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RESEARCH ARTICLE

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Debaryomyces hansenii as a new biocatalyst in the asymmetric reduction of substituted acetophenones

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ABSTRACT

Chiral secondary alcohols are convenient mediator for the synthesis of biologically active compounds and natural products. In this study fifteen yeast strains belonging to three food originated yeast species *Debaryomyces hansenii*, *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* were tested for their capability for the asymmetric reduction of acetophenone to 1-phenylethanol as biocatalyst microorganisms. Of these strains, *Debaryomyces hansenii* P1 strain showed an effective asymmetric reduction ability. Under optimized conditions, substituted acetophenones were converted to the corresponding optically active secondary alcohols in up to 99% enantiomeric excess and at high conversion rates. This is the first report on the enantioselective reduction of acetophenone by *D. hansenii* P1 from pastırma, a fermented Turkish meat product. The preparative scale asymmetric bio reduction of 3-methoxy acetophenone **1g** by *D. hansenii* P1 gave (*R*)-1-(3-methoxyphenyl) ethanol **2g** 82% yield, and >99% enantiomeric excess. Compound **2g** can be used for the synthesis of (+)-NPS-R-568 [3-(2-chlorophenyl)-N-[(1R)-1-(3-methoxyphenly) ethyl] propan-1-amine] which have a great potential for the treatment of primary and secondary hyper-parathyroidism. In addition, *D. hansenii* P1 successfully reduced acetophenone derivatives. This study showed that this yeast can be used industrially to produce enantiomerically pure chiral secondary alcohols, which can be easily converted to different functional groups.

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Introduction

Biocatalysts has earned major importance in the asymmetric synthesis of optically pure compounds owing to its high environmental convenience, high enantioselectivity and very slight reaction conditions (Yamada and Shimizu 1988; Wichmann and Vasic-Racki 2005; Patel 2008; Huang et al. 2010; Strohmeier et al. 2011). Biocatalysts have many superiorities compared to chemical catalysts. Chemical catalysts generate toxic waste while biocatalysts provide a clean and environment friendly way to perform chemical reaction if biocatalysts have specific safety properties such as GRAS status. The exploration of new biocatalysts to produce optically pure compounds in mild conditions with high selectivity is an issue of great interest. One of the most popular enantioselective synthesis is the asymmetric reduction of prochiral ketones to secondary alcohols. Optically pure secondary alcohols are useful as important intermediates in the synthesis of pharmaceutical and agricultural chemicals at low production cost in industry. For example, (*R*)-phenylephrine (Legnani and Morandi 2016), (+)-NPS-R-568 (Cohen and Silverberg 2002; Joy et al. 2006; Nagano 2006) and (*S*)-orphenandrine (Wujkowska et al. 2016) (Figure 1) are pharmacologically active compounds based upon optically pure secondary alcohols. Optically pure 1-phenylethanol is a chiral building block used for the synthesis of pharmaceuticals such as inhibitors of cholesterol intestinal absorption (Kurbanoglu et al. 2007). Besides, (*S*)-1-(2-cholorophenyl)ethanol has been used as a key intermediate for the synthesis of anti-asthma compound L-cloprenaline (Kurbanoglu et al. 2009).

Hence, preparation of optically pure secondary alcohols has received more and more attention. The significance of these compounds have resulted in the stunning need for new, efficient, and enantioselective ways for making them (Comasseto et al. 2004; Patel et al. 2004; Murzin et al. 2005). Optically pure phenyl ethanol and its derivatives can be converted without racemization into other functional groups (Pollard and Woodley 2006). Fungus or yeast are often used as reducing agents because of the fact that they are cheap and easily available. For this purpose, either

