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One-pot synthesis of (*R*)-1-(1-naphthyl)ethanol by stereoinversion using *Candida parapsilosis*

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ABSTRACT

(*R*)-1-(1-Naphthyl)ethanol is an essential chiral substrate for the synthesis of nonactin and dihydro-[1*H*]-quinoline-2-one derivatives. Stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by whole cell biocatalysis, using *Candida parapsilosis*, is reported here. *Candida parapsilosis* possesses a requisite redox system for the stereoinversion of secondary alcohol. The reaction conditions (temperature, time, pH, organic solvent, etc.) significantly influenced the stereoinversion process. Optimum conditions were found to be the reaction temperature of 30 °C, a cellmass concentration of 200 mg/mL, pH 7 (phosphate buffer, 50 mM), a shaking speed of 200 rpm, and a 12 h reaction time. Under these optimum conditions, (*R*)-1-(1-naphthyl)ethanol was obtained in 100% ee_R and 88% yield.

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Dihydro-[1*H*]-quinoline-2-one derivatives are retinoid X receptor (RXR) agonists having a role in the treatment of dyslipidemia, hypercholesteremia, and diabetes.[1] (*R*)-1-(1-Naphthyl)ethanol is a key chiral substrate used in the synthesis of dihydro-[1*H*]-quinoline-2-one derivatives.[1] It is also used as a precursor for the synthesis of nonactin used as antibiotic.[2] Biocatalysis provides the enantiopure secondary alcohols using various methods such as kinetic resolution by lipases,[3] stereoselective oxidases,[4] and reductases.[4a,5–9] The synthesis of enantiopure (*S*)-1-(1-naphthyl)ethanol has been reported by the kinetic resolution[10] and dynamic kinetic resolution[11] of racemic 1-(1-naphthyl)ethanol using lipase.[3a,b] Enantiopure (*S*)-1-(1-naphthyl)ethanol has also been produced by the asymmetric reduction of 1-acetonaphthone using Baker's yeast, *Rhizopus arrhizus*,[9] *Merulius tremellosus* ono991,[12] *Daucus carota*,[13] *Geotrichum candidum*,[14] and *Candida vishwaathii*.[5,15] Few racemic alcohols are cheaper or more readily available than their prochiral ketones.[16] For such cases the reduction of prochiral ketones may not be suitable to obtain enantiopure secondary alcohols.[17] Stereoinversion of racemic alcohols remains the only alternative to obtain 100% theoretical yield,[18] as kinetic resolution has a limitation of 50% yield.[17,19] Stereoinversion and dynamic kinetic resolution are used for deracemization.[18] Stereoinversion requires two redox systems of opposite stereo-preferences.[20] Any one of the enantiomers from the racemic mixture undergoes stereoselective oxidation into prochiral ketone while other enantiomer remains unreacted. The prochiral ketone is then stereoselectively reduced to another enantiomer. The theoretical yield of the desired enantiomer can be attained to 100% by stereoinversion.[18,21] It can be carried out by isolated enzymes or by the whole cells of microorganisms.[22] Biocatalysis using whole cells circumvents the isolation and purification of relevant enzymes, and eliminates the addition of cofactors.[22] Stereoinversion using a few microorganisms like *Nocardia* sp.,[18] *Geotrichum candidum*[23] has been reported in the literature.[4,24] Voluminous research is going on with *Candida parapsilosis* as a putative organism for stereoinversion.[25,26] Enantiopure secondary alcohols,[19] 1,2-diols,[20] α-hydroxy esters,[27] aromatic β-hydroxy esters,[28] allylic alcohols,[29] and propargylic esters[30] were obtained by stereoinversion. Here we report the optimization conditions for the one-pot synthesis of (*R*)-1-(1-naphthyl)ethanol by stereoinversion using *Candida parapsilosis* (Scheme 1).

Three different strains of *Candida parapsilosis* (*Candida parapsilosis* MTCC 1965, *Candida parapsilosis* MTCC 4448, and *Candida parapsilosis* MTCC 2511) were screened for the experiments.[31,32] Stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol was observed only with *Candida parapsilosis* MTCC 1965. HPLC analysis of the reaction mixture at different time intervals showed the decline in the concentration of (*S*)-1-(1-naphthyl)ethanol with the concomitant increase in the concentration of (*R*)-1-(1-naphthyl)ethanol. Intermediate occurrence of 1-acetonaphthone confirmed the mechanism is stereoinversion.

