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Highly Enantioselective Production of Chiral Secondary Alcohols with *Candida Zeylanoides* as a New Whole Cell Biocatalyst

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The increasing demand for biocatalysts in synthesizing enantiomerically pure chiral alcohols results from the outstanding characteristics of biocatalysts in reaction, economic, ecological issues. Herein fifteen yeast strains belonging to three food originated yeast species *Candida zeylanoides*, *Pichia fermentans* and *Saccharomyces uvarum* were tested for their capability for asymmetric reduction of acetophenone to 1-phenylethanol as biocatalysts. Of these strains, *Candida zeylanoides* P1 showed an effective asymmetric reduction ability. Under optimized conditions, substituted acetophenones were converted to corresponding optically active secondary alcohols in up to 99% enantiomeric excess and at high yield. The preparative scale asymmetric bioreduction of 4-nitro acetophenone **1m** by *Candida zeylanoides* P1 gave (*S*)-1-(4-nitrophenyl) ethanol **2m** 89% yield, and >99% enantiomeric excess. Compound **2m** has been obtained in an enantiomerically pure and inexpensive form. Additionally, these results indicate that *Candida zeylanoides* P1is a promising biocatalyst for the synthesis of chiral alcohols in industry.

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Introduction

Production of single enantiomers of different chiral compounds is one of the main targets of different industries including pharmaceutical industry as majority of the drugs in the market are optically pure compounds at least bearing one chiral center [1-5]. Chiral secondary alcohols are good examples of chiral compounds that can be used in the production of different drugs. For instance, isoproterenol[6] which is used for the treatment of bradycardia, prozac[7] which is used in the treatment of depression and neobeodine[8] which display antihistaminic and anticholinergic action are synthesized from chiral secondary alcohols (Figure 1). These enantioselective products can be produced with different methodologies although biotransformation is one of the most preferred methodologies among them that have many advantages compared to chemical catalysts [9-11]. For instance, biocatalysts are providing clean and eco-friendly way to perform chemical reactions under mild conditions and high selectivity for the substrate whereas chemical catalysts produce toxic waste and a large amount of waste product [12,13]. Biotransformation generally relies on the use of isolated enzymes or whole cell microorganism as both methods have some advantages and drawbacks. Isolated enzymes are extremely selective and recyclable catalysts, but are usually very expensive and not readily accessible due to the limited availability of cosubstrates and cofactors of the biocatalyst [14]. On the other hand, whole cells are small factories, especially advantageous since they are equipped with all the metabolic ways for the regenerations of necessary cofactors, well protected within their natural cellular environment and several different microorganisms were successfully shown to be used for these biotransformation reactions [15-18]. These examples showed that different microorganisms belonging to bacteria, yeast or fungi can act as different biotransformation agents with different final yields and ee values and finding new microbial species with biotransformation potential is crucial. So three different yeast species *Candida zeylanoides*, *Pichia fermentans* and *Saccharomyces uvarum*, were isolated from fermented pastırma, orange juice and sourdough, respectively as potential whole cell biotransformation agents. These yeast species were further tested for the asymmetric reduction of acetophenone and its derivatives in order to investigate the percent conversion and

enantiomeric excess. A *Candida zeylanoides* strain showed good characteristics among the tested strains and reaction conditions including pH, incubation temperature, time, agitation level was optimised to achieve higher conversion and ee values. Following the optimisation, this strain was used as a new biocatalyst for production of a chiral secondary alcohol **2m** which has not been obtained enantio pure in the gram scale previously. Importantly, this compound can be easily and inexpensively converted to (*S*)-1-(4-nitrophenyl) ethanamine in future works which was previously reported to be producible in an expensive way[19]. Our findings clearly showed the usage of whole cells as a cheap and effective methodology to produce different secondary alcohols including (*S*)-1-(4-nitrophenyl) ethanol.

