Biocatalysis and Biotransformation, 2013; 31(6): 323–328

informa
healthcare

ORIGINAL ARTICLE

Asymmetric bioreduction of *p*-haloacetophenones by *Mucor*

ZUOHUI ZHANG[1,2], XIONGMIN LIU[1], LI MA[1], MEIFANG ZHUO[1] & FANG SHEN[1]

[1]*College of Chemistry and Chemical Engineering, Guangxi University, Nanning, P. R. China and* [2]*Guangxi Institute of Fisheries, Nanning, P. R. China*

Abstract

A number of *p*-haloacetophenones were asymmetrically bioreduced to their corresponding (S)-alcohols by *Mucor* sp. CG10 with good conversion and excellent enantioselectivity. The results showed that the electronic effects of the halogen substituent (X-group) affected the conversion of the substrates and the enantioselectivity of the reaction. The trend observed was as the X-group at the *para*-position became more electron donating from F, to Cl, Br and I, the conversion of substrates decreased, while the enantioselectivity increased.

Keywords: *p-haloacetophenones, chiral alcohols, Mucor, whole-cell*

Introduction

Enantiomerically pure alcohols are useful building blocks and key intermediates for the synthesis of bioactive compounds such as pharmaceuticals, flavour, agricultural chemicals and speciality materials (Hanson et al. 2005). There are many methods to produce enantiomerically pure alcohols, for example, chromatographic techniques (McConnell et al. 2007), organocatalyzed direct asymmetric aldol reactions (Matsuda et al. 2009) and the enzymatic kinetic resolution of racemic alcohols (Singh et al. 2010). In addition to these methods, the asymmetric bioreduction of prochiral ketones (Goldberg et al. 2007a,b; Singh et al. 2009; Barros-Filho et al. 2009) is the most attractive because it can result in enantiomerically pure alcohols and can give 100% theoretical yield (Yang et al. 2007).

Whole-cells of different fungi have been used in asymmetric bioreduction because these are inexpensive and readily accessible. For example, *Aspergillus terreus* and *Rhizopus oryzae* were found to reduce fluoroacetophenones to corresponding alcohols in good yield and high enantioselectivity (Comasseto et al. 2003). *Aspergillus niger* could reduce substituted acetophenones to the corresponding optically active alcohol (Kurbanoglu et al. 2007). *Rhodotorula rubra* and *Geotrichum candidum* were used to reduce 4-bromoacetophenone to (S)-4-bromophenylethanol and (R)-4-bromophenylethanol (Lopes et al. 2011), etc. However, no microorganism that could reduce the four kinds of *p*-haloacetophenones into the corresponding (S)-alcohols with good conversion and high enantioselectivity have been reported.

Mucor has been reported to produce a carbonyl reductase which utilizes only conjugated polyketones as substrates (Shimizu et al. 1988). Previously we have reported the reduction of acetophenone to (S)-1-phenylethyl alcohol, using *Mucor* sp.JX23, with high stereoselectivity (Ma et al. 2011). Herein, we reported another ketone reductase-producing strain *Mucor* sp. CG10 for the reduction of *p*-haloacetophenones 1a–1d into the corresponding (S)-alcohols 2a–2d with good conversion and high enantioselectivity (Figure 1). The enantioselectivities were affected by the electronic effects of the halogen substituent.

Methods

Reagents and solvents

1a (99%), 1b (99%), 1c (99%) and 1d (99%) were purchased from Fluka; The racemic alcohols 2a–2d were prepared by reduction of the corresponding ketones 1a–1d with sodium borohydride in methanol;

Correspondence: X. M. Liu, College of Chemistry and Chemical engineering, Guangxi University, Nanning 530000, P. R. China. Tel: + 86-771-3270732. E-mail: xmliu1@gxu.edu.cn

(Received 19 January 2013; revised 18 June 2013; accepted 21 October 2013)

ISSN 1024-2422 print/ISSN 1029-2446 online © 2013 Informa UK, Ltd.
DOI: 10.3109/10242422.2013.858710

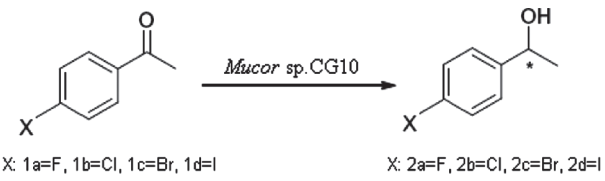

Figure 1. Reduction of 4-haloacetophenones with whole cells of *Mucor* sp.CG10.

