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Candida viswanathii as a novel biocatalyst for stereoselective reduction of heteroaryl methyl ketones: a highly efficient enantioselective synthesis of (*S*)-α-(3-pyridyl)ethanol

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Abstract—The enantioselective reduction of various heteroaryl methyl ketones, such as 2-, 3-, and 4-acetyl pyridines, 2-acetyl thiophene, 2-acetyl furan, and 2-acetyl pyrrole, was carried out with the resting cells of a novel yeast strain *Candida viswanathii*. Excellent results were obtained with acetyl pyridines. Moderate conversion took place with 2-acetyl thiophene, but no significant reduction was observed with 2-acetyl furan and 2-acetyl pyrrole. In the case of acetyl pyridines, the bioreduction was found to be sensitive toward the nature of substitution on the pyridine nucleus and the conversion followed the order 4-acetyl pyridine > 3-acetyl pyridine > 2-acetyl pyridine. Reduction of 3-acetyl pyridine with a high conversion (>98%) and excellent enantioselectivity (ee >99%) provided the biocatalytic preparation of (*S*)-α-(3-pyridyl)ethanol, a key intermediate of pharmacologically interesting alkaloids–akuamidine and heteroyohimidine. Finally, preparative scale reduction of 3-acetyl pyridine has been carried out with excellent yield (>85%) and almost absolute enantioselectivity (ee >99.9%).
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1. Introduction

Chiral pyridyl ethanols are important intermediates in the synthesis of a variety of pharmaceuticals including pharmacologically active alkaloids such as akuamidine and heteroyohimidine.[1] These chiral alcohols are useful as dopants, which give spiral structures for liquid crystal molecules in liquid crystal compositions.[2] Apart from this, chiral 1-pyridyl ethanols are useful chiral auxiliaries as they serve as an efficient catalyst in a number of asymmetric addition reactions.[3,4] The asymmetric reduction of heteroaryl ketones is a straightforward approach to prepare this class of compounds.

A number of chemical reducing agents that provide chiral pyridyl alcohols in good yield have been developed.[5,6] Nevertheless, none of these reagents afford a product of high enantiopurity. Furthermore, the preparation of these chiral alcohols with the use of hydrolytic enzymes, such as lipases, has also been attempted.[7–9] However, these reduction processes cannot provide conversions more than 50% as commonly observed in such biocatalytic resolutions. Recently, there have been reports of the synthesis of these chiral alcohols using microbial[10–14] and certain plant cells.[15] Good yields and excellent enantiopurities (ee >99%) were observed, however they suffered due to long reaction times (1–17 days) required to carry out the reaction. Recently, we have reported a highly efficient stereoselective biocatalytic reduction of *N*,*N*-dimethyl-3-keto-3-(2-thienyl)-1-propanamine using *Candida viswanathii*.[16,17] Encouraged by these results and taking into consideration the importance of enantiopure heteroaryl ethanols, we carried out the reduction of different heteroaryl methyl ketones, such as 2-, 3-, and 4-acetyl pyridines, 2-acetyl thiophene, 2-acetyl furan, and 2-acetyl pyrrole.

2. Results and discussion

2.1. Selection of the best biocatalyst

To obtain the best biocatalyst, about 50 oxidoreductase-producing soil isolates were tried for the reduction of

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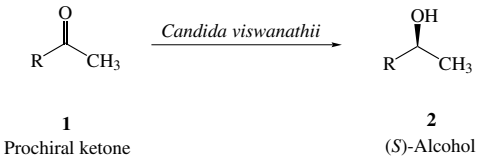
ketones **1a**,**d**–**f**, (Fig. 1) in sodium phosphate buffer (0.2 M, pH 7.0). The reduction of 2-acetyl pyridine **1a** took place with 81–85% conversion. Though 2-acetyl thiophene **1d** was reduced to some extent (9–12% conversion), negligible reduction of 2-acetyl furan **1e**, and 2-acetyl pyrrole **1f** was observed.



1
Prochiral ketone

2
(*S*)-Alcohol

a: R = 2-Pyridyl; **b**: R = 3-Pyridyl; **c**: R = 4-Pyridyl;
d: R = 2-Thienyl; **e**: R = 2-Furyl; **f**: R = 2-Pyrrolyl

Figure 1. Biocatalytic reduction of heteroaryl methyl ketones.

Three yeast species *Candida viswanathii*, *Candida parapsilopsis*, and *Candida melibiosa* were found to possess appreciable reductive properties (Table 1). The stereoselectivity of the bioreduction was found to follow Prelog's rule in all the cases. 2-Acetyl pyridine **1a** was the best substrate as its bioreduction occurred more rapidly when compared to the other ketones, yielding the alcohol, (*S*)-(−)-**2a** with high conversion rate (>80%), and excellent enantiomeric excess (>99%).