Results and Discussion

Fifteen different strains belonging to three different yeast species that were isolated from fermented pastırma, orange juice and sourdough namely, *Candida zeylanoides, Pichia fermentans* and *Saccharomyces uvarum* were tested for the bioreduction of acetophenone **1a** to 1-phenylethanol **2a** (Table 1). The bioreduction ability of these strains were determined by addition of 5 ml wet yeast cells to the 50 ml culture environment in which 1 mmol substrate **1a** was added and cells with substrate were incubated at 30^{o}C for 24 h under 100 rpm agitation conditions. The yield and the conversion rate of the reaction were determined by ^{1}H-NMR using internal standard. The enantiomeric specificity of the final product was determined by chiral HPLC. As can be seen in Table 1, the strains produced (*S*)-1-phenylethanol **2a** (41-74% ee). The good results for the bioreduction of acetophenone was obtained when *Candida zeylanoides* P1 was used as a whole cell biocatalyst. The conversion rate to (*S*)-1-phenylethanol by *Candida zeylanoides* P1 was at moderate conversion (67%) and the ee 74%. *Candida zeylanoides* P1 was thus selected for further studies, which includes reduction of acetophenone derivatives.

Firstly, we investigate the effects of reaction conditions on the product's enantioselectivity and conversion. Reaction conditions are important in the successful production of a secondary chiral alcohols [20]. Thus, reaction conditions such as pH, temperature, incubation period and agitation speed were optimized using *Candida zeylanoides* P1 as a biocatalyst. The results of these optimizations can be seen in Table 1. Different pH ranges (4.5-8.0) were chosen to monitor the progress of the bioreduction reaction in the reaction conditions of 30^{o}C, 100 rpm and 24 h. The

highest conversion (81%) and enantiomeric excess (89%) were obtained when the medium pH was controlled at 6.5. This effect of pH on conversion and enantioselectivity can be due to the fact that the three-dimensional structures of the enzymes can vary depending on the pH which affects both enantioselectivity and conversion as previously discussed [15]. The effects of several temperatures were investigated by carrying out the fermentation process within different temperature ranges (25-34^{o}C) in the reaction condition of 100 rpm, 24h and pH 6.5. The highest conversion (81%) and ee (89%) were obtained at 30^{o}C. When the temperature rises above 30 degrees, both the conversion and the ee decreased significantly. For instance, the lowest conversion (40%) and ee (60%) were obtained at 34^{o}C. These results show that the increase in temperature above 30 ^{o}C has negative effects on conversion and enantioselectivity which might be related with optimum temperature range of the corresponding enzyme as the optimum temperature conditions for yeast species is around 30 ^{o}C. Therefore, the temperature of the reduction reaction was chosen to be 30°C. Several incubation times were selected to follow the progress of the bioreduction reaction in the reaction conditions of pH 6.5 and 30^{o}C. The highest conversions (94%) and (97%) were obtained after 72 and 96 hours, respectively, but the enantioselectivity (81% and 77%) decreased during these periods. The increase in the incubation period positively affected the conversion, but negatively affected the enantioselectivity. This decrease in the enantioselectivity might be due to the degradation of the yeast cells as a result of the long incubation period [21]. The period of incubation was chosen to be 48 hours because the best enantioselectivity (90%) was obtained during this time. Different agitation speeds (100-250 rpm) were selected to monitor the progress of the bioreduction reaction in the reaction conditions of pH 6.5, 30^{o}C and 48 h. The highest values for both ee (>99%) and conversion (>99%) were achieved at 150 rpm and thus this agitation speed was chosen as optimum for fermentation. Complete conversion of **1a** was obtained at 200 and 250 rpm, but enantioselectivity dropped sharply at these agitation speeds. This decrease might be owing to the effect of stress on the yeast cells as well as on the enzyme structure [15].