In stereoinversion, (*S*)-1-(1-naphthyl)ethanol is stereoselectively oxidized to prochiral 1-acetonaphthone, and the synthesized 1-acetonaphthone is then stereoselectively reduced to (*R*)-1-(1-naphthyl)ethanol. The maximum percentage of enantiomeric excess and yield would be attained only when both the stereoselective oxidation, and the reduction operate efficiently in tandem.

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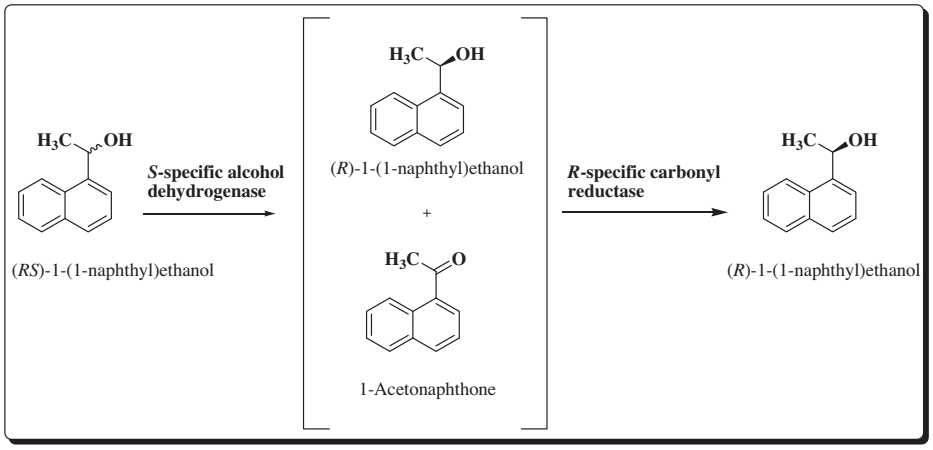

Scheme 1. Stereoinversion of (*S*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*.

Different reaction conditions were studied to attain the optimum stereoinversion. For optimization studies, one condition was changed at a time, while others were kept constant. Optimum condition in one step was used for the subsequent studies.

Temperature affects the rate of reaction, the stability of the biocatalyst, the enantioselectivity of the catalyst,[15] and the solubility of the substrate and its products.[8] In stereoinversion, two enzyme systems are being used in tandem. Temperature may hamper the activity of either one or both of the enzymes. The enantiomeric excess values (% ee_R) did not change with the increasing of temperature up to 35 °C, thereafter, the values decreased drastically (Table 1). Maximum percentage yield of (*R*)-1-(1-naphthyl)ethanol was 87% at 30 °C, and decreased thereafter. Hence, for all the subsequent reactions, 30 °C was used as the optimum reaction temperature.

Reaction progress was monitored by analyzing the reaction mixture at different time intervals.[21,30] Concentration of (*R*)-1-(1-naphthyl)ethanol increased with the concurrent decrease of (*S*)-1-(1-naphthyl)ethanol concentration in the reaction mixture. Formation of intermediate prochiral ketone with the advent of stereoinversion was observed (Fig. 1). At 12 h, there was a maximum percentage ee_R, and a maximum percentage yield of (*R*)-1-(1-naphthyl)ethanol (Table 2). All the subsequent reactions were run for 12 h.pH alters the ionic state of the enzyme protein molecule, therefore pH plays an important role in its reactivity.[8,33] For stereoinversion, the effect of pH on the activity of either oxidase or reductase, or both would be important. The oxidoreductase system of *C. parapsilosis* showed a change in the conversion with respect to pH. To determine the optimum pH for the stereoinversion reaction, the reaction was performed at different pHs (50 mM) using citrate buffer for a pH range 3–6, phosphate buffer for a pH range 7–8 and Tris–HCl for a pH range 9–10. It is evident from Figure 2 that with the increasing reaction pH, both the enantiomeric excess and the yield increased. Maximum percentage ee_R and percentage yield were obtained at pH 7. From pH 7 onwards, the percentage ee_R remained the same, while the percentage yield of (*R*)-1-(1-naphthyl)ethanol started decreasing. It may be due to a drastic decline in the stereoselective reductase activity of *C. parapsilosis* at alkaline pH. At pH 7, the maximum enantiomeric excess (100% ee_R) and yield (88%) of (*R*)-1-(1-naphthyl)ethanol were observed.

