J Ind Microbiol Biotechnol (2012) 39:1253–1259
DOI 10.1007/s10295-012-1130-0

BIOCATALYSIS

Influence of hydroxypropyl-β-cyclodextrin on phytosterol biotransformation by different strains of *Mycobacterium neoaurum*

Yan-Bing Shen · Min Wang · Hua-Nan Li · Yi-Bo Wang · Jian-Mei Luo

Received: 29 August 2011 / Accepted: 12 April 2012 / Published online: 22 May 2012

Abstract Cyclodextrins (CDs) can improve productivity in the biotransformation of steroids by increasing conversion rate, conversion ratio, or substrate concentration. However, little is known of the proportion of products formed by multi-catabolic enzymes, e.g., via sterol side chain cleavage. Using three strains with different androst-1,4-diene-3,17-dione (ADD) to androst-4-ene-3,17-dione (AD) ratios, *Mycobacterium neoaurum* TCCC 11028 (*MNR*), *M. neoaurum* TCCC 11028 M1 (*MNR* M1), and *M. neoaurum* TCCC 11028 M3 (*MNR* M3), we found that hydroxypropyl-β-cyclodextrin (HP-β-CD) can appreciably increase the ratio of ADD to AD, the reaction rate, and the molar conversion. In the presence of HP-β-CD, conversion of 0.5 g/L of phytosterol (PS) was 2.4, 2.4, and 2.3 times higher in the *MNR*, *MNR* M1, and *MNR* M3 systems, respectively, than in the controls. The ADD proportion increased by 38.4, 61.5, and 5.9 % compared with the control experiment, which resulted in a strong shift in the ADD/AD ratio in the ADD direction. Our results imply that the three PS-biotransforming strains cause efficient side chain degradation of PS, and the increased conversion of PS when using HP-β-CD may be associated with the higher PS concentration in each case. A similar solubilizing effect may not induce a prominent influence on the ADD/AD ratio. However, the different activities of the Δ^1-dehydrogenase of PS-biotransforming strains result in different incremental percentage yields of ADD and ADD/AD ratio in the presence of HP-β-CD.

Keywords Phytosterols · Hydroxypropyl-β-cyclodextrin · Side chain degradation · Bioconversion · Δ^1-dehydrogenase

Y.-B. Shen · M. Wang (✉) · H.-N. Li · Y.-B. Wang · J.-M. Luo
Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, No. 29, 13th Avenue, TEDA, Tianjin 300457, People's Republic of China
e-mail: minw@tust.edu.cn

Introduction

Androst-4-ene-3,17-dione (AD) and androst-1,4-diene-3,17-dione (ADD) are very important biological intermediates which can be obtained via the side chain cleavage of natural phytosterol (PS) by microorganisms [6, 14, 17]. Microbial transformations of sterols are severely limited by the poor water solubility of such hydrophobic substrates and the inhibition or toxicity that both substrate and product exert upon the microorganism [8]. Many techniques have been used to deal with these shortcomings, including the use of surfactants, ionic liquids, liquid polymers, cyclodextrin (CD), liposome, and water-miscible and water-immiscible organic solvents [1, 2, 4, 15, 22, 25, 26]. CD is an effective medium for enhancing the biotransformation of steroid compounds [8, 11]. This enhancement is generally attributed to the increased solubility of steroids through the formation of inclusion complexes with CDs [13, 18]. Several groups have demonstrated enhanced steroid biotransformation in the presence of natural and chemically modified CDs [3, 7, 15, 16, 20], including the authors' previous work [12, 13, 24, 29]. Numerous studies have been reported on biocatalysis in a CD medium; however, most of these studies focused on catalysis with improved steroid productivity by means of increasing conversion rate, conversion ratio, or substrate

concentrations. Furthermore, not much data are