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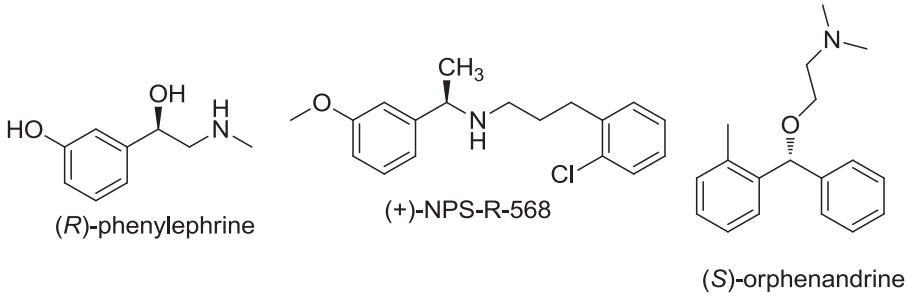


Figure 1. Some examples of pharmaceutical compounds.

pure enzyme or whole cell microorganism is used as a biocatalyst. Actually, there are many benefits of using whole cell microorganism as biocatalysts instead of pure enzymes. Pure enzymes are very costly and hardly accessible; there is a limited availability of enzymes and they need co-substrates like cofactors. The use of whole cell is especially favorable to achieve the desired reduction since they do not need addition of cofactors for their renewal and enantioselective reaction can be carried out with inexpensive equipment (Patel et al. 2004; Murzin et al. 2005; Pollard and Woodley 2006). But, some unconventional biocatalysts can also be used for these purposes (Zilbeyaz et al. 2010) which should also be in consideration during finding new biocatalysts as microorganisms.

Herein, we report the potential of fifteen yeast strains belonging to three yeast species *Debaryomyces hansenii*, *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii*, isolated from fermented meat product, sourdough and orange juice, respectively as whole cell biocatalysts to explore the bioreduction of prochiral ketones, with the aim to screen the biocatalyst substrate specificities and the enantioselectivities depend on molecular structure of the prochiral ketone to be reduced. Importantly, as food isolates, these strains have great potential to be used safely for the industrial purposes which increase their value as biocatalyst.

The objective of this study was to find successful yeast strain in terms of percent conversion and enantiomeric excess, for the asymmetric reduction of prochiral ketones. Besides, we demonstrate the use of *Debaryomyces hansenii* P1 (*D. hansenii* P1) as a new biocatalyst for production of a chiral secondary alcohol on a preparative scale.

Materials and methods

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 $CDCl_3$ (Supplementary file). Enantiomeric excesses were determined by chiral HPLC analysis using OD-H, OD and AS chiral columns. Enantiomeric excess was determined directly from the areas under the curve (Supplementary file). 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 prepared using preparative TLC.

Microorganisms identification and culture conditions

All yeast species used in this study were previously isolated from different food products. *Debaryomyces hansenii* strains were previously isolated from pastırma, a fermented Turkish meat product (Ozturk 2015), and *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* strains were isolated from sourdough and orange juice, respectively, in this study. The genotypic identification of the yeast strains was conducted by PCR analysis. Briefly, each isolate was grown in YPD medium (Merck) and the 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 with 1µl of DNA template, 10µl of 5x PCR buffer, 0.4 µl dNTPs, 1µl 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 (Yilmaz et al. 2016) and later BLAST search of the obtained sequence of 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

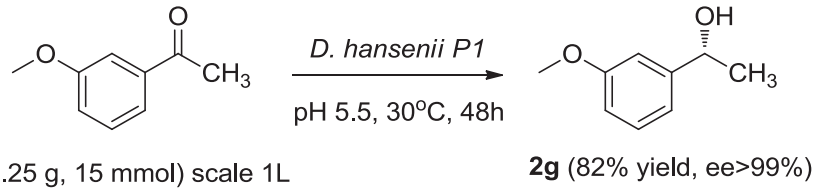


Figure 2. A gram scale production of (*R*)-1-(3-methoxyphenyl) ethanol (**2g**).

growth at 30 °C. From this culture, exponentially grown yeast cell was inoculated to 50 ml YPD medium at 10% concentration and pH was adjusted to 5.5. This was shaken for 2 hours, then 1 mmol substrate was directly added to the medium and incubated on a shaker (150 rpm) at 30 °C for 48 h. 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 was saturated with $NaCl_2$, then extracted with diethyl ether. Diethyl ether extracts were combined and was dried over Na_2SO_4. After removal of the solvent under reduced pressure the crude product was identified by NMR analysis. The absolute configuration was determined by sign of specific rotation and comparison with the literature. The enantiomeric excess of the secondary alcohol was determined by chiral HPLC analysis.

