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Asymmetric reduction of acetophenone analogues by *Alternaria alternata* using ram horn peptone

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Abstract—*Alternaria alternata* EBK-4 fungus isolated from a plant sample was evaluated for the asymmetric reduction of acetophenone analogues. In a previous study, this isolate was used for the reduction of acetophenone to 1-phenylethanol in excellent enantiomeric excess. The substituted acetophenones were converted to the corresponding optically active alcohol by *A. alternata* EBK-4 under optimized conditions in up to >99% enantiomeric excess (ee). This is the first report on the enantiomeric reduction of acetophenone analogues by *A. alternata* using ram horn peptone from waste material.

1. Introduction

Chiral alcohols are important as bioactive compounds and as starting materials for the synthesis of various biologically active compounds. The need for optically active drugs has increased in pharmaceutical and agrochemical fields in the recent years. Therefore, the asymmetric reduction of ketones to their corresponding alcohols is useful in organic synthesis.[1,2] Optically active phenylethanol and its derivatives are useful building blocks for the synthesis of complex molecules, because the alcohol functionality can be easily transformed without racemization into other useful functional groups.[3] There has been much interest in the use of fungus or yeast for the enantioselective reduction of aromatic ketones. They are used as a reducing agent because they are easily available and cheap. There are many advantages to using microorganisms as biocatalysts instead of purified enzymes. Microorganisms are generally much less expensive, and in some cases, enzymes are more stable within the cell, thus extending the life of the biocatalyst. In addition, the use of microbial cells is particularly advantageous for carrying out the desired reduction since they do not require the addition of cofactors for their regeneration.[4–6] On the other hand, biotechnology opens up future prospects in the chemical field for the synthesis of complex compounds and combines inexpensive raw materials with environmentally friendly processes.[7] The fermentation medium can represent almost 30% of the cost for a microbial fermentation, with micronutrients representing the most significant cost of production.

By-products can supply unique micronutrients to replace expensive peptone and yeast extract. The consistency of ingredients used in commercial medium formulations and significant increase in product yield or cost reduction are critical for industrial fermentation utilization of any by-product.[8]

Recently, we reported that ram horn peptone (RHP) can be utilized as a source of peptone for microbial growth media in chiral alcohol synthesis.[9f] Herein, we report a study on the potential of *Alternaria alternata* EBK-4 as an asymmetric reducing agent for acetophenone analogues by using the RHP in fermentation medium.

2. Results and discussion

A series of acetophenone derivatives were targeted for the biotransformation using *A. alternata* isolate in RHP medium, and *ortho*-, *meta*- and *para*-substituted fluoro, chloro, bromo, methyl methoxy and phenyl acetophenones **1b–l** were reduced to the corresponding (*R*)- or (*S*)-alcohols.