Under the optimized conditions (pH 6.5, temperature 30^{o}C, time 48 h and agitation speed 150 rpm) asymmetric reduction of the derivatives of acetophenone by *Candida zeylanoides* P1 were investigated. The stereoselectivity of the reaction was investigated for the acetophenones bearing electron-donating groups (Me, OMe and phenyl) and electron-withdrawing groups (CI, Br, NO_2) at the *ortho*-, *metha*- and *para*-position (Table 3). The bioreduction of *ortho*-, and *metha*-

substituted chloro, bromo, methoxy and nitro acetophenones, as well as the *para*-nitro acetophenones were transformed to the corresponding alcohols with excellent enantioselectivity (Table 3, entry 2-7, 12). However, depending on the steric and electronic effects of these groups, the conversions are slightly reduced especially in the electron-donating methoxy group (Table 3, entry 4, 7). The reduction of 4-nitroacetophenone **1m** occurred with complete conversion, yielding the corresponding (*S*)-secondary alcohol with excellent ee (>99%) (Table 3, entry 12).

Electron-donating substituents methyl and phenyl groups at the *para* position on the aromatic ring let to a slight decrease in enantiomeric excess and conversion (Table 3, entry 11, 13). The reduction of 4-methoxyacetophenone **1j** occurred with good enantiomeric excess (96%) and lowest conversion (65%) (Table 3, entry 10). The *Candida zeylanoides* P1 reduced *para* halogenated acetophenones **1i** with good enantiomeric excess (97%) and moderate conversion. Similar selectivity was obtained in the reduction of *ortho*- and *para*-nitro isomers highlighting the importance of electronic effects for the conversion and selectivity of bioreduction, with a negligible steric effects of the substituent. The enantioselectivity of the acetophenone derivatives were dependent on the position of substitutes at benzene ring [22]. Since several acetophenone derivatives demonstrated perfect enantioselectivities for the asymmetric reduction on small scale, we decided to conduct the asymmetric reduction of **1m** to (*S*)-**2m** on a large scale to prove the applicable of the current system as industrially feasible. The asymmetric bioreduction in a 1 L Erlenmeyer with 12 mmol of 4-nitoacetophenone **1m** was converted to the (*S*)-1-(4-nitophenyl) ethanol **2m** with 89% yield and >99% enantiomeric excess (Figure 2). This result demonstrates and confirms that the system is feasible for industrial purpose. In this way, (*S*)-**2m** was obtained in gram scale. So in this study *Candida zeylanoides* P1 has been shown to be effective as a whole cell biocatalyst for the reduction of acetophenone and its derivatives.

Conclusions

In this study, different acetophenones **1a-n** were reduced corresponding chiral secondary alcohols **2a-n** with high enantiosectivity using *Candida zeylanoides* P1 as yeast species. We have showed a new yeast strain to obtain enantiopure chiral secondary alcohols. The present study has different benefits, because enantioselectivity and conversion of the bioreduction are controlled by the substituent position and electronic effect on phenyl ring, and this yeast can be used to obtain

gram scale chiral secondary alcohols. This system shows perfect enantioselectivity and can be carried out for the synthesis of valued enantiopure secondary alcohols. By using *Candida zeylanoides* P1 yeast whole cell as an alternative to other classic enantioselective catalysts that frequently required the use of costly ligands or metal complexes, an easy and productive process has been developed for the preparation of chiral secondary alcohols in excellent enantiomeric excess. This new biocatalyst has high selectivity for the asymmetric reduction reaction in a biocatalytic process that offers higher yields and optically pure products. *Candida zeylanoides* P1 can be considered to be an outstanding catalyst for asymmetric synthesis of various important chiral alcohols industrially.

Experimental Section

General

The chemical reagents were obtained from Sigma-Aldrich in the highest purity available. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 spectrometer in $CDCl_3$. Enantiomeric excesses were determined by chiral HPLC analysis using chiral columns OD-H, OD and AS. Enantiomeric excess was determined directly from the areas under the curve. The racemic **2a-n** were obtained from corresponding **1a-n** with $NaBH_4$ in methanol at room temperature used as a standard for the determination of the (*R*) or (*S*)-enantiomers. Reactions were monitored by thin layer chromatography (TLC) using aluminum-backed Merck Silica-Gel 60 F254 plates. For analysis purpose, a small fraction of the product was by preparative TLC.