Production of gram scale of 2 g

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 YPD medium in 2L fermenter and following 15 mmol **1g** was directly added to the reaction medium and incubated for 48 h. Then the mixture was extracted with diethyl ether and was dried over Na_2SO_4. After evaporation of the solvent, the product **2g** was purified on silica gel column (Figure 2) and obtained as a optically pure secondary alcohol (1.87 g, 12.3 mmol, 82% yield).

***1-Phenylethanol* (2a)** (Wei and Du 2014; Perna et al. 2016)

Colorless oil, Yield 89%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.38–7.33$ (m, 4H), 7.31–7.26 (m, 1H), 4.84 (q, $J = 6.45$ Hz, 1H), 2.62 (bs, OH: exchanges with D_2O), 1.48 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 146.0$, 128.4, 127.3, 125.5, 70.2, 25.2; $[\alpha]_D^{25} = -45.3$ (c 1.1, $CHCl_3$) 95% enantiomeric excess (ee); retention time: 7.5 min (minor), 8.7 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 1.0 mL/min, 210 nm.

***1-(2-Chlorophenyl) ethanol* (2b)** (Zhang et al. 2011; Liu et al. 2015)

Colorless oil, Yield 91%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.58–7.56$ (m, 1H), 7.32–7.17 (m, 3H), 5.27 (q, $J = 6.4$ Hz, 1H), 2.25 (bs, OH: exchanges with D_2O), 1.47 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 143.0$, 131.6, 129.4, 128.4, 127.2, 126.4, 66.9, 23.5; $[\alpha]_D^{25} = +66.1$ (c 1.0, $CHCl_3$) 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.

***1-(2-Bromophenyl) ethanol* (2c)** (Zhang et al. 2011; Li et al. 2014)

Colorless oil, Yield 90%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.56–7.54$ (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, OH: exchanges with D_2O), 1.44 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 144.7$, 132.6, 128.7, 127.8, 126.7, 121.6, 69.1, 23.6; $[\alpha]_D^{25} = +56.6$ (c 1.0, $CHCl_3$); 99% ee; retention time: 11.7 min (minor), 13.3 min (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1.0 mL/min, 220 nm.

***1-(2-Methoxyphenyl) ethanol* (2d)** (Zhang et al. 2011)

Colorless oil, Yield 93%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.36–7.43$ (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 (bs, OH: exchanges with D_2O), 1.50 (d, $J = 7.1$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 156.5$, 133.4, 128.3, 126.3, 120.8, 110.4, 66.4, 55.2, 22.9; $[\alpha]_D^{25} = -11.2$ (c 1.1, $CHCl_3$) 45% ee; retention time: 16.9 min (major), 18.0 min (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1.0 mL/min, 220 nm.

***1-(2-Nitrophenyl) ethanol* (2e)** (Vieira et al. 2010)

Colorless oil, Yield 87%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.84$ (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, OH: exchanges with D_2O), 1.51 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 147.7$, 141.0, 133.6, 128.0, 127.5, 124.2, 65.4, 24.2; $[\alpha]_D^{25} = -29.8$ (c 0.4, $CHCl_3$); 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.

***1-(3-Chlorophenyl) ethanol* (2f)** (Wei and Du 2014)

Colorless oil, Yield 93%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.34–7.19$ (m, 4H), 4.82 (q, $J = 6.4$ Hz, 1H), 2.43 (bs, OH: exchanges with D_2O), 1.45 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 147.8$, 134.3, 129.8, 127.5, 125.6, 123.5, 69.7, 25.2; $[\alpha]_D^{25} = -35.6$ (c 0.60, $CHCl_3$);

92% ee; retention time: 22.1 min (major), 23.6 min (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 99:1, flow rate of 1.0 mL/min, 210 nm.

