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Enantioselective reduction of substituted acetophenones by *Aspergillus niger*

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Abstract—Fourteen strains of *Aspergillus niger* were isolated from soil samples. These isolates were evaluated for the reduction of acetophenone to 1-phenylethanol. Among the tested isolates, whole cells of the *A. niger* EBK-9 isolate were found to be an effective biocatalyst for the enantioselective reduction of acetophenone. Under optimized conditions substituted acetophenones were converted to the corresponding optically active alcohol in up to 99% ee.

1. Introduction

Chiral alcohols are useful starting materials for the synthesis of various biologically active compounds. The need for optically active drugs has increased in pharmaceutical and agrochemical fields in recent years and thus, the demand for chiral alcohols has increased.[1] Among the current methodologies to obtain chiral alcohols are the biocatalytic reduction[2] of the corresponding ketones, and kinetic resolution[3] with lipases of the racemic alcohols via esterification or hydrolysis of the appropriate esters. The use of microbial whole cells as biocatalysts is particularly advantageous for carrying out the desired reduction, since they contain multiple dehydrogenases that are able to accept a broad spectrum of unnatural substrates, as well as all the necessary co-factors and metabolic pathways for their generation. Furthermore, all the enzymes and co-factors are well protected within their natural cellular environment.[4,5] In this way screening for new microorganisms strains from different natural environments can be used in the search for efficient enzymatic systems, which can promote the biotransformation with no need for external additives. Recently, we reported that ram horn hydrolysate (RHH) can be utilized as a source of peptone for microbial growth media.[6] Herein, we report a study on the potential of *A. niger* strains isolated from soil samples as enantioselective reducing agents for substituted acetophenones.

2. Results and discussion

Fourteen different isolates of *A. niger* isolated from soil samples were evaluated for reduction of acetophenone **1a** to 1-phenylethanol **2a**. The microbial reduction was performed by suspending the wet cells in a 50 ml fresh medium, **1a** was then added and the mixture was incubated for 48 h in an orbital shaker at 28 °C. The reaction progress was monitored by 400 MHz ^{1}H NMR spectroscopy and a chiral HPLC column was used for the determination of the enantiomeric excess of **2a**. As can be observed in Table 1, the strains under study produced (*R*)-1-phenylethanol[7] **2a** ranging from 41% to 75% ee. The best results for the bioreduction were obtained when whole cells of *A. niger* EBK-9 were used. The reaction occurred with moderate conversion and produced **2a** in 75% ee (Table 1, entry 9).

Reaction conditions, such as reaction time, the amount of wet biocatalyst, and pH were investigated using *A. niger* EBK-9 under the standard conditions. The best ee (80%) and yield (44%) were observed before 48 h, but an extension of the reaction time led to a decrease in ee, but an increase in the conversion (entry 15). The optimum pH for the enantioselective reduction was found to be 7.0, which yielded **2a** with 82% ee (entry 16). Under the optimum

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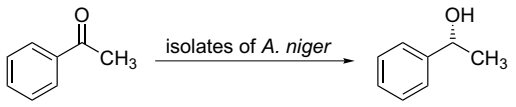
Table 1. Screening for *Aspergillus niger* isolates in the bioreduction of acetophenone **1a**[a]



1a **2a**

Entry	Isolates of *A. niger*	Conversion[b] (%)	Yield[c] (%)	ee of (*R*)-**2a** (%)
1	EBK-1	30	23	64
2	EBK-2	28	15	41
3	EBK-3	35	23	46
4	EBK-4	36	27	67
5	EBK-5	35	26	67
6	EBK-6	36	24	57
7	EBK-7	38	30	65
8	EBK-8	41	34	70
9	EBK-9	46	39	75
10	EBK-10	41	32	64
11	EBK-11	18	14	48
12	EBK-12	36	28	46
13	EBK-13	34	28	48
14	EBK-14	39	52	65
15[d]	EBK-9	53	44	80
16[e]	EBK-9	57	47	82
17[f]	EBK-9	63	54	87