Microorganisms, identification and culture conditions

Candida zeylanoides strains were previously isolated from pastırma a fermented Turkish meat product[23] and *Pichia fermentans* and *Saccharomyces uvarum* strains were isolated from orange juice and sourdough, respectively. The genotypic identification of the latter two species was conducted by PCR analysis. Briefly, each isolate was grown in YPD medium (Merck) and DNA of the yeast samples were isolated by phenol-chloroform methodology. For the identification, the D1/D2 domain of 26S rDNA region was PCR amplified using the primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′).

The PCR mix was prepared with1 μL of DNA template, 10 μL 5× PCR buffer, 0.4 μL dNTPs, 1 μL of 20 mM primers NL1 and NL4, 0.25 μL 5U Taq polymerase (Promega) and up to 50 μL of sterile H_2O and PCR conditions were conducted as described previously[24] and after BLAST search of the obtained sequence both species were identified with 97–100% similarity criterion.

General Bioreduction Reactions

Yeast strain was propagated from their glycerol stocks by inoculation to 10 ml YPD medium (1% yeast extract, 1% peptone from casein, 2% Glucose) followed by 2 d growth at 30°C. From this cultures, exponentially grown yeast cell was inoculated to 50 ml YPD medium at 10% concentration and pH was adjusted 6.5 and shaken 2 hours than 1 mmol substrate was directly added to the medium and incubated on a shaker (150 rpm) at 30°C for 48h. At the end of the incubation period, the cells were separated by centrifugation at 6000 × g for 5 min at 4°C and the supernatant saturated with NaCl, then extracted with diethyl ether. Diethyl ether extracts were combined and dried over Na_2SO_4. After removal of the solvent under reduced pressure the crude product identified by NMR analysis. The absolute configuration was determined by sign of specific rotation and comparison with the literature. The enantiomeric excess of the seconder alcohol was determined by chiral HPLC analysis.

Production of gram scale of 2m

The same transformation was performed on the preparative scale in 2000 mL flask. Yeast strain was propagated from stock by incubation to 100 mL YPD medium followed by 2 d growth at 30 °C, then grown yeast cell was inoculated to 1000 mL sterilized fresh YPD medium in 2 L flask and following 12 mmol **1m** was directly added to the reaction medium and incubated for 48 h. Then the mixture was extracted with EtOAc and dried over Na_2SO_4. After evaporation of the solvent the product **2m** was purified on silica gel column (Figure 2) and obtained as an optically pure secondary alcohol (1.78 g, 10.68 mmol, 89% yield)

Spectral data of secondary alcohols

(*S*)-1-phenylethanol (2a)[25]

Colourless oil, Yield 89%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.38-7.33 (m, 4H), 7.31-7.26 (m, 1H), 4.84 (q, J = 6.45 Hz, 1H), 2.62 (bs, 1H), 1.48 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 146.0, 128.4, 127.3, 125.5, 70.2, 25.2; $[\alpha]_D^{25}$ = -42.0 (c 1.1, $CHCI_3$), >99% ee; retention time:

9.3 min (minor), 11.0 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 1.0 mL/min, 210 nm.

(*R*)-1-(2-chlorophenyl) ethanol (2b)[26,27]

Colourless oil, Yield 91%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.58-7.56 (m, 1H), 7.32-7.17 (m, 3H), 5.27 (q, J = 6.4 Hz, 1H), 2.25 (bs, 1H), 1.47 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 143.0, 131.6, 129.4, 128.4, 127.2, 126.4, 66.9, 23.5; $[\alpha]_D^{25}$ = +66.1(c 1.0, $CHCI_3$), 97% ee; retention time: 15.1 min (major), 16.9 min (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1.0 mL/min, 220 nm.

(*R*)-1-(2-bromophenyl) ethanol (2c)[27,28]

Colourless oil, Yield 90%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.56-7.54 (m, 1H), 7.50-7.48 (m, 1H), 7.33-7.29 (m, 1H), 7.12-7.08 (m, 1H), 5.39 (q, J = 6.4 Hz, 1H), 2.79 (bs, 1H), 1.44 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 144.7, 132.6, 128.7, 127.8, 126.7, 121.6, 69.1, 23.6; $[\alpha]_D^{25}$ = +56.6 (c 1.0, $CHCI_3$), >99% ee; retention time: 13.6 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1.0 mL/min, 220 nm.