***1-(3-Methoxyphenyl) ethanol* (2g)** (Zhang et al. 2011)

Colorless oil, Yield 94%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.27–7.23$ (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: exchanges with D_2O), 1.46 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 159.7$, 147.6, 129.5, 117.7, 112.8, 110.9, 70.2, 55.2, 25.1; $[\alpha]_D^{25} = +58.8$ (c 0.9, $CHCl_3$); 99% ee; retention time: 12.6 min (minor), 20.4 (major), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 210 nm.

***1-(4-Chlorophenyl) ethanol* (2h)** (Wei and Du 2014; Liu et al. 2015)

Colorless oil, Yield 95%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.29–7.22$ (m, 4H), 4.79 (q, $J = 6.5$ Hz, 1H), 2.77 (bs, OH: exchanges with D_2O), 1.41 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 144.2$, 132.9, 128.5, 126.8, 69.6, 25.2; $[\alpha]_D^{25} = -34.6$ (c 1.0, $CHCl_3$); 74% ee; retention time: 13.3 min (major), 14.9 (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 98:2, flow rate of 1 mL/min, 220 nm.

***1-(4-Bromophenyl) ethanol* (2i)** (Singh and Chopra 2016)

Colorless oil, Yield 85%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.43–7.40$ (m, 2H), 7.18–7.15 (m, 2H), 4.75 (q, $J = 6.5$ Hz, 1H), 2.93 (bs, OH: exchanges with D_2O), 1.39 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 144.7$, 131.5, 127.2, 121.0, 69.6, 25.2; $[\alpha]_D^{25} = +32.8$ (c 1.0, $CHCl_3$); 82% 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.

***1-(4-Methoxyphenyl) ethanol* (2j)** (Singh and Chopra 2016)

Colorless oil, Yield 88%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.30–7.27$ (m, 2H), 6.89–6.85 (m, 2H), 4.83 (q, $J = 6.4$ Hz, 1H), 3.79 (s, 3H), 2.11 (bs, OH: exchanges with D_2O), 1.46 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 158.9$, 138.0, 126.7, 113.8, 69.9, 55.3, 25.0; $[\alpha]_D^{25} = +3.7$ (c 1.0, $CHCl_3$); 6% ee; retention time: 14.9 min (major), 16.6 (minor), Chiralcel OD-H, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 220 nm.

1-(p-Tolyl) ethanol (2k) (Wakeham et al. 2015)

Colorless oil, Yield 95%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.27$ (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, OH: exchanges with D_2O), 2.37 (s, 3H), 1.48 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 142.9$, 137.0, 129.1, 125.4, 70.1, 25.1, 21.1; $[\alpha]_D^{25} = +53.8$ (c 1.0, $CHCl_3$) 90% 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.

***1-(4-Nitrophenyl) ethanol* (2m)** (Vieira et al. 2010)

Colorless oil, Yield 91%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.10–8.14$ (m, 2H), 7.51–7.48 (m, 2H), 5.00–4.94 (m, 1H), 2.75 (bs, OH: exchanges with D_2O), 1.47 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 153.3$, 147.0, 126.1, 123.7, 69.4, 25.4; $[\alpha]_D^{25} = -25.4$ (c 0.5, $CHCl_3$) 99% ee; retention time: 13.3 min (minor), 15.4 (major), Chiralcel AS, *n*-hexane/*i*-PrOH, 95:5, flow rate of 0.8 mL/min, 220 nm.