Analytical grade solvents for extraction and GC were purchased from Fisher Scientific.

Microorganism and cultures

Mucor sp.CG10 (Zhang et al. 2010) used for the bioreduction of *p*-haloacetophenones was cultivated in a culture medium composed of 50 g dextrin, 24 g peptone, 0.5 g NaCl, 0.5 g $MgSO_4$, 1 g K_2HPO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, per litre (pH 6.0).

Conditions for growth

Mucor sp.CG10 from a slant was inoculated into a 500 ml conical flask containing 100 ml of culture medium and allowed to grow at 30°C under shaking condition (180 rpm) for 72 h. After that period, mycelia were separated from the culture broth by filtration and wet mycelia were then obtained.

Bioreductions of 1a–1d

2.4 g wet mycelia were put into a 500 ml conical flask containing 100 ml of fresh culture medium and the substrates 1a–1d (20 mM diluted in 2 ml of 99% ethanol) were added to the medium. Bioreductions were performed at pH 4, 30°C, 180 rpm for 72 h and the asymmetric reaction mixtures were then recovered.

Analytical methods

The asymmetric reaction mixtures were extracted from the filtered broth with ethyl acetate and concentrated under vacuum. Gas Chromatography analyses were performed using a Shimadzu GC-2010 Plus, equipped with a Cyclodex-B capillary chiral column (30 m × 0.32 mm, Agilent) and a FID, for the determination of the conversion and enantiomeric exces (ee) of the products formed. The chromatographic conditions were carrier gas nitrogen 60 kPa; injector temperature 220°C; detector temperature 220°C; injector split ratio 30:1; temperature program 120°C–148°C with a rate of 5°C per minute, then to 150°C with a rate of 1°C per minute, and then to 166°C with a rate of 8°C per minute, to 170°C with a rate of 1°C per minute, to 190°C with a rate of 5°C per minute, then maintained for 5 min. The absolute configurations were identified by comparing the GC retention times with those of corresponding racemic mixtures and the optical rotations of raw 2a–2d products were measured; the $[\alpha]_D$ were all of negative value.

Results and discussion

Effect of growth conditions on the wet weight of Mucor sp.CG10

Growth conditions are important for the growth of microorganisms and they lead to different concentrations of cell mass. The growth conditions for *Mucor* sp.*CG10*, such as temperature and pH were investigated. The optimum temperature for the growth of *Mucor* sp. CG10 was found to be 30°C (Figure 2), at which 2.2 g wet mycelia were obtained. Under the optimum temperature (30°C), the effect of pH was investigated. The highest yield of wet mycelia 2.4 g was obtained at pH 5.0 (Figure 3). Thus, a cultivation temperature of 30°C and a culture medium of pH 5.0 for *Mucor* sp. CG10 were chosen.

Effect of pH on bioreduction of 1a–1d

One of the important parameters in industrial bioproduction is medium pH and its control. In this study, the influence of pH was investigated, the bioreductions were carried out in the range of pH 3.0–8.0. As shown in Figure 3, *Mucor* sp. CG10 showed similar reduction profiles for all substrates, the conversion of 1a–1d was significantly affected at low pH, while the ee of 2a–2d were slightly affected by the pH values tested. The conversion increased rapidly when increasing pH from pH 3.0 to pH 4.0, and decreased slowly from pH 4.0 to pH 6.0. At higher pH values (7.0 and 8.0), the conversion decreased rapidly once again. The effect of pH on the reduction was roughly similar to that on the growth of *Mucor sp.CG10*, except the slightly difference in the optimum values.