(*S*)-1-(2-methoxyphenyl) ethanol (2d)[27]

Colourless oil, Yield 93%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.36-7.43 (m, 1H), 7.28-7.23 (m, 1H), 6.97 (t, J = 7.5 Hz, 1H), 6.88 (d, J = 8.20 Hz, 1H), 5.13-5.07 (m, 1H), 3.86 (s, 1H), 2.78 (d, J = 4.3 Hz, 1H), 1.50 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 156.5, 133.4, 128.3, 126.3, 120.8, 110.4, 66.4, 55.2, 22.9; $[\alpha]_D^{25}$ = -24.7 (c 1.1, $CHCI_3$), >99% ee; retention time: 17.0 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1.0 mL/min, 220 nm.

(*S*)-1-(2-nitrophenyl) ethanol (2e)[29]

Colourless oil, Yield 87%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.84 (dd, J = 8.2, 1.1 Hz, 1H), 7.79 (dd, J = 7.9, 1.1 Hz, 1H), 7.63-7.59 (m, 1H), 7.40-7.36 (m, 1H), 5.35 (q, J = 6.4 Hz, 1H), 2.85 (bs, 1H), 1.51 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 147.7, 141.0, 133.6, 128.0, 127.5, 124.2, 65.4, 24.2; $[\alpha]_D^{25}$ = -29.8 (c 0.4, $CHCI_3$), 99% ee; retention time: 19.1 min (minor), 20.3 min (major), Chiralcel AS, *n*-hexane/*i*-PrOH, 97:3, flow rate of 0.8 mL/min, 220 nm.

(*R*)-1-(3-chlorophenyl) ethanol (2f)[25]

Colourless oil, Yield 93%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.34-7.19 (m, 4H), 4.82 (q, J = 6.4 Hz, 1H), 2.43 (bs, OH), 1.45 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 147.8, 134.3,

129.8, 127.5, 125.6, 123.5, 69.7, 25.2; $[\alpha]_D^{25}$ = +38.20 (c 0.60, $CHCI_3$), >99% ee; retention time: 22.0 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 99:1, flow rate of 1.0 mL/min, 210 nm.

(*R*)-1-(3-methoxyphenyl) ethanol (2g)[27]

Colourless oil, Yield 94%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.27-7.23 (m, 1H), 6.93-6.91 (m, 2H), 6.81-6.78 (m, 1H), 4.83 (q, *J* = 6.4 Hz, 1H), 3.79 (s, 3H), 2.41 (bs, OH), 1.46 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 159.7, 147.6, 129.5, 117.7, 112.8, 110.9, 70.2, 55.2, 25.1; $[\alpha]_D^{25}$ = +58.8 (c 0.9, $CHCI_3$), 99% ee; retention time: 12.1 min (minor), 20.1 (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 210 nm.

(*R*)-1-(4-chlorophenyl) ethanol (2h)[25,26]

Colourless oil, Yield 95%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.29-7.22 (m, 4H), 4.79 (q, *J* = 6.5 Hz, 1H), 2.77 (bs, 1H), 1.41 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 144.2, 132.9, 128.5, 126.8, 69.6, 25.2; $[\alpha]_D^{25}$ = +39.8 (c 1.0, $CHCI_3$), 88% ee; retention time: 13.3 min (minor), 16.1 (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1 mL/min, 220 nm.

(*R*)-1-(4-bromophenyl) ethanol (2i)[30]

Colourless oil, Yield 85%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.43-7.40 (m, 2H), 7.18-7.15 (m, 2H), 4.75 (q, *J* = 6.5 Hz, 1H), 2.93 (bs, 1H), 1.39 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 144.7, 131.5, 127.2, 121.0, 69.6, 25.2; $[\alpha]_D^{25}$ = +38.9 (c 1.0, $CHCI_3$), 97% ee; retention time: 8.7 min (major), 9.6 (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 1 mL/min, 220 nm.