***1-(p-Biphenyl) ethanol* (2n)** (Zilbeyaz et al. 2012)

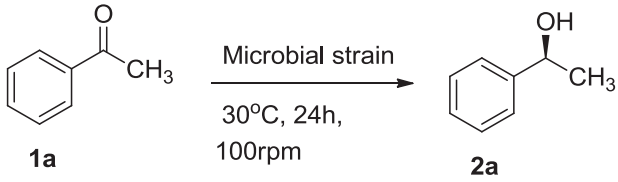
White solid, m.p. 92–94 °C, yield 92%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.63–7.60$ (m, 4H), 7.49–7.45 (m, 4H), 7.40–7.36 (m, 1H), 4.95 (qd, $J = 6.4$, 2.9 Hz, 1H), 2.24 (bs, OH: exchanges with D_2O), 1.56 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 144.9$, 140.9, 140.4, 128.8, 127.3, 127.3, 127.1, 125.9, 70.1, 25.2; $[\alpha]_D^{25} = -46.9$ (c 0.55, CH_2Cl_2); 90% ee; retention time: 16.5 min (major), 17.9 (minor), Chiralcel OD, *n*-hexane/*i*-PrOH, 90:10, flow rate of 0.6 mL/min, 220 nm.

Results and discussion

Fifteen different strains belonging to three different yeast species that were isolated from fermented pastırma, sourdough and orange juice namely *D. hansenii*, *S. cerevisiae* and *H. guilliermondii* were tested for the bioreduction of acetophenone **1a** to 1-phenylethanol **2a** (Table 1). The bioreduction ability of these strains were determined by the addition of 5 ml wet yeast cells to the 50 ml culture environment in which 1 mmol substrate of **1a** was added and cells with substrates were incubated at 30 °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** (39–71% ee). Good results for the bio reduction of acetophenone was obtained when *D. hansenii* P1 was used as a whole cell biocatalyst for further studies. The conversion rate to (*S*)-1-phenylethanol by *D. hansenii* P1 was a moderate conversion (60%) and the ee was 71%. *D. hansenii* P1 was thus selected for further studies which includes the reduction of acetophenone derivatives.

Firstly, we investigated 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 (Pereira 1988). Hence, reaction conditions such as pH, reaction time and agitation speed were optimized using *D. hansenii* P1 as a biocatalyst. The results of

Table 1. Screening of yeast strains for the bio reduction of acetophenone.



1a 2a

Microorganism[a]	Conversion[b] (%)	M.B.[b,c] %	ee[d] % (*S*)
Saccharomyces cerevisiae SD1	Weak growth		
Saccharomyces cerevisiae SD2	35	67	54
Saccharomyces cerevisiae SD3	47	77	41
Saccharomyces cerevisiae SD4	38	69	46
Saccharomyces cerevisiae SD5	17	88	57
Debaryomyces hansenii P1	60	87	71
Debaryomyces hansenii P2	47	91	58
Debaryomyces hansenii P3	39	83	48
Debaryomyces hansenii P4	46	89	51
Debaryomyces hansenii P5	54	83	48
Hanseniaspora guilliermondii OJ1	51	89	41
Hanseniaspora guilliermondii OJ2	28	91	55
Hanseniaspora guilliermondii OJ3	57	80	39
Hanseniaspora guilliermondii OJ4	No growth		
Hanseniaspora guilliermondii OJ5	61	88	47

Reaction conditions: biocatalyst 5 ml, substrate: 1mmol, temperature: 30 °C, time: 24 h, pH: 6.5, 100 rpm
[a]Comparison of the best microorganism.
[b]The conversions and mass balances (MB) were determined by ^{1}H NMR analysis with diphenyl methane 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.