2.2. Reduction of acetyl pyridine

The reduction of 2-, 3-, and 4-acetyl pyridines **1a**–**c** was carried out with *C. viswanathii* and it was observed that the conversion followed the order **1c** > **1b** > **1a** (Fig. 2). This might be due to the steric hindrance exerted by the proximity of the basic nitrogen, which is heavily hydrated in the aqueous medium.[18]

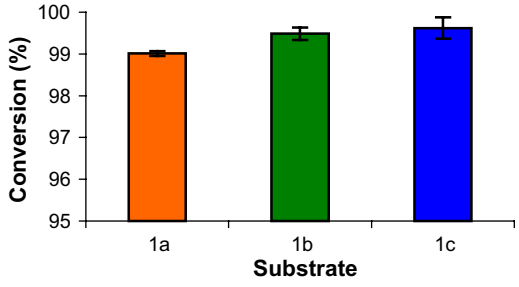


Figure 2. Effect of position of acetyl group on the microbial reduction. (The bioreduction was carried out using 166 g/L of cells for reduction of 2 g/L of ketones at 30 °C, 200 rpm for 12 h.)

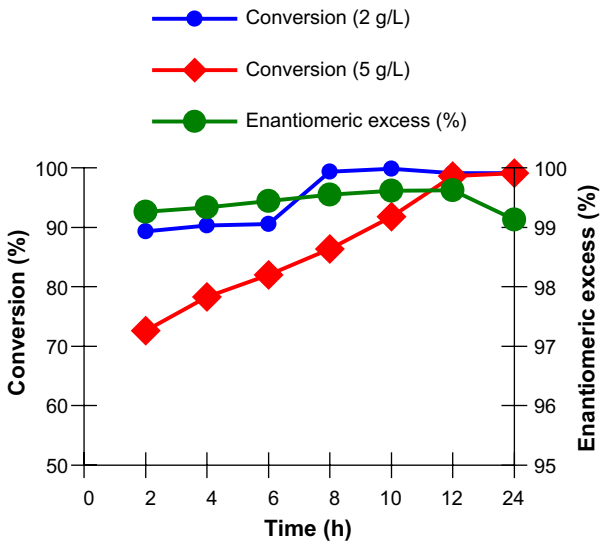


Figure 3. Time course of reduction of **1b** using resting cells of *C. viswanathii*. (The reaction was carried out using 166 g/L of cells at 30 °C, 20 rpm.)

We focussed our attention on the reduction of 3-acetyl pyridine **1b** as (*S*)-α-(3-pyridyl)ethanol **2b** is a key intermediate of pharmacologically interesting alkaloids–akuamidine and heteroyohimidine. Reduction of **1b** was carried out at a substrate concentration of 2 g/L and a resting cell concentration of 166 g/L. Excellent conversion (about 98%) was achieved after 12 h with an enantiomeric excess of >99% (Fig. 3). As a result, all further reactions were carried out for 12 h.

In order to scale up the bioreduction, various concentrations of **1b** ranging from 1 to 20 g/L were tried (Fig. 4). The conversion was >98% up to 5 g/L, beyond which a decrease in the reduction was observed, indicating a detrimental effect of the ketone to the microbial cells at a higher concentration. A 91% conversion was achieved at a substrate concentration of 10 g/L. However, the conversion was 21% with a substrate concentration of 20 g/L.

Keeping the substrate concentration constant (5 g/L), the reduction was carried out at different concentrations of resting cells (50–300 g/L) and it was observed that a resting cell concentration of 150 g/L was sufficient enough to achieve excellent conversion (Fig. 5). A further increase in the resting cell concentration had no significant effect on the reductive potential of the resting cells.

In order to find the optimum temperature, the bioreduction of **1b** was carried out at different temperatures. A significant increase in the conversion was observed when

Table 1. Screening results of heteroaryl methyl ketone reduction

Microorganisms	2-Acetyl pyridine **1a**	2-Acetyl thiophene **1d**	2-Acetyl furan **1e**	2-Acetyl pyrrole **1f**
C. viswanathii	85.39	12.08	3.07	2.13
C. parapsilopsis	81.05	8.95	—	—
C. melibiosa	83.38	10.98	—	—

Reaction conditions: Resting cells (166 g/L) in phosphate buffer (pH 7.0, 0.2 M) and ketone concd in reaction (2 g/L); reaction for 12 h at 30 °C (200 rpm).

