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Letter

Enantioselective bioreduction of acetophenone and its analogous by the fungus *Trichothecium* sp.

Deendayal Mandal [a], Absar Ahmad [b], M. Islam Khan [b], Rajiv Kumar [a,*]

[a] *Catalysis Division, National Chemical Laboratory, Pune 411008, India*
[b] *Biochemical Sciences Division, National Chemical Laboratory, Pune 411008, India*

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Abstract

Whole cells of the fungus *Trichothecium* were found to be an effective biocatalyst for the enantioselective bioreduction of acetophenone and its analogous compounds to their corresponding (*R*)-alcohols with good to excellent enantiomeric excesses (90–98%). The easy availability of the biocatalyst besides simple reaction conditions suggests the possible use of the present method for preparing important chiral alcohols.

Keywords: Bioreduction; Trichothecium; Acetophenone; Fungus

Significant attention has been paid to enantioselective synthesis of optically pure compounds or chiral synthons, having increasing demand for the development of modern drugs and agrochemicals. Among the chiral compounds, enantiomerically pure alcohols are particularly useful as building blocks for the synthesis of natural products, pharmaceuticals and agricultural chemicals.

Biotransformation is a convenient method for preparing chiral compounds, including chiral alcohols [1,2]. The use of whole microbial cells is particularly advantageous for carrying out the desired reduction since they do not require addition of cofactors for their regeneration.

Bakers' yeast, widely used for bioreduction of prochiral ketones, generally gives the (*S*) alcohol according to Prelog's rule. Simple aromatic ketones are usually very poor substrates for Bakers' yeast mediated reductions, often furnishing very low yields of desired chiral alcohol [3,4]. Whole cells of the fungus *Rhizopus arrhizus* also reduces aromatic ketones to corresponding (*S*)-alcohols [5,6]. Asymmetric reduction of prochiral ketones was also catalysed by the fungus *Geotrichum candidum* IFO 4597 to produce (*S*)-alcohols, with poor [7] to excellent ee [8–10]. However, another strain of *Geotrichum* (*Geotrichum* sp. G38) reduces oxo-esters and aliphatic diketones to produce corresponding (*R*)-alcohol [11]. Very recently, *Rhizopus oryzae* and *Aspergillus terreus* were found to reduce fluoroacetophenone to corresponding alcohols with good to excellent ee [12]. Both enantiomers of secondary alcohols from aromatic ketones could be obtained using the fungus *G. candidum* IFO 5767 by modifying the reaction conditions [13]. Pure enzymatic method has also been applied for the production of (*R*)-1-phenylethanol and the crystal structure of (*R*)-specific alcohol dehydrogenase has been well studied [14,15].

In this communication, we report the use of whole cells of fungi *Trichothecium* sp. to produce mainly (*R*)-alcohols from aromatic ketones, for the first time.

The fungus was maintained on potato-dextrose agar (PDA) slants at 25 °C. The medium MGYP (malt–glucose–yeast–peptone) was prepared by mixing malt extract powder (0.3%), glucose (1.0%), yeast extract powder (0.3%), and peptone (0.5%) in 100 ml distilled water in 500 ml Erlenmeyer flask. The fungus from PDA slant was incubated in the sterilised medium and allowed to grow at 25–28 °C under shaking condition (200 rpm) for 72 h. After 72 h of fermentation, mycelia were separated from the culture broth by centrifugation (5000 rpm) at 10 °C for 20 min and settled mycelia were washed twice with sterile distilled water. The harvested mycelial mass was then resuspended in 100 ml sterile distilled water in 500 ml flasks at pH 5.5–6.0 and the bio-transformation was started by adding the substrate

* Corresponding author. Tel.: +91-20-5893300x2272; fax: +91-20-5893761.
E-mail address: rajiv@cata.ncl.res.in (R. Kumar).

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Table 1
Screening of fungi for the bioreduction of acetophenone

Entry number	Fungal strain	Conversion (mol%)	Time (h)	ee (%)[a]
1	*Fusarium oxysporum* f. sp. *ciceri* NCIM 1282	100	42	74 (*S*)
2	*Verticillium* AAT-TS-3	78.0	48	41.0 (*R*)
3	*Fusarium* sp. NCIM 1075	25.5	24	51.5 (*R*)
4	*Aspergillus flavus* NCIM 542	100	48	67.7 (*R*)
5	*Aspergillus oryzae* NCIM 649	100	48	81.5 (*R*)
6	*Trichothecium* sp.	85.0	72	93.5 (*R*)

Reaction conditions: substrate, 100 mg; room temperature; shaker speed, 200 rpm.
[a] ee was determined by HP chiral (20% permethylated β-cyclodextrin) capillary column.

(100 mg) which was predissolved in ethanol (500 mg) under sterile conditions. The whole mixture was put into a shaker at 28 °C (200 rpm).