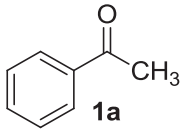
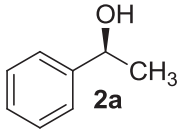
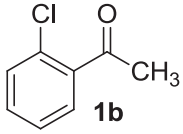
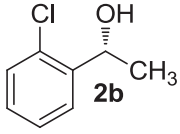
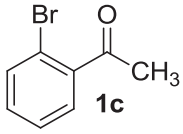
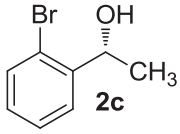
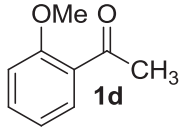
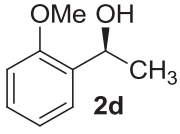
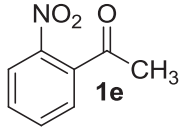
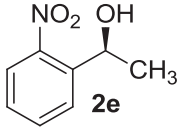
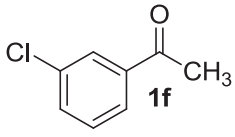
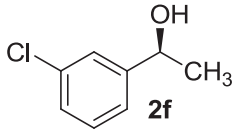
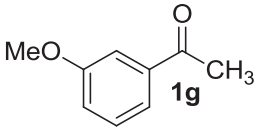
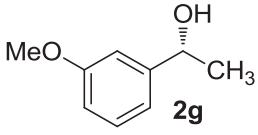
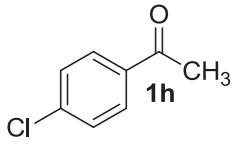
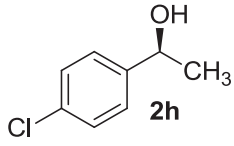
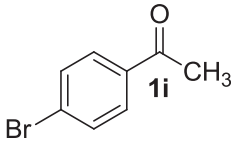
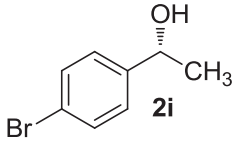
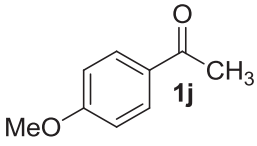
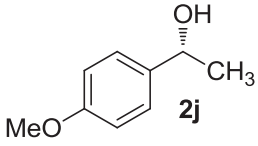
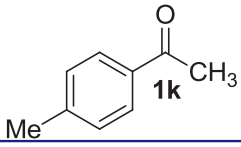
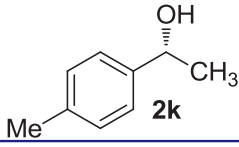
Table 2. Optimization of parameters for the bioreduction of acetophenone **1a** with *D. hansenii* P1.

	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	38	92 (*S*)	25	65	83 (*S*)	24	60	71 (*S*)	100	60	71 (*S*)
5	50	91 (*S*)	28	68	90 (*S*)	48	90	95 (*S*)	150	97	95 (*S*)
5.5	78	95 (*S*)	30	80	95 (*S*)	72	87	83 (*S*)	200	98	67 (*S*)
6	55	90 (*S*)	32	65	87 (*S*)	96	97	65 (*S*)	250	99	60 (*S*)
6.5	60	92 (*S*)	34	45	60 (*S*)						
7	40	88 (*S*)									
7.5	38	84 (*S*)									
8[e]	0										

[a]Substrate: 1 mmol.
[b]The conversion was determined by ^{1}H NMR analysis with diphenyl methane 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.

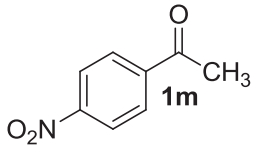
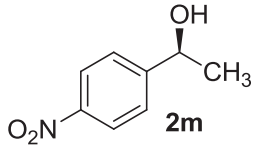
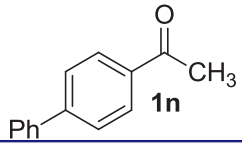
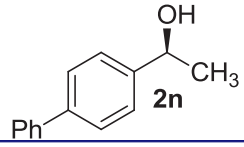
optimizations are shown in Table 2. Several pH's (4.5–8) were selected to monitor the progress of the asymmetric reduction reaction in the reaction conditions of 100 rpm, 30 °C and 24h. The highest conversion (78%) and ee (95%) were obtained when the pH was controlled at 5.5. The impacts of different culture temperatures were investigated by using fermentation processes at different temperatures (25–34 °C) in the reaction conditions of pH = 5.5, 100 rpm and 24h. The highest conversion (80%) and ee (95%) were obtained at 30 °C. When the temperature increased over 30 °C, not only ee but also conversion dropped drastically. For instance, low ee (60%) and conversion (45%) were seen at 34 °C. This suggest that high temperature had a negative action on conversion of reduction and enantioselectivity. Thus, we continued the study with 30 °C. Various incubations times (24–96 h) were selected to observed the progress of the reduction reaction in the reaction conditions of pH = 5.5, 100 rpm and 30 °C. The highest conversion (97%) was observed at 96 h, but the ee (65%) was decreased. As incubations time increased, the conversions of bio reduction increased but ee decreased substantially. Hence, the best time chosen was 48 hours because the best ee was obtained in this process. Finally, the rate of agitation speeds was investigated for the best conversion and ee in the reaction conditions of pH = 5.5, 30 °C and 48h. As seen in Table 1, when the agitation speed is increased, conversion of the reaction increased, but the enantioselectivity reduced