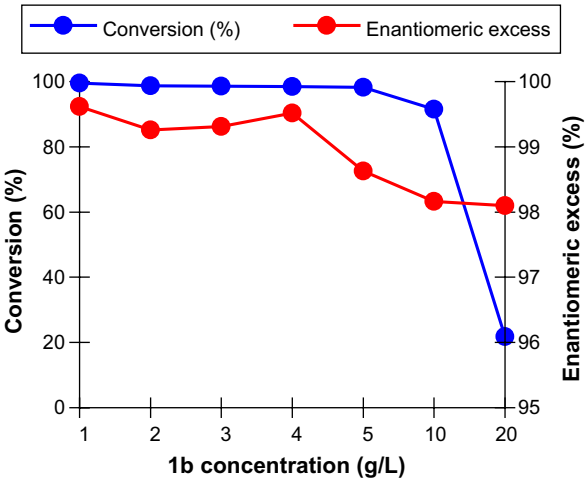


Figure 4. Effect of substrate concentration on the conversion as well as the enantiomeric excess in the microbial reduction of **1b**. (The reaction was carried out using 166 g/L of cells at 30 °C, 200 rpm for 12 h.)

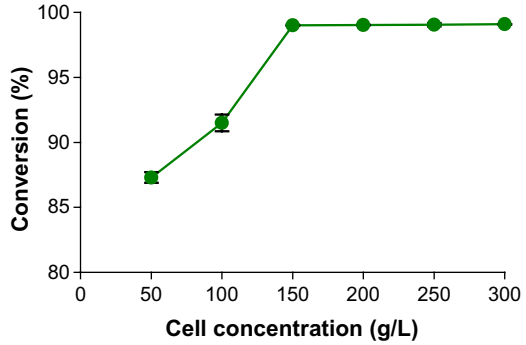


Figure 5. Effect of cell concentration on the microbial reduction of **1b** by *C. viswanathii*. (The bioreduction was carried out using 5 g/L of **1b** at 30 °C, 200 rpm for 12 h.)

the reaction temperature was increased from 25 to 30 °C, beyond which no increase in conversion was observed (Fig. 6).

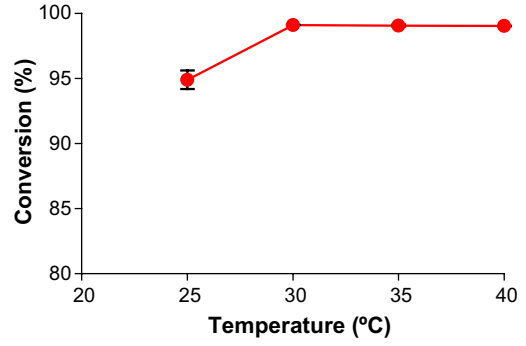


Figure 6. Effect of temperature on the microbial reduction of **1b** by *C. viswanathii*. (The bioreduction was carried out using 150 g/L of cells for the reduction of 5 g/L of **1b** for 12 h.)

The effect of pH on the bioreduction was also studied. This microbial strain was found to carry out the stereoselective reduction of **1b** at a wide pH range (4–9) giving excellent results (conversion > 93%, ee >99%) (data not shown). However, all subsequent experiments were carried out in sodium phosphate buffer (0.2 M) at pH 7.

Finally, preparative scale bioreduction of **1b** was carried out by subjecting 0.8 g of the ketone to reduction by resting cell suspension of *C. viswanathii* in phosphate buffer (0.2 M, pH 7.0) at 30 °C (200 rpm). After 12 h, 685 mg of (*S*)-α-(3-pyridyl)ethanol **2b** was obtained with 85% yield and almost absolute enantioselectivity (ee >99%).

3. Conclusion

It has been shown that *C. viswanathii* acts as a novel biocatalyst for stereoselective reduction of heteroaryl methyl ketones. High substrate selectivity following the order pyridyl ≫ thienyl > furan ~ pyrrolyl was observed for reduction of various heteroaryl methyl ketones. In the case of pyridyl methyl ketones, the selectivity toward the substrate followed the order 4-pyridyl > 3-pyridyl > 2-pyridyl. A highly efficient enantioselective synthesis of (*S*)-α-(3-pyridyl)ethanol **2b**, a key intermediate of pharmacologically interesting alkaloids–akuamidine and heteroyohimidine, was achieved in 98% yield with an ee of >99%. The high yield and excellent enantiomeric excess of **2b** obtained in the present method should offer a simple, efficient, and environment-friendly protocol for synthetically useful quantities of this chiral alcohol. Recently a patent application has been filed for the same.[19] Studies on the purification and characterization of the carbonyl reductase of this microorganism are currently underway. Further exploiting the potential of this microorganism for synthesis of other optically active alcohols is also in progress.