[a] Reaction conditions: biocatalyst 5 g (wet weight); acetophenone: 1 mmol; temperature: 28 °C; time: 24 h; pH: 6.8.
[b] Conversion was determined by ^{1}H NMR analysis with diphenylmethane as an internal standard; error ca. ±5% of the stated values.
[c] Isolated yields.
[d] Biocatalyst: 5 g (wet weight); time: 48 h; temperature: 28 °C; pH: 6.8.
[e] Biocatalyst: 5 g (wet weight); time: 48 h; temperature: 28 °C; pH: 7.0.
[f] Biocatalyst: 7.5 g (wet weight); time: 48 h; pH: 7.0.

conditions (pH 7.0, 48 h), the effect of a wet biocatalyst amount on the conversion and enantioselectivity of acetophenone **1a** reduction was investigated. The best ee (87%) and moderate conversion (63%) were obtained when 7.5 g biocatalyst was applied (entry 17). A biocatalyst amount higher than 7.5 g led to complete conversion of **1a** to **2a**, but with very low ee (27%). The experiments were conducted with *A. niger* EBK-9, which gave the best results in terms of conversion and enantiomeric excess.

After successfully optimizing the reaction conditions, we turned our attention to the biocatalytic reduction of substituted acetophenones **1b**–**1k**. Acetophenone derivatives with different substituents (chlorine, bromine, methyl, methoxy and phenyl groups) on the benzene ring were selected to assess the efficiency and stereoselectivity of the ketone functionality bioreduction by the dehydrogenase present in the enzymatic system of *A. niger* EBK-9. As can be seen in Table 2, an enantioselectivity of more than 96% ee was obtained for the reduction of *o*-chloro and *o*-bromo acetophenones **1b** and **1c**, which are transformed into (*S*)-alcohols[8] **2b** and **2c** with high enantioselectivity and in low conversion for 48 h. However, the bioreduction of *m*-chloro and *m*-bromo derivatives **1d** and **1e** led to the formation of alcohols **2d** and **2e** with moderate ee in low conversion for 48 h. The low conversions observed for substrates **1b**–**1e** show that the rate of the reduction depends on the steric and electronic effects of the bromine or chlorine atom at the *ortho*- and *meta*-position on the aromatic ring. The *A. niger* EBK-9 reduced *para* halogenated acetophenones **1f** and **1g** to the *anti*-Prelog (*R*)-*p*-bromo- and *p*-chloro-arylethanols[9] **2f** and **2g** with complete conversion and excellent enantiomeric excess (99%). Electron donating substituents methyl, methoxy and phenyl groups at the *para* position on the aromatic ring led to a slight decrease in the conversion and enantiomeric excess. Thus, **1h**–**k** were converted to the corresponding (*R*)-alcohols[10] **2h**–**k** with 95, 90, 60% ee, respectively. Since several acetophenones afforded excellent enantioselectivities for the reduction on a small scale, we decided to conduct the transformation of **1f** to (*R*)-**2f** on a large scale to demonstrate the viability of the present system as industrially feasible. The bioreduction in a 2 L Erlenmeyer flask with 10 mmol of 1-(4-chloro-phenyl)ethanone **1f** produced (*R*)-**2f** in 70% yield and 99% enantiomeric excess (Scheme 1). In this way, (*R*)-**2f**[7] was isolated in gram amount. The data concerning the observed stereoselectivities for the substrates clarified that *A. niger* EBK-9 produced (*R*)-alcohols **2f**–**k** from *para*-substituted acetophenone derivatives **1f**–**k** and (*S*)-alcohols **2b**–**e** from *ortho*- and *meta*-substituted acetophenone derivatives **1b**–**e**. This observation can be explained in terms of steric hindrance of the substituents. The bioreduction of sterically unhindered aryl alkyl ketones **1a** and **1f**–**k** was very fast the *anti*-Prelog-(*R*) enantiomer predominated, but acetophenones **1b**–**e** with substituent proximate to the carbonyl group were converted to the Prelog product (*S*)-enantiomer.[11]