Effect of temperature on bioreduction of 1a–1d

The bioreductions were performed at 20, 25, 30, 35 and 40°C. It was observed that the conversion significantly increased as the temperature increased from 20 to 30°C and decreased at higher temperature. No significant change was observed for the enantioselectivity (Figure 2). It seemed that the optimum reaction temperature was 30°C, according

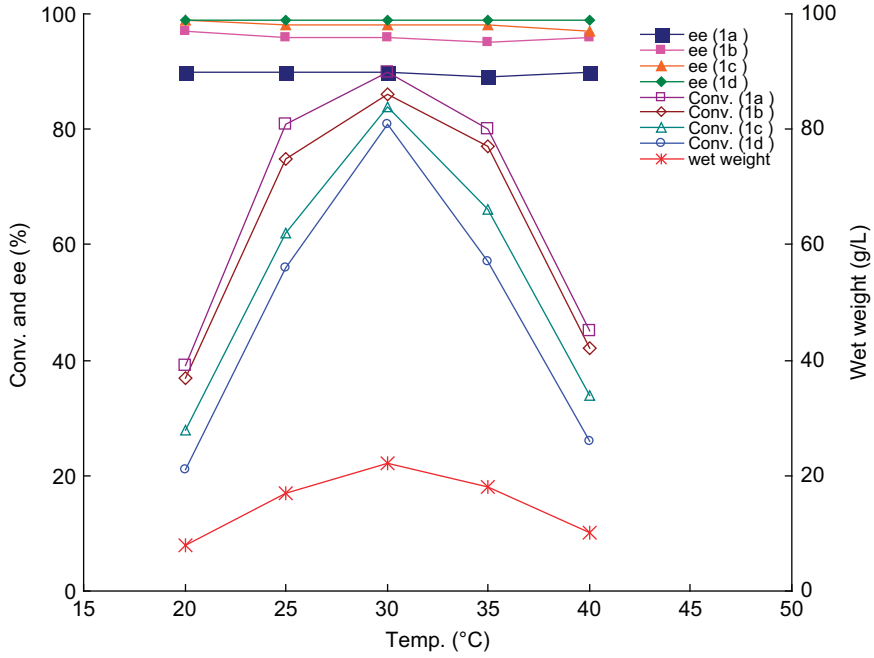

Figure 2. Effect of temperature on the wet weight of *Mucor sp.*CG10 and the bioreduction of 1a–1d.

with its optimum growth temperature of *Mucor* sp. CG10.

Effect of glucose as a co-substrate

Cofactor recycling is one of the key issues in asymmetric bioreductions (Nie et al. 2004), and cofactor regeneration with glucose is attractive because it is plentiful and cheap. In order to investigate the effect of glucose on the bioreduction, different concentrations of 1a–1d were reduced in the presence of different concentrations of glucose. It can be seen that the effect of glucose was significant (Table I), the conversion of the reactions with different concentrations (10–40 g/L) of glucose were higher than that without glucose, and the effect

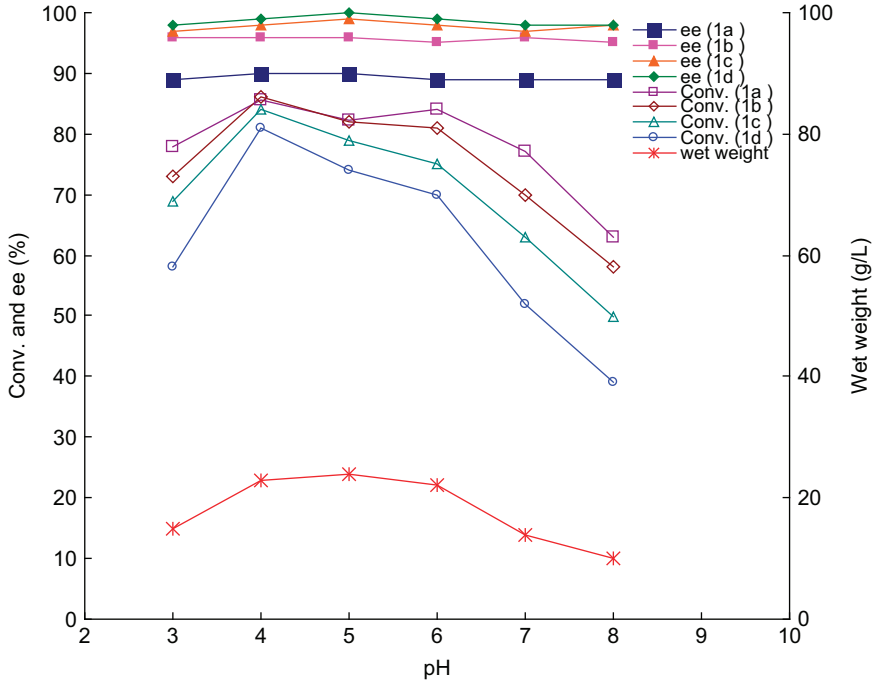

Figure 3. Effect of pH on the wet weight of *Mucor sp.*CG10 and the bioreduction of 1a–1d.