Table 3. Enantioselective reduction of acetophenone derivatives **1a–n** by *D hansenii* P1.

Entry	Substrate	Product	ee (%)[b,c]	Conversion (%)[d]	M.B. (%)[d]
1	 **1a**	 **2a**	95 (*S*)	97	94
2	 **1b**	 **2b**	97 (*R*)	90	87
3	 **1c**	 **2c**	99 (*R*)	87	91
4	 **1d**	 **2d**	45 (*S*)	73	88
5	 **1e**	 **2e**	99 (*S*)	93	94
6	 **1f**	 **2f**	92 (*S*)	93	90
7	 **1g**	 **2g**	99 (*R*)	85	89
8	 **1h**	 **2h**	74 (*S*)	95	93
9	 **1i**	 **2i**	82 (*R*)	91	92
10	 **1j**	 **2j**	6 (*R*)	77	94
11	 **1k**	 **2k**	90 (*R*)	72	90

(*continued*)

Table 3. Continued

Entry	Substrate	Product	ee (%)[b,c]	Conversion (%)[d]	M.B. (%)[d]
12			99 (*S*)	>99	92
13			90 (*S*)	80	93

[a]Bio reduction conditions: Substrate: 1 mmol, temperature: 30 °C, time: 48 h, pH: 5.5, 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 literature values.
[d]The conversions and mass balances (MB) were determined by ^{1}H NMR analysis with diphenylmethane as an internal standard.

drastically. The highest ee (95%) and conversion (97%) rate was obtained at 150 rpm and therefore this agitation speed was selected for optimum conditions.