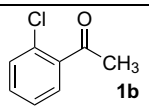
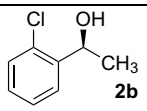
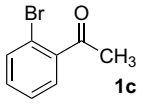
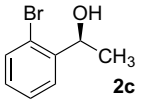
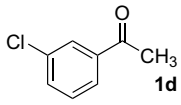
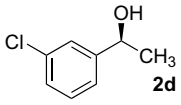
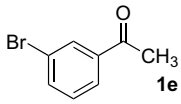
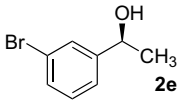
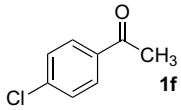
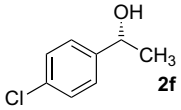
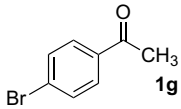
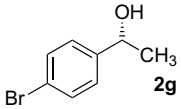
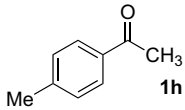
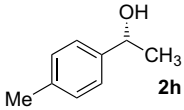
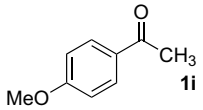
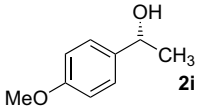
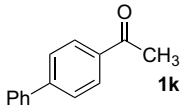
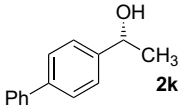
3. Conclusions

In conclusion, 14 strains of *Aspergillus niger* were isolated from soil samples. The *para*- and *ortho*-substituted acetophenones are reduced by whole cells of the *A. niger* EBK-9 to the corresponding chiral alcohols with high enantiomeric excess (up to 99%). The observed Prelog and *anti*-Prelog enantioselectivity depends on the substituents position in the benzene ring. The present bioreduction protocol would be applicable to the production of some enantiomerically pure alcohols.

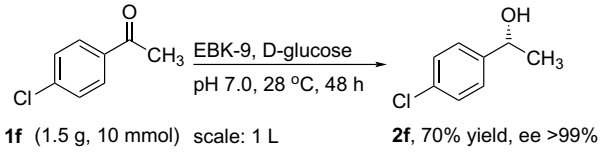
4. Experimental

Ram horns were obtained from the slaughterhouse of Erzurum, Turkey. The other components of the culture media and the chemical reagents were obtained from Acros Chemical and from Sigma in the highest purity available. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 spectrometer in $CDCl_3$. Enantiomeric excesses were determined by HPLC analysis using a chiral column (Chiralcel OD) with eluent *n*-hexane–*i*-PrOH, 90:10, flow rate of 0.6 ml/min, detection performed at 254 nm. Optical rotation was measured with a Bellingham + Stanley, ADP220, 589 nm spectropolarimeter. A polarimetric Chiralyser detector was also employed to assess the configuration of the enantiomer formed. The racemic **2a**–**k** were obtained by reacting the corresponding **1a**–**k** with $NaBH_4$ in methanol at rt.

Table 2. Enantioselective reduction of substituted acetophenones **1b**–**1k**[a]

Substrate	Product	ee[b,c] (%)	Conversion[d] (%)	Yield[e] (%)
 **1b**	 **2b**	>99 (*S*)	16	12
 **1c**	 **2c**	96 (*S*)	11	7
 **1d**	 **2d**	57 (*S*)	25	19
 **1e**	 **2e**	66 (*S*)	21	16
 **1f**	 **2f**	>99 (*R*)	100	87
 **1g**	 **2g**	>99 (*R*)	100	89
 **1h**	 **2h**	95 (*R*)	60	47
 **1i**	 **2i**	90 (*R*)	33	28
 **1k**	 **2k**	65 (*R*)	67	52

[a] Reaction conditions: biocatalyst 7.5 g (wet weight); substrate: 1 mmol; temperature: 28 °C; time: 48 h; pH: 7.0.
[b] Determined by HPLC using Chiralcel OD column.
[c] Absolute configurations were assigned by comparison of the sign of optical rotations relative to the literature values.
[d] Conversion was determined by ^{1}H NMR analysis with diphenylmethane as an internal standard; error ca. ±5% of the stated values.
[e] Isolated yields.