Table I. Asymmetric bioreductions of 1a–1d at different glucose concentrations by *Mucor* sp.CG10.[a]

Conc. of substrate (mM)	Conc. of glucose (g/L)	Conv.[b] (%)				ee[c] (%) - Configuration			
		1a	1b	1c	1d	2a	2b	2c	2d
10	0	97	95	93	91	90-S	96-S	98-S	99-S
10	10	99	99	97	96	93-S	97-S	99-S	>99-S
10	20	99	98	98	98	92-S	96-S	99-S	>99-S
10	30	98	98	98	98	92-S	96-S	>99-S	>99-S
10	40	98	97	98	98	91-S	96-S	>99-S	>99-S
20	0	90	86	84	81	90-S	96-S	98-S	99-S
20	10	97	94	90	88	92-S	96-S	>99-S	>99-S
20	20	99	95	94	93	92-S	96-S	>99-S	>99-S
20	30	98	95	94	94	92-S	96-S	>99-S	>99-S
20	40	96	94	94	94	92-S	96-S	>99-S	>99-S
30	0	82	52	38	29	89-S	94-S	97-S	98-S
30	10	91	60	47	40	91-S	95-S	99-S	99-S
30	20	97	64	57	49	92-S	95-S	>99-S	>99-S
30	30	94	66	58	51	92-S	95-S	>99-S	>99-S
30	40	90	70	59	52	92-S	95-S	99-S	>99-S
40	0	39	19	11	8	86-S	94-S	97-S	98-S
40	10	46	25	19	15	88-S	94-S	98-S	>99-S
40	20	54	28	27	24	89-S	95-S	>99-S	>99-S
40	30	56	30	28	24	90-S	95-S	>99-S	>99-S
40	40	50	31	28	23	90-S	95-S	99-S	>99-S
50	0	11	8	6	5	86-S	94-S	95-S	97-S
50	10	15	12	9	8	88-S	95-S	97-S	99-S
50	20	18	15	14	12	88-S	96-S	>99-S	>99-S
50	30	21	16	15	13	89-S	96-S	99-S	>99-S
50	40	19	17	16	13	89-S	95-S	99-S	99-S

[a]Reaction conditions: pH 4, temperature 30°C, time 72 h, agitation 180 rpm.
[b]The conversions were determined by GC analysis.
[c]Enantiomeric excess (ee) was determined by chiral GC analysis, and the absolute configurations were determined by measuring the optical rotation of raw 2a–2d obtained.

became greater as the concentrations of substrate increased. Moreover, at the same concentrations of the substrates, the lowest amount of glucose was required when 1a was reduced to its maximum conversion, compared with 1b, 1c and 1d.

Effect of the electronic effects of substituents

We began with a research for the bioreduction of 1b by *Mucor* sp. CG10, and extended this to the other *p*-haloacetophenones 1a, 1c and 1d to investigate the influence of electronic effects of substituents on the bioreduction by *Mucor* sp. CG10 (Figure 4). The results of bioreduction assays with different concentrations of 1a–1d are listed in Table I. Usually, because of the toxicity of aromatic ketones, only very low substrate concentrations (typically 10 mM) can be used in biocatalytic reactions using whole-cells as biocatalysts (Xie et al. 2009). Kurbanoglu et al. (2010) described the reductions of 1a–1c and obtained the corresponding (S)-alcohols 2a-2c with 100% conversion and higher than 99% ee at low