(*R*)-1-(4-methoxyphenyl) ethanol (2j)[30]

Colourless oil, Yield 88%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.30-7.27 (m, 2H), 6.89-6.85 (m, 2H), 4.83 (q, *J* = 6.4 Hz, 1H), 3.79 (s, 3H), 2.11 (bs, 1H), 1.46 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 158.9, 138.0, 126.7, 113.8, 69.9, 55.3, 25.0; $[\alpha]_D^{25}$ = +58.6 (c 1.0, $CHCI_3$), 96% ee; retention time: 10.8 min (major), 12.0 (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 220 nm.

(*R*)-1-(p-tolyl) ethanol (2k)[31]

Colourless oil, Yield 95%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.27 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 4.84 (q, *J* = 6.5 Hz, 1H), 2.42 (bs, 3H), 2.37 (s, 3H), 1.48 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 142.9, 137.0, 129.1, 125.4, 70.1, 25.1, 21.1; $[\alpha]_D^{25}$ = +54.4 (c 1.0,

$CHCI_3$), 91% ee; retention time: 21.1 min (minor), 22.9 (major), Chiralcel OD, *n*-hexane/*i*-PrOH, 90:10, flow rate of 0.5 mL/min, 220 nm.

(*S*)-1-(4-nitrophenyl) ethanol (2m)[29]

Colourless oil, Yield 91%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.10-8.14 (m, 2H), 7.51-7.48 (m, 2H), 5.00-4.94 (m, 1H), 2.75 (d, J = 3.4 Hz, 1H), 1.47 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 153.3, 147.0, 126.1, 123.7, 69.4, 25.4; MS: *m/z* 168 $(M+1)^+$; $[\alpha]_D^{25}$ = -25.4 (c 0.5, $CHCI_3$), >99% ee; retention time: 13.2 min (minor), 15.4 (major), Chiralcel AS, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 220 nm.

(*S*)-1-(p-biphenyl) ethanol (2n)[32]

White solid, mp 92-94^{o}C, yield 92%, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.63-7.60 (m, 4H), 7.49-7.45 (m, 4H), 7.40-7.36 (m, 1H), 4.95 (qd, J = 6.4, 2.9 Hz, 1H), 2.24 (d, J = 2.8 Hz, 1H), 1.56 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 144.9, 140.9, 140.4, 128.8, 127.3, 127.3, 127.1, 125.9, 70.1, 25.2; $[\alpha]_D^{25}$ = -44.3 (c 0.55, CH_2CI_2), 85% ee; retention time: 16.8 min (major), 18.2 (minor), Chiralcel OD, *n*-hexane/*i*-PrOH, 90:10, flow rate of 0.6 mL/min, 220 nm.

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Supplementary data

Copies of ^{1}H, ^{13}C NMR spectra and chiral HPLC profiles of **2a-n** related to this article can be found at supplementary data

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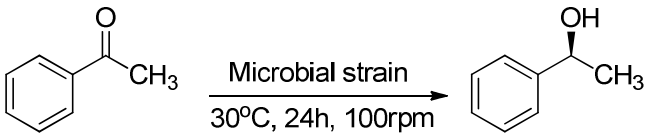
Tables and Figures

Table 1.



1a **2a**

Microbial strain[a]	Conversion[b] (%)	M.B.[b,c] %	ee[d] % (*S*)
Saccharomyces uvarum S1	No growth	--	--
Saccharomyces uvarum S2	54	89	41
Saccharomyces uvarum S3	51	94	46
Saccharomyces uvarum S4	43	97	57
Saccharomyces uvarum S5	39	89	59
Pichia fermentans O1	Weak growth	--	--
Pichia fermentans O1	59	94	49
Pichia fermentans O1	37	90	64
Pichia fermentans O1	29	91	53
Pichia fermentans O1	51	88	61
Candida zeylanoides P1	67	93	74
Candida zeylanoides P2	56	95	58
Candida zeylanoides P3	54	97	49
Candida zeylanoides P4	50	89	54
Candida zeylanoides P5	41	90	48

Reaction condition: acetophenone 1 mmol, temperature: 30 °C, time 24 h, 100 rpm

[a] Comparison of the best micobial strain.