Under the optimum conditions (pH 5.5, temperature 30 °C, time 48h and agitation speed 150 rpm) asymmetric reduction of acetophenone derivatives **1a–n** with *D. hansenii* P1 were investigated, since the corresponding chiral secondary alcohols **2a–n** are important starting materials in the synthesis of pharmacological and bioactive compounds. The bio reduction products demonstrated in Table 3. The stereoselectivity of the reaction was initially studied for acetophenones bearing electron-donating groups (Me and OMe) and electron-withdrawing groups (Cl, Br and NO_2) at the *ortho*-, *meta*- and *para*-position on aromatic benzene ring. As can be seen Table 3, the enantioselectivity of more than 99% was obtained from reduction of *o*-nitro and *o*-bromo acetophenones **1c** and **1e**, which are converted into corresponding alcohols **2c** and **2e** with highest ee (99%) and high conversions 93 and 87% respectively (Table 3, entry 3,5). *o*-chloro acetophenone **1b** was reduced into the (*R*)-alcohol **2b** with high ee (97%) and conversion (90%) (Table 3, entry 2). However, *o*-methoxy acetophenone **1d** was reduced into the (*S*)-alcohol **2d** with low ee (45%), and conversion (73%) (Table 3, entry 4). The bioreduction of *m*-chloro **1f** led to the observe of alcohol **2f** with high ee (92%) and conversion (93%) (Table 3, entry 6). *m*-methoxy acetophenone **1g** was reduced in excellent ee (99%) and moderate conversion (75%) (Table 3, entry 7). Reduction of 4-chloro acetophenone **1h** and 4-bromo acetophenone **1i** occurred with high conversions (95%, 91%), and ee (74%, 82%) respectively (Table 3, entry 8, 9). However, 4-nitroacetophenone **1m** was reduced in the (*S*)-alcohol **2m** with excellent conversion (99%) and ee (99%) (Table 3, entry 12). Electron-donating group methyl at the para position on the aromatic ring led to a slight decrease in the conversion and enantiomeric excess (Table 3, entry 11). 1-(*p*-biphenyl) ethanol **2n** was obtained with moderate conversion rate and enantiomeric excess (Table 3, entry 13). However, the lowest enantioselectivity (6%) and low conversion rate (77%) were obtained in the bio reduction of *p*-methoxy acetophenone **1j** (Table 3, entry 10). The lowest decrease in conversions was observed for substrates **1b** and **1c,** which suggests that the rate of the reduction depends on the steric and electronic effects of bromine and chlorine atom at the *ortho*-position on the phenyl ring. Enantioselectivity decreases as chlorine and bromine groups move away from the reaction center. The decrease in bromine was less because bromine was larger than the chlorine in terms of volume. This shows that the steric effect of chlorine and bromine is important in enantioselectivity. Clearly, the electron-deficient substrate showed higher selectivity, while electron-rich substrate showed moderate selectivity except for *m*-methoxy derivative **1g** (Table 3, entry 7). The methoxy group provides more electrons in the *ortho* and *para* positions while it cannot provide more electrons in the *meta* position. Therefore, the methoxy group in the *meta* position has significantly increased enantioselectivity. It can be said that electronic effect of the methoxy group is greater than steric effect. Similar selectivity in the reductions of the *ortho*- and *para*-nitro derivatives was observed which highlighted the importance of electronic effects for the selectivity of bio reduction with a negligible steric effects on the substituent for these samples. The enantioselectivity of the acetophenone derivatives were dependent on the position of substitutes at benzene ring (Prelog 1964). Since several acetophenone derivatives demonstrated perfect enantioselectivities for the asymmetric reduction on small scale, we decided to conduct the asymmetric reduction of **1g** to (*R*)-**2g** on a large scale to prove the applicability of the current system as

industrially feasible. The asymmetric bioreduction of 3-methoxyacetophenone **1g** (15 mmol) was carried out in a 1 L Erlenmeyer and (*R*)-1-(3-methoxyphenyl) ethanol **2g** was obtained in 82% yield and 99% enantiomeric excess, which is a good result for a preparative scale (Figure 2). In this way, (*R*)-**2g** which can be used for treatment of hyper-parathyroidism was obtained in gram scale. Transformations using isolated enzyme as biocatalysts for reduction of prochiral ketones can produce excellent yields and enantioselectivity. But, these bioreduction reactions require very costly cofactors such as NADPH. These cofactors can be regenerated by using whole cell as biocatalysts throughout the bioreduction reaction (Bradshaw et al. 1992; Kataoka et al. 1997; Chen et al. 2010). In this study, similar to the bio reduction method, the substrate was directly added to the reaction medium without using cofactors and buffers. *D. hansenii* P1 was used effectively as a whole cell biocatalyst for reduction of acetophenone and its derivatives.

Conclusion

In this study, different acetophenones **1a–m** were reduced to the corresponding chiral secondary alcohols **2a–m** with high enantioselectivity using *D. hansenii* P1 yeast strain. We have showed a new yeast to obtain enantiopure chiral secondary alcohols. The present study has many 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 is suitable and shows perfect enantioselectivity which can be used to carried out synthesis of valued enantiopure secondary alcohols. An easy and productive process has been developed for the preparation of chiral secondary alcohols in excellent enantiomeric excess by using *D. hansenii* P1 yeast whole cell as an alternative to other classic enantioselective catalysts that frequently required the use of costly ligands or metal complex. *D. hansenii* P1 can be beneficial for the production of important building blocks in pharmaceutical synthesis.

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Disclosure statement

No potential conflict of interest was reported by the author.

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