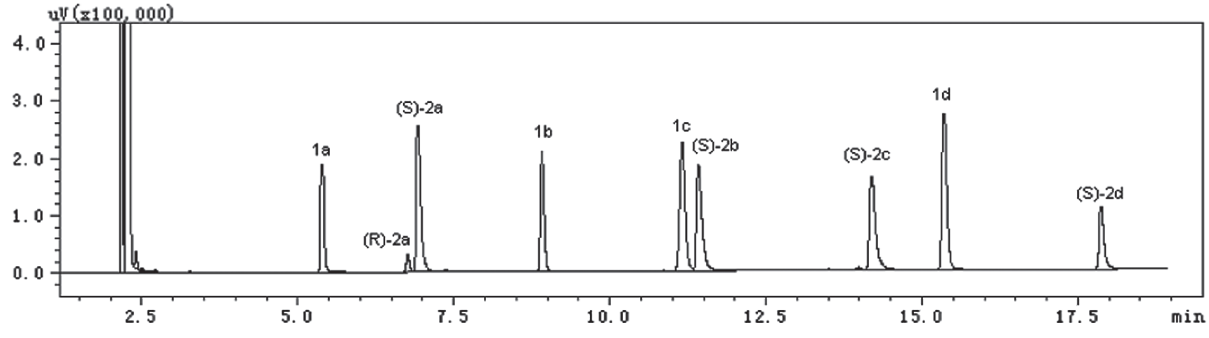

Figure 4. GC chromatography of 1a–1d catalyzed by *Mucor* sp.CG10.

concentration of substrates (1 mM). In our work, 4-haloacetophenones were asymetrically reduced by *Mucor* sp. CG10, followed the Prelog rule, at a relatively high concentration (10–30 mM). As Table I shows, when the substrates concentration was increased to 20 mM (glucose 20 g/L), satisfactory results were obtained, with conversions of 1a–1d (>93%) and ee of 2a–2d (>92%). When the concentration of 1a was further increased up to 30 mM (glucose 20 g/L), the reaction conversion achieved 97% and ee 92%, indicating the potential for industrial production of 2a. Kurbanoglu et al. (2011) observed a decrease in conversion for the analogues containing electron-donating substituents (methyl, methoxy and phenyl) at the *para*-position. Mandal et al. (2004) found that conversion with a methyl substitution (+I effect) at *para*-position was lower than that of *p*-chloro substitution (−I effect) over the same reaction time. Here, as shown in Table I, the conversion of 1a was the highest among the four *p*-haloacetophenones. The results indicated that the electron-withdrawing substituents were beneficial to the conversion. On the other hand, the optical purity of 2a was the lowest, only in the range of 86–93%, with the optical purities of 2b, 2c and 2d increasing in this order. This indicated that the electronic effects of substituents in *p*-substituted haloacetophenones significantly affected the enantioselectivity of reactions. As the X-group became more electron donating going from F, to Cl, Br and I, the enantioselectivity increased.

Conclusion

An efficient method for the reduction of *p*-haloacetophenones to the corresponding alcohols with whole cells of *Mucor* sp. CG10 has been developed. *Mucor* sp. CG10 used glucose for cofactor regeneration and proved to be an excellent biocatalyst in the reduction of *p*-haloacetophenones to their corresponding (S)-alcohols. The results showed that the electronic effects of the halogen substituent (X-group) in *p*-substituted haloacetophenones greatly affected the conversion and enantioselectivity of the reaction. As the *p*-substituent became more electron donating from F, to Cl, Br and I, the conversion of substrates decreased, while the enantioselectivity increased. Further studies with identification and characterization of the enzymes in *Mucor* sp. CG10 are currently in progress within our research group.

Acknowledgements

None.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

This research was financially supported by Scientific Research & Technology Development Project of Guangxi Zhuang Autonomous Region (No: 1099061-2).

References

Barros-Filho BA, de Oliveira MCF, Lemos TLG, de Mattos MC, de Gonzalo G, Gotor-Fernández V, Gotor V. 2009. *Lentinus strigellus*: a new versatile stereoselective biocatalyst for the bioreduction of prochiral ketones. Tetrahedron Asymmetry 20:1057–1061.

Comasseto JV, Omori ÁT, Andrade LH, Porto AM. 2003. Bioreduction of fluoroacetophenones by the fungi *Aspergillus terreus* and *Rhizopus oryzae*. Tetrahedron Asymmetry 14: 711–715.