[b] The conversions and mass balances (MB) were determined by ^{1}H NMR analysis with diphenyl methan as an internal standard.

[c] MB refers to the sum of the yields of the characterized adduct **2a**, and the unreacted acetophenone **1a**.

[d] Determined by HPLC using Chiralcel OD-H column.

Table 2.

pH			Temperature			Incubation period			Agitation speed		
	Conv. (%)[b]	ee (%)[c,d]	°C	Conv. (%)[b]	ee (%)[c,d]	h	Conv. (%)[b]	ee (%)[c,d]	rpm	Conv. (%)[b]	ee (%)[c,d]
4.5	54	65 (*S*)	25	67	71 (*S*)	24	81	89 (*S*)	100	88	90 (*S*)
5	67	74 (*S*)	28	70	72 (*S*)	48	88	90 (*S*)	150	99	>99 (*S*)
5.5	71	79 (*S*)	30	81	89 (*S*)	72	94	81 (*S*)	200	99	55 (*S*)
6	70	82 (*S*)	32	77	81 (*S*)	96	97	77 (*S*)	250	99	35 (*S*)
6.5	81	89 (*S*)	34	40	60 (*S*)						
7	65	84 (*S*)									
7.5	60	81 (*S*)									
8[e]	0	-									

[a] Substrate 1mmol.

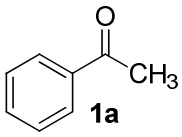
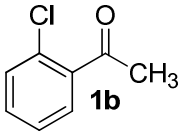
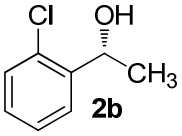
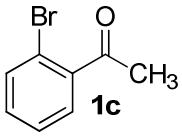
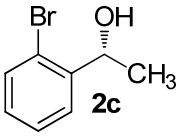
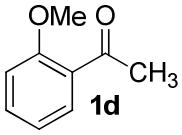
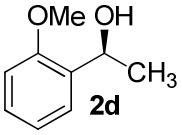
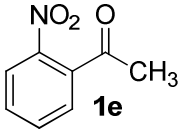
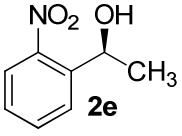
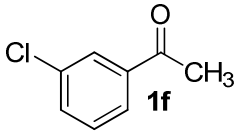
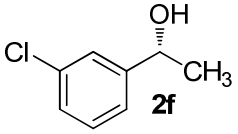
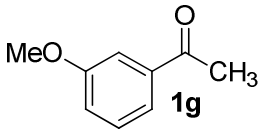
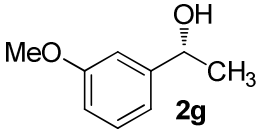
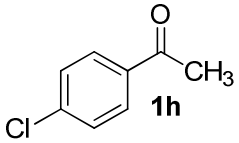
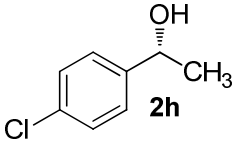
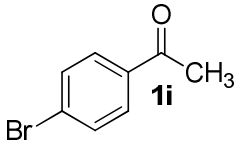
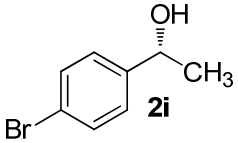
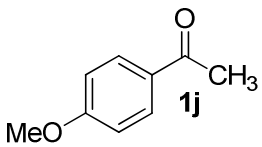
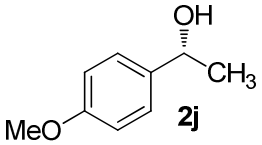
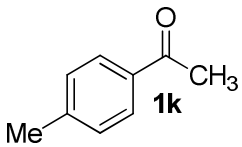
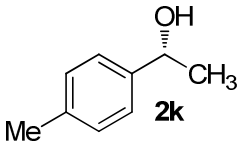
[b] The conversion was determined by 1H NMR analysis with diphenylmethane as an internal standard.