Table 1
Effect of temperature on the stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*

Temperature (°C)	% ee_R	% Yield
25	100	67
30	100	87
35	100	55
40	22	49
45	9	49

To increase the substrate solubility both polar and non polar organic solvents were selected. Water miscible organic solvents were thought to aid in the solubility by the phenomenon of co-solvency, however water immiscible organic solvents aid by forming a biphasic reaction mixture. Different organic solvents of varying Log*P* values were selected to study the effect of organic solvents on biocatalytic stereoinversion.[7,15] It was observed that in most of the cases, both the stereoselective oxidation and the reduction were severely affected. Among these, percentage ee was better with 1,4-dioxane, hexane, and dimethyl sulphoxide. The reactions were carried out with a 2% (v/v) organic solvent. The maximum stereoinversion >99% (ee_R) was obtained in hexane with a 55.78 percentage yield of (*R*)-1-(1-naphthyl)ethanol (Table 3). Whole cell biocatalysis in organic solvent has a limitation due to the toxic effect of the solvent on enzyme activity. Kansal et al. studied the effect of organic solvent on the activity of ketone reductase from *Candida viswanathii* and found that there is no direct correlation between solvent polarity (Log*P*) and enzyme activity.[15] Similar results were obtained while studying stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by *C. parapsilosis*. The higher values of enantiomeric excess with hexane, DMSO, and dioxane might be due the absorption of these organic solvents inside the membrane leading to a change in membrane fluidity and ease in substrate uptake resulting in activity retention, while the other solvents might have deactivated reductase enzyme. Retention of enzyme activity in DMSO and hexane was also reported by Zhu et al. during the asymmetric reduction of aryl ketones by *Pyrococcus furiosus*.[7] The results of stereoinversion in organic solvents were found to be inferior to those in an aqueous system. Significant conversion to ketone compared to ee_R in the presence of organic solvents was observed. This indicates that organic solvents have a detrimental effect on the activity of reductase.

In conclusion, *Candida parapsilosis* MTCC 1965 contains oxidoreductase system for stereoinversion. Reaction conditions were optimized for the stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol. In the present study, about 100 percentage ee_R of (*R*)-1-(1-naphthyl)ethanol with 88 percentage yield was achieved at the optimum reaction condition. The yield may be increased by the further manipulation of reaction conditions. Similar oxidoreductase activity was not detected in other two organisms of the same species. The oxidoreductase system seems to be very