The biotransformations were routinely monitored by periodic sampling of aliquots (2 ml), which were extracted with dichloromethane and analysed by GC. After completion of the biotransformation the mycelia were removed by centrifugation (5000 rpm) and the supernatant was extracted with dichloromethane. Extracted solvent was dried and concentrated under vacuum. Crude reaction mixture was analysed by GC (Agilent 6890 series) using HP chiral (20% permethylated β-cyclodextrin) (30MX0.32X0.25) capillary column. The products were also confirmed by GC–MS (Shimadzu, GCMS-QP 2000A) and ^{1}H NMR.

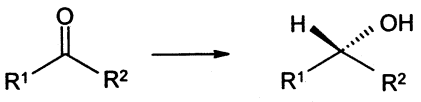
The products (alcohols) were purified by preparative TLC using petroleum ether/ethyl acetate (5:1) solvent system. Optical rotations of purified alcohols were recorded on a JASCO DIP-1020 digital polarimeter and absolute configuration was determined by comparison with literature [16,17].

Among the strains screened (Table 1), *Trichothecium* (entry **6**) was found to yield very high ee followed by *Aspergillus* (entry **4** and **5**) for the enantioselective reduction of acetophenone. As far as *Fusarium* strains are concerned, one strain (entry **1**) could reduce acetophenone to (*S*)-1-phenylethanol in quantitative yield with good enantioselectivity (74%), whereas another strain *Fusarium* sp. NCIM 1075 (entry **3**) produced (*R*)-1-phenyl ethanol with moderate ee (51.5%) and the conversion was also rather low (25.5%). *Verticillium* (entry **2**) also gave moderate ee (41%) at 78% conversion. Both strains of *Aspergillus* reduce acetophenone to (*R*)-1-phenylethanol in the range of moderate to good ee (67.7%, entry **4** and 81.5%, entry **5**).

The fungus *Trichothecium* was also applied to reduce some other prochiral ketones for investigating the substrate specificity. The results are summarised in Table 2. As it may be recalled that acetophenone (entry **1**) could be reduced to (*R*)-1-phenylethanol with 93.5% ee, there was no reaction in the case of ketones where $R^2 = CF_3$ (entry **2**), and cyclopropyl (entry **3**) and R^1 = Ph.

Further, the role of substituents in the aromatic moiety (R1) on the course of the biotransformation was also studied (entry **5**-**6**). In this case, *p*-substituted (electron donating as well as electron withdrawing) phenylethanones were consistently converted to the corresponding (*R*)-alcohols. Introduction of an electron withdrawing substituents is expected to assist the reaction. Indeed, the *p*-chloro-substituted acetophenone was reduced with high enantioselectivity possibly due to the $-I$ effect of the substituent. Acetophenone gets reduced 56% in 24 h by *Trichothecium*, whereas upon methyl substitution ($+I$ effect) at para position (entry **5**)

Table 2
Bioreduction of different ketones by *Trichothecium*



1- 8 **1a – 8a**

Substrate	R1	R2	Product	Yield[a] (mol%)	Time (h)	ee (%)[a]
1	Ph	CH_3	**1a**	85	72	93.5 (*R*)
2	Ph	CF_3		No reaction		
3	Ph	Cyclopropyl		No reaction		
4	Cyclohexyl	CH_3	**4a**	85	96	97.5 (*R*)
5	*p*-MeC_6H_4	CH_3	**5a**	45.5	24	90.5 (*R*)
6	*p*-ClC_6H_4	CH_3	**6a**	72	24	98.5 (*R*)
7	*p*-OHC_6H_4	CH_3		No reaction		
8	*p*-$MeOC_6H_4$	CH_3		No reaction		

Reaction conditions: Biocatalyst, 0.7 g (dry weight); substrate, 100 mg; room temperature; shaker speed, 200 rpm.
[a] Yield and ee were determined by GC analyses by using HP chiral (20% permethylated β-cyclodextrin) capillary column. Absolute configuration was determined by measuring optical rotation and compared with literature.

the reaction becomes slow (conversion 45.5% in 24 h). In contrast, *p*-chloro substitution (entry **6**) accelerates the reaction resulting 72% conversion during same reaction time of 24 h. Two additional electron donating substrates namely *p*-hydroxy acetophenone and *p*-methoxy acetophenone (entry **7**, **8**) were used as reactants. But, there was almost negligible conversion.

The bioreduction of acetophenone was also performed using the solvent DMF instead of ethanol. It was observed that conversion remained same indicating that ethanol was not used for cofactor regeneration.

The effect of different surfactants (SDS, CTAB, IGEPAL, Tritan, Tween 20, Tween 80) was studied to find out whether it could increase the conversion of acetophenone reduction. But interestingly, they did not work.

In conclusion, a simple, effective and inexpensive procedure has been developed for the preparation of (*R*)-alcohols from ketones in good enantiomeric purity.

Further studies to apply the method to other substrates are in progress in our laboratories.

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