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In a previous study, *A. alternata* fungus isolated from a plant sample was evaluated for reduction of acetophenone **1a** to 1-phenylethanol **2a**.[9] The asymmetric reductions of **1a** derivatives with the same fungus were performed under previously determined optimum fermentation conditions. 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 **1a** derivatives. As can be seen from Table 1, the fungus was found to catalyze the asymmetric reduction of **1a** derivatives, such as **1b**–**l**, to give the corresponding chiral alcohols. The experiments were carried out on a millimole scale in 100 mL culture medium and the corresponding chiral alcohols according to substrate conversion rate were isolated in 17–78% yields. Reduction of *o*-, *m*-fluoro, *o*-, *m*-chloro and *o*-, *m*-bromo acetophenones provided excellent enantioselectivity (>99%). In contrast, reduction of *p*-chloro, *p*-bromo, *p*-nitro, methoxy and phenyl acetophenones was observed in low yields (17–68) and enantioselectivity (29–90%). The results obtained from **1b**–**g** demonstrated the applicability of this process and the fungus in the preparation of **2b**–**g**. Acetophenone derivatives with different substituents, such as 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. alternata* EBK-4. As can be seen in Table 1, an enantioselectivity of more than 99% ee was obtained for the reduction of *o*-fluoro, *o*-chloro, *o*-bromo, *m*-fluoro, *m*-chloro and *m*-bromo acetophenones **1b**–**g**, which are transformed into the (*S*)-alcohols[10] **2b**–**g** with high enantioselectivity in complete conversion. However, the bioreduction of *para* halogenated acetophenones *p*-chloro and *p*-bromo derivatives **1h** and **1i** led to the formation of alcohols **2h** and **2i** with moderate ee in low conversion. The low conversions or ees observed for the substrate **1h**–**l** testify that the rate of the reduction depends on the steric and the electronic effects of the bromine or chlorine atom at the *ortho*-, *meta*- or *para*-position on the aromatic ring. Electron donating or withdrawing substituents nitro, methoxy and phenyl groups at the *para*-position on the aromatic ring led to a dramatical decrease in the conversion and enantiomeric excess. Thus, **1h**, **1i** and **1k** were converted to the corresponding (*R*)-alcohols[11] **2h**, **2i** and **2k** with 29, 49 and 49% ee, respectively. It was found that the electronic effect of the substituents on the aromatic ring has a defined role in the enantioselectivity and the configuration of the reaction products. The data concerning the observed stereoselectivities for the substrates clarified that *A. alternata* EBK-4 produced (*S*)-alcohols **2b**–**g** from *o*- and *m*-substituted acetophenone derivatives **1b**–**g** and (*R*)-alcohols **2h**,**i**,**k** from *para*-substituted acetophenone derivatives **1h**,**i**,**k**. This observation can be explained in terms of steric hindrance of the substituents. The bioreduction of sterically unhindered aryl alkyl ketones **1b**–**g** and **1j**,**l** was very fast; the Prelog-(*S*) enantiomer predominated, but the acetophenones **1h**,**i**,**k** with a substituent proximate to the carbonyl group were converted to the *anti*-Prelog product (*R*)-enantiomer.[12] These results suggest that the reduction of *o*-, *m*-fluoro, chloro and bromo acetophenones by *A. alternata* by using RHP will be very useful for the practical preparation of (*S*)-alcohols. The *A. alternata* fungus catalyzed the reactions in an eco-friendly environment when compared to chemical reactions. Moreover, the RHP from waste material as an inexpensive substrate for microbial growth was used. Meat industry wastes are an important environmental contamination source. The importance of this research must be high because of the formation of little waste, use of acceptable solvents, transformation of waste materials into valuable products and the highly asymmetric synthesis of the many desired products.

3. Conclusions

In conclusion, *A. alternata* EBK-4 isolated from plant sample is the first report regarding the use of a biocatalyst for the asymmetric reduction of acetophenone derivatives. The *ortho*- and *meta*-substituted acetophenones are reduced in submerged culture of the *A. alternata* EBK-4 to the corresponding chiral alcohols with high enantiomeric excess (>99%). The observed Prelog and *anti*-Prelog enantioselectivity depends on the substituent's position in the benzene ring. In previous study, (*S*)-1-phenylethanol was successfully produced on a gramme scale with the present process.[9] This bioreduction protocol is applicable to the production of some enantiomerically pure alcohols.

4. Experimental

4.1. Materials

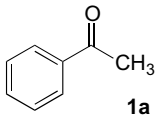
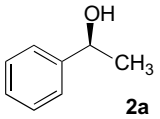
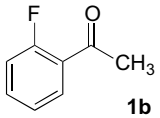
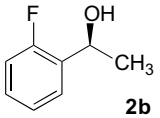
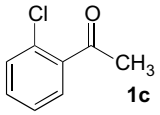
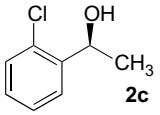
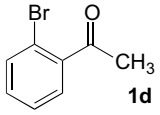
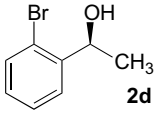
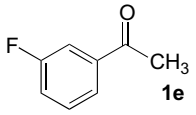
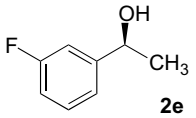
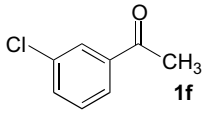
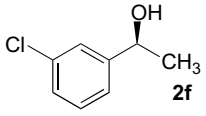
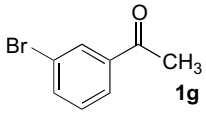
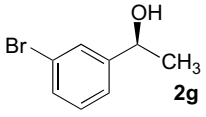
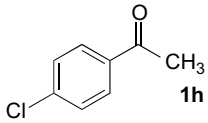
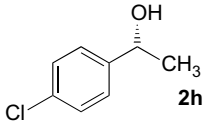
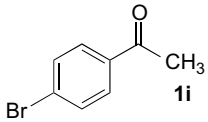
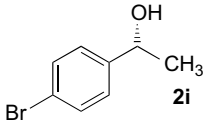
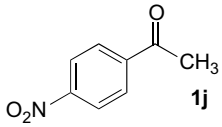
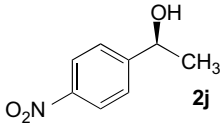
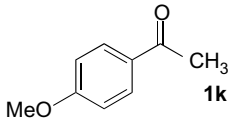
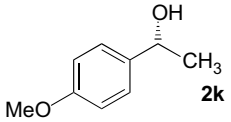
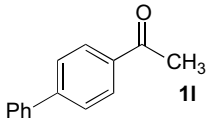
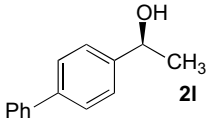
Ram horns were obtained from the Slaughterhouse of Erzurum, Turkey. The other components of the culture media and the chemical reagents were obtained from Merck and Sigma in the highest purity available. The production of ram horn peptone was carried out with the method of Kurbanoglu and Kurbanoglu.[9]