4. Experimental

4.1. General

Heteroaryl methyl ketones **1a**–**f**, were obtained from Lancaster Synthesis Ltd, UK. Conversion of the biocatalytic reduction of the ketones was monitored by RP-HPLC performed on a Shimadzu 10AVP Instrument equipped with UV detector. Ketones **1a**–**f** and the racemic alcohols **2a**–**f** were analyzed by reverse-phase C_{18} (ODS) HPLC column (4 mm × 25 mm, 5 μm, Merck, Germany) using a mobile phase of 10 mM phosphate buffer (pH 7.0) and acetonitrile (85:15, v/v) at a flow rate of 1 mL/min and detected at 254 nm. Chiral alcohols (*S*)-**2d**–**f** were analyzed using Chiralcel OJH column (0.46 mm × 250 mm, 5 μm, Diacel) with hexane–isopropyl alcohol in the ratio of 98:2 (v/v) as the mobile phase (0.5 mL/min) and detected at 254 nm. The enantiomeric excess of (*S*)-**2a**–**c** was determined by GC–MS on GC–MS–QS Shimadzu system. The ee was defined as the ratio of [S] − [R] / [S] + [R] × 100%, where [S] and [R] are the concentrations of the (*S*)- and (*R*)-enantiomers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 NMR spectrometer.

Chemical shifts were reported in parts per million (ppm, δ) using TMS as an internal standard. IR spectra were recorded on a Nicolet spectrometer. Optical rotations were measured on a Rudolph polarimeter (Rudolph Research Autopol IV).

4.2. GC–MS method

To determine the enantiomeric excess of (*S*)-**2b**, a β-column (30 m × 0.25 mm, 0.25 μm, Sigma–Aldrich, USA) with a chiral stationary phase of polysiloxine was used. The temperature program used was 100 °C/2 min—5 °C/min—200 °C/5 min. Retention time for (*R*)-**2b** was 15.9 min and for (*S*)-**2b** was 16.1 min and was ascertained on the basis of optical rotation of (*S*)-**2b** obtained by bioreduction of **1b**.

4.3. Synthesis of reference compounds (*rac*-alcohols)

The racemic alcohols **2a**–**f** were synthesized by reduction of the corresponding ketones **1a**–**f** with $NaBH_4$ following standard procedure.[18] On each occasion, the product was characterized by IR, ^1H and ^{13}C NMR, and GC–MS. The two enantiomers exhibited distinctly different retention times on chiral GC (for **2a**–**c**) and chiral HPLC (for **2d**–**f**).

4.4. General procedure for the microbial reduction of heteroaryl ketones

The microorganism, *C. viswanathii*, was isolated by enrichment and isolation techniques, using acetophenone as the sole source of carbon and energy.[20] A sterilized nutrient broth (100 mL) was inoculated with 5% (v/v) of the preculture (24 h old). The mixture was incubated in an orbital shaker (200 rpm) for 48 h at 30 °C. The cells were harvested by centrifugation (7000*g*, 20 min, 4 °C) and washed thoroughly with phosphate buffer (0.2 M, pH 7.0) and finally resuspended in the same buffer. To the cell suspension (12 mL), an appropriate prochiral ketone (25 mg) in ethanol was added. After 12 h of further incubation, an aliquot of the reaction mixture was centrifuged to separate out the cells. The supernatant as well as the cells was extracted twice with ethyl acetate. The combined ethyl acetate extracts were dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was then analyzed by HPLC.

4.5. Preparative scale bioreduction

The reaction was repeated on a preparative scale by adding 0.8 g of **1b** (as a 100 mg/mL solution in EtOH) to 400 mL of the resting cell suspension (150 g/L) of the yeast cells. The enzymatic reaction was allowed to proceed for 12 h (at 30 °C), after which the reaction mixture was centrifuged to separate out the cells. The supernatant and the cells were extracted three times using ethyl acetate as the solvent, which was evaporated under reduced pressure. The organic solvent was dried over anhydrous sodium sulfate to obtain an oily mass (760 mg). This crude reaction mixture was analyzed by HPLC and GC–MS to determine conversion and enantiomeric excess. Chromatography (silica, CH_3OH 4% in $CHCl_3$ as eluent) of this oily mass afforded the chiral alcohol (*S*)-**2b** (685 mg, isolated yield 85.6%) with >99.9% enantiomeric excess determined by GC–MS on a chiral column. $[\alpha]_D^{20} = -38.4$ (*c* 1.0, EtOH) {lit. $[\alpha]_D^{24} = -38.9$ (*c* 1.02, EtOH)}.[18] MS (ApcI): *m*/*z* = 124 $(M+1)^+$; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (d, 3H), 4.91 (q, 4H), 5.39 (br s, 1H), 7.22 (dd, 1H), 7.73 (d, 1H, 7.97), 8.33 (d, 1H, 4.82), 8.41 (s, 1H); ^{13}C NMR (300 MHz, MeOD): δ = 25.04, 67.14, 76.61, 77.03, 77.45, 123.45, 133.59, 141.92, 146.69, 147.55; IR (neat): ν = 3351.2 cm^{-1}.

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