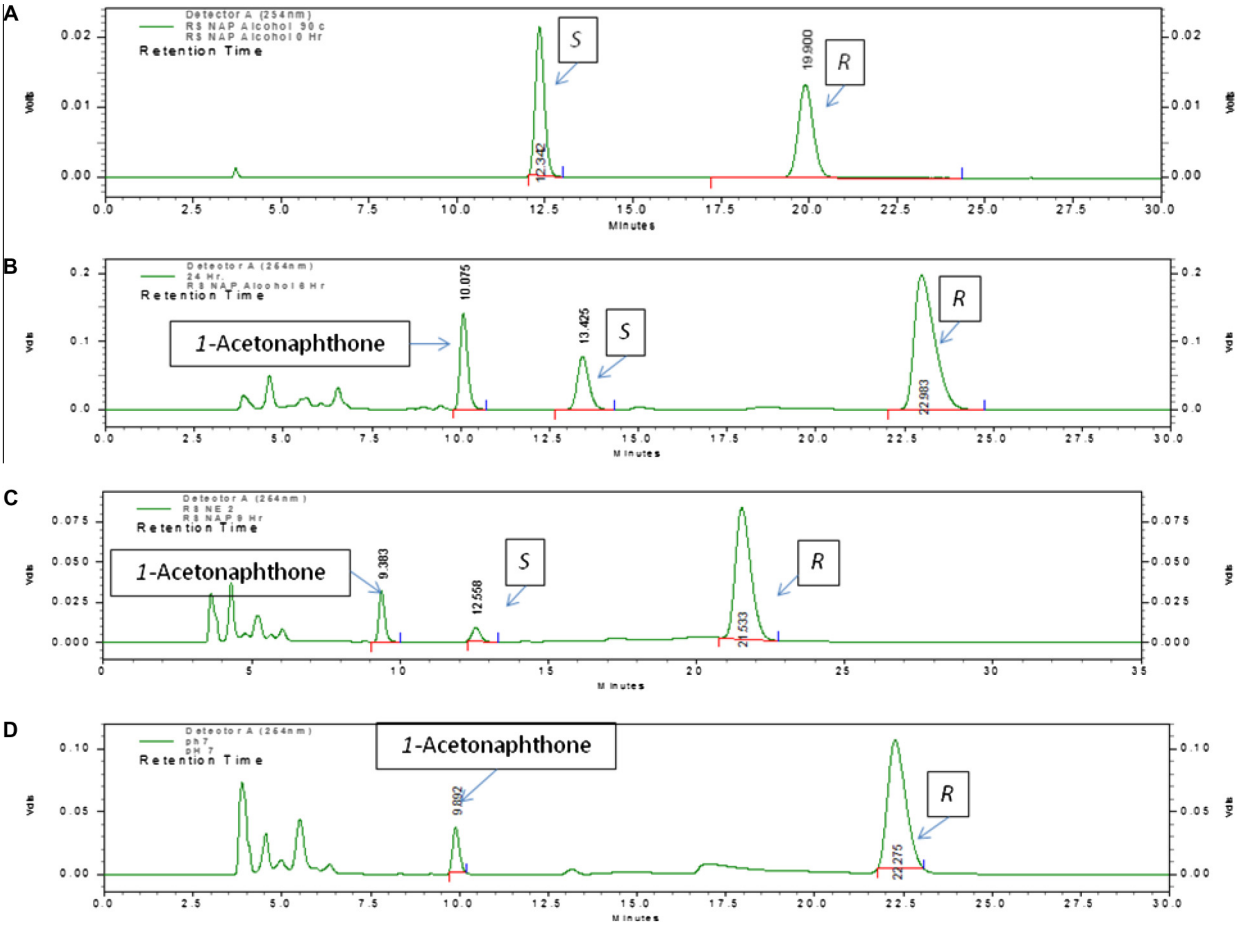

Figure 1. HPLC chromatograms for stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*. (A) 0 h, (B) 6 h, (C) 9 h, (d) 12 h.

Table 2
Course of stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*

Time (h)	% ee_R	% Yield
0	0.14	50
3	1	51
6	63	66
9	89	83
12	100	88

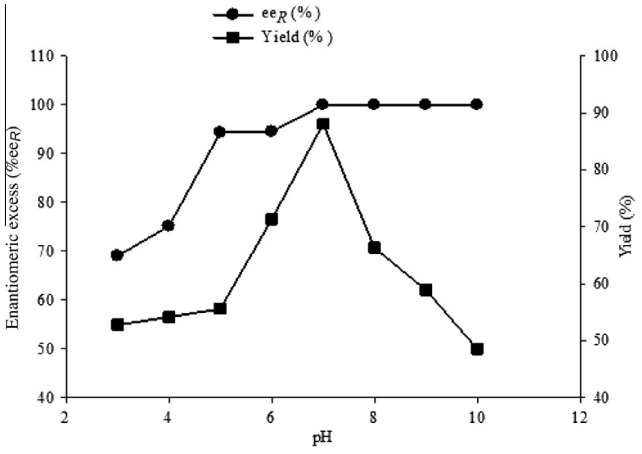

Figure 2. Effect of pH on the stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*.

Table 3
Effect of organic solvent on the stereoinversion of (*S*)-1-(1-naphthyl)ethanol to (*R*)-1-(1-naphthyl)ethanol by the whole cells of *Candida parapsilosis*

Solvent	% ee_R	% Yield
Dioxane	100	52
Ethanol	8	49
Hexane	100	56
2-Propanol	20	48
Pyridine	1	26
Toluene	12	48
Dimethyl sulphoxide	100	50

specific. This may be a very good technique for obtaining 100% pure alcohol from the racemic mixture.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.04.051.

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31. *Cultivation of microorganisms*: *Candida parapsilosis* MTCC 1965, *Candida parapsilosis* MTCC 4448, and *Candida parapsilosis* MTCC 2511 were individually precultured for 12 h at 25 °C with shaking (200 rpm) in yeast malt broth medium that contained 5 g/L peptone, 3 g/L malt extract, 3 g/L yeast extract and 10 g/L dextrose. From the precultured growth, 10% (v/v) inoculum was transferred individually to 500 mL Erlenmeyer flasks containing 100 mL medium. The cultures were grown on a rotatory shaker at 25 °C (200 rpm) for 14 h. The cells were harvested at mid-exponential phase by centrifugation (7000×g, 20 min) at 4 °C and washed thrice with distilled water.
32. *Synthesis of (R)-1-(1-naphthyl) ethanol using whole cells of Candida parapsilosis*: Reaction mixture (20 mL) was prepared by suspending 4 g (wet weight) of cell pellets in phosphate buffer (pH 7, 50 mM). The final substrate concentration was 2 mM racemic alcohol in the reaction mixture. This was incubated at 30 °C (200 rpm) for 12 h. Ethyl acetate extraction was carried out and the organic layer was dried with anhydrous sodium sulphate and evaporated under vacuum. Product obtained was solubiliszd in IPA and analyzed by Chiral HPLC.
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