The microorganism used in this study was isolated from a plant sample. The isolation process was performed by the serial dilution of the samples according to standard techniques. Taxonomic identification of filamentous fungi was identified in-house by using mature cultures on standard potato dextrose agar (PDA) in order to ensure a good development of taxonomically relevant features. The fungus used was maintained on PDA slants, incubated at 25 °C and stored at 4 °C. The conidia from 10 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 to break the clumps of conidia.[9f]

4.2. Culture conditions and reduction of acetophenone analogues

The culture conditions were prepared according to optimum fermentation parameters found for *A. alternata* in a previous study.[9] The per liter fermentation medium contained (g/L): glucose 20, yeast extract 3 and RHP 4. The initial pH of the culture medium was adjusted to 6.5 with 1 M HCl and 1 M NaOH and sterilized at 121 °C for

Table 1. Asymmetric reduction of acetophenone analogues **1b–l** by *A. alternata* EBK-4[a]

Substrates	Products	ee[b,c] (%)	Conversions[d] (%)	Yields (%)
 **1a**	 **2a**	(*S*)-> 99[e]	100[e]	86[e]
 **1b**	 **2b**	(*S*)- >99	100	78
 **1c**	 **2c**	(*S*)- >99	100	76
 **1d**	 **2d**	(*S*)- >99	100	74
 **1e**	 **2e**	(*S*)- >99	100	72
 **1f**	 **2f**	(*S*)- >99	100	73
 **1g**	 **2g**	(*S*)- >99	100	74
 **1h**	 **2h**	(*R*)-29	92	68
 **1i**	 **2i**	(*R*)-49	85	61
 **1j**	 **2j**	(*S*)-20	70	52
 **1k**	 **2k**	(*R*)-49	36	27
 **1l**	 **2l**	(*S*)-90	25	17

[a] Bioreduction conditions: substrate: 1 mmol; temperature: 28 °C; time: 24 h; pH: 6.5, 150 rpm.
[b] Determined by HPLC using Chiralcel OD and OB columns.
[c] Absolute configurations were assigned by comparison of the sign of specific rotations relative to the literature values.
[d] Conversion was determined by ^{1}H NMR analysis with diphenylmethane as internal standard; error ca ±5% of the stated values.
[e] From Ref. 9f.

15 min. 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 150 rpm, 28 °C for 48 h. After the growth of the fungus, acetophenone derivatives (1 mmol) were directly added to each medium and then the incubation continued on a reciprocal shaker at 150 rpm, 28 °C for 24 h.

4.3. Purification of products and analytical processes

After the specified time, the mycelium was separated by filtration, and the filtrate was saturated with sodium chloride and then extracted with diethyl ether. The mycelia were also extracted with diethyl ether. The diethyl ether extracts were combined; the ether was dried over Na_2SO_4, and evaporated to dryness. For analysis, a small fraction of the product was separated by preparative silica-gel TLC. The ees of the products were determined by HPLC with OD and OB columns using eluent *n*-hexane–*i*-PrOH, 90:10, flow rate of 0.6 mL/min, detection performed at 220 nm. The crude product was purified by silica gel column chromatography. ^1H and ^{13}C NMR spectra were recorded on a Varian 400 spectrometer in $CDCl_3$. Purified chiral alcohols were identified by spectral data (^1H and ^{13}C NMR). Conversions and yields were determined by ^1H NMR analysis with diphenylmethane as internal standard; error ca ±5% of the stated values. The racemic alcohols **2b**–**l** were obtained by reacting the corresponding **1b**–**l** with $NaBH_4$ in methanol at rt.

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