butan-2-ol from 42% to 50%. For the first time, the biocatalyst mediated asymmetric reduction of various α-nitro ketones is reported here. The substrate scope of the biocatalyst revealed that the size of the alkyl groups is important for the absolute configuration of the resulting product alcohol; thus substrates with ethyl, propyl, isopropyl groups give (R)-alcohols (2a’-c’) while butyl, pentyl and hexyl groups give the (S)-alcohols (2d’-g’). Steric factors (e.g. substrates with hexyl (1f) and cyclohexyl (1g) groups) influence the ee as seen in 2f’ (ee: 59%) and 2g’ (ee: 11%) respectively. The asymmetric reduction of β- and γ-nitro ketones gave nitro alcohols (2h’ and 2i’) with (S)-configuration with a excellent ee (up to >99%) in substantially lesser time (4 h) compared to the earlier studies (2-4 days).

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Notes and references