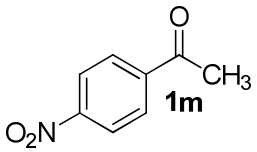
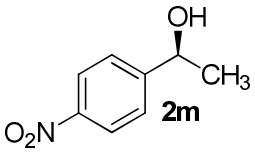
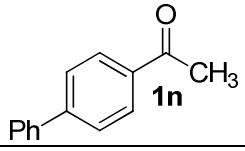
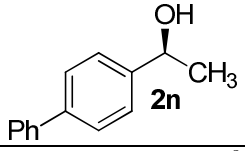
[c] Determined by HPLC using Chiralcel OD-H column.

[d] Absolute configuration were assigned by comparison of the sign of optical rotations relative to the values in literature

[e] weak growth

Table 3.

Entry	Substrate	Product	ee (%)[b,c]	Conversion (%)[d]	M.B. (%)[d]
1	 **1a**	 **2a**	>99 (*S*)	>99	94
2	 **1b**	 **2b**	97 (*R*)	77	87
3	 **1c**	 **2c**	>99 (*R*)	75	91
4	 **1d**	 **2d**	>99 (*S*)	70	90
5	 **1e**	 **2e**	99 (*S*)	97	93
6	 **1f**	 **2f**	>99 (*R*)	87	89
7	 **1g**	 **2g**	99 (*R*)	77	93
8	 **1h**	 **2h**	88 (*R*)	94	94
9	 **1i**	 **2i**	97 (*R*)	87	92
10	 **1j**	 **2j**	96 (*R*)	65	88
11	 **1k**	 **2k**	91 (*R*)	70	94

12	 **1m**	 **2m**	>99 (*S*)	>99	95
13	 **1n**	 **2n**	85 (*S*)	77	94

[a] Bireduction conditions: Substrate: 1 mmol, temperature: 30 °C, time: 48 h, pH: 6.5, 150 rpm
[b] Determined by HPLC using Chiralcel OD-H, OD and AS columns.
[c] Configurations were assigned by comparison with the sign of specific rotation relative to the literatüre values.
[d] The conversions and mass balances (MB) were determined by ^{1}H NMR analysis with diphenylmethane as an internal standard.

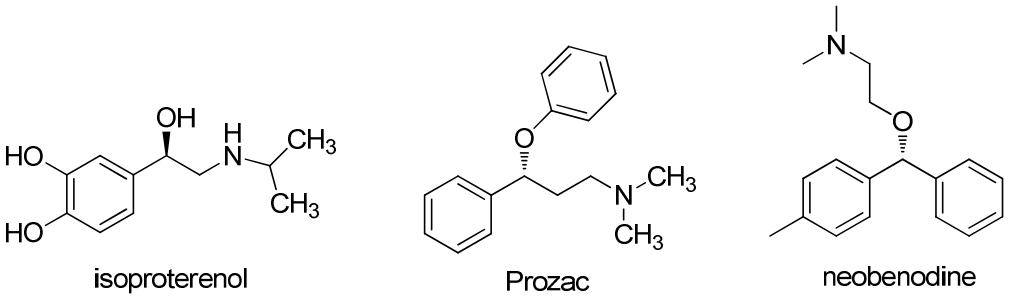


Figure 1.

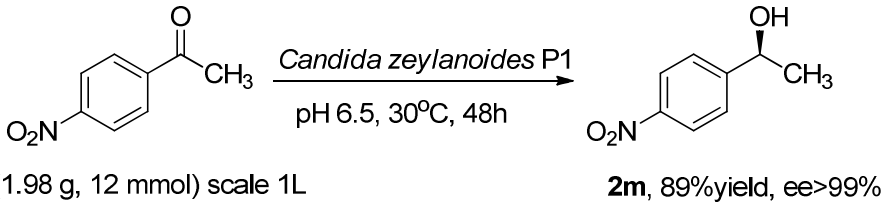


Figure 2.