Scheme 1. Bioreduction of **1f** to **2f**.

4.1. Isolation of microorganisms, identification and inoculation

All the microorganisms used in this study were isolated from soil samples collected from around Erzurum, Turkey. The isolation process was performed by serial dilution of the samples according to standard techniques.[12] Taxonomic identification of filamentous fungi were identified in-house by using mature cultures on standard potato dextrose agar (PDA) in order to ensure a good development of taxonomically relevant features, and following the identification keys provided by von Arx[13] and Domsch et al.[14] These cultures were maintained on PDA slants, incubated at 30 °C and stored at 4 °C. The conidia from 5 days old cultures were used for inoculation. The conidial suspension was prepared in sterilized 10 ml distilled water by gently scratching conidia with a sterile wire loop and then it was shaken vigorously for breaking the clumps of conidia.

4.2. Mycelium production from *A. niger* strains and culture conditions

The per liter fermentation medium contained 15 g glucose, 3 g yeast extract and 30 ml RHH. Ram horn hydrolysate was prepared as described previously.[6] The initial pH of

the culture medium was adjusted to 7.0 with 1 M HCl and 1 M NaOH. All the cultures were grown in 250 ml flasks containing 100 ml of medium. One ml of conidial suspension was added to each flask. Flasks were incubated on a reciprocal shaker at 200 rpm, 28 °C for 48 h.

4.3. General procedure for bioreduction of ketones 1a–k

After 48 h of fermentation, mycelia were separated from the culture broth by filtration and cell pellets were washed twice with sterile 0.1 M KH_2PO_4 (pH 7.0). Approximately 7.5 g wet mycelia were put in a 250 ml conical flask containing 50 ml of the fresh culture medium and incubated at 28 °C for 24 h under static conditions. After the growth of the fungus, the substrate (1 mmol) was added directly to medium and then the incubation continued on a reciprocal shaker at 200 rpm, 28 °C for 48 h. At the end of the incubation period, the mycelium was separated by filtration, and the filtrate was saturated with sodium chloride and then extracted with diethyl ether. The mycelia were washed with diethyl ether. Diethyl ether extracts were combined and dried over Na_2SO_4. After removal of the solvent under reduced pressure (25 °C, 100 Torr) the crude product was purified by silica gel column chromatography and identified by NMR analysis. The absolute configuration was determined by the sign of the specific rotation and comparison with the literature. The enantiomeric excess of the alcohol was then determined by chiral HPLC analysis.

4.4. Preparative scale bioreduction of 1f

After 48 h of fermentation, mycelia were separated from the culture broth by filtration and cell pellets were washed twice with sterile 0.1 M KH_2PO_4 (pH 7.0). Approximately 75 g wet mycelia were put in a 2 L conical flask containing 500 ml of the fresh culture medium and incubated at 28 °C for 24 h under static conditions. After the growth of the fungus, 1-(4-chlorophenyl)ethanone **1f** (1.54 g, 10 mmol) was added directly to the medium and then the incubation continued on a reciprocal shaker at 200 rpm, 28 °C for 48 h. At the end of the incubation period, the mycelium was separated by filtration, the filtrate saturated with sodium chloride and then extracted with diethyl ether. The mycelia were washed with diethyl ether. The diethyl ether extracts were combined and dried over Na_2SO_4. After removal of the solvent under reduced pressure (25 °C, 100 Torr) (*R*)-1-(4-chlorophenyl)ethanol **2f** was purified by silica gel column chromatography and identified by NMR analysis (1.1 g, 7 mmol, 70% yield). The absolute configuration was determined by the sign of the specific rotation and comparison with the literature. The enantiomeric excess of the alcohol was then determined by chiral HPLC analysis.

Acknowledgements

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