Goldberg K, Schroer K, Lütz S, Liese A. 2007a. Biocatalytic ketone reduction-a powerful tool for the production of chiral alcohols-part I:processes with isolated enzymes. Appl Microbiol Biotechnol 76:237–248.

Goldberg K, Schroer K, Lütz S, Liese A. 2007b. Biocatalytic ketone reduction-a powerful tool for the production of chiral alcohols-part II: whole-cell reductions. Appl Microbiol Biotechnol 76:249–255.

Hanson RL, Goldberg S, Goswami A, Tully TP, Patel RN. 2005. Purification and cloning of a ketoreductase used for the preparation of chiral alcohols. Adv Synth Catal 347: 1073–1080.

Kurbanoglu EB, Zilbeyaz K, Kurbanoglu NI, Kilic H. 2007. Enantioselective reduction of substituted acetophenones by Aspergillus niger. Tetrahedron Asymmetry 18:1159–1162.

Kurbanoglu EB, Zilbeyaz K, Ozdal M, Taskin M, Kurbanoglu NI. 2010. Asymmetric reduction of substituted acetophenones using once immobilized *Rhodotorula glutinis* cells. Bioresour Technol 101:3825–3829.

Kurbanoglu EB, Zilbeyaz K, Kurbanoglu NI. 2011. *Cryptococcus laurentii* as a new biocatalyst for the asymmetric reduction of substituted acetophenones. Tetrahedron Asymmetry 22: 345–350.

Lopes RO, Ribeiro JB, Ramos AS, Miranda LSM, Leal ICR, Leite SGF, Souza ROMA. 2011. Highly enantioselective bioreduction of 4-bromoacetophenone. Tetrahedron Asymmetry 22:1763–1766.

Ma L, Liu X, Liang J, Zhang Z. 2011. Biotransformations of cinnamaldehyde, cinnamic acid and acetophenone with *Mucor*. World J Microbiol Biotechnol 27:2133–2137.

Mandal D, Ahmad A, Khan MI, Kumar R. 2004. Enantioselective bioreduction of acetophenone and its analogous by the fungus *Trichothecium sp*. J Mol Catal B Enzym 27:61–63.

Matsuda T, Yamanaka R, Nakamura K. 2009. Recent progress in biocatalysis for asymmetric oxidation and reduction. Tetrahedron Asymmetry 20:513–557.

McConnell O, Bach A, Balibar C, Byrne N, Cai Y. 2007. Enantiomeric separation and determination of absolute stereochemistry of asymmetric molecules in drug discovery-building chiral technology toolboxes. Chirality 19:658–682.

Nie Y, Xu Y, Mu XQ. 2004. Highly enantioselective conversion of racemic 1-phenyl-1,2-ethanediol by stereoinversion involving a novel cofactor-dependent oxidoreduction system of *Candida parapsilosis* CCTCC M203011. Org Process Res Dev 8:246–251.

Shimizu S, Hattori S, Hata H, Yamada H. 1988. A novel fungal enzyme, NADPH dependent carbonyl reductase, showing high specificity to conjugated polyketones. Eur J Biochem 174: 37–44.

Singh A, Bhattacharyya MS, Banerjee UC. 2009. Purification and characterization of carbonyl reductase from *Geotrichum candidum*. Process Biochem 44:986–991.

Singh M, Singh RS, Banerjee UC. 2010. Enantioselective transesterification of racemic phenyl ethanol and its derivatives in organic solvent and ionic liquid using Pseudomonas aeruginosa lipase. Process Biochem 45:25–29.

Xie Y, Xu JH, Lu WY, Lin GQ. 2009. Adzuki bean: a new resource of biocatalyst for asymmetric reduction of aromatic ketones with high steroselectivity and substrate tolerance. Bioresour Technol 100:2463–2468.

Yang Y, Zhu D, Piegat TJ, Hua L. 2007. Enzymatic ketone reduction:mapping the substrate profile of a short-chain alcohol dehydrogenase (YMR226c) from Saccharomyces cerevisiae. Tetrahedron Asymmetry 18:1799–1803.

Zhang Z, Liu X, Ma L, Chen X. 2010. Hydrogenation of cinnamyl alcohol to 3-phenylpropanol by biocatalysis. Chem Ind Eng Progr (China); 29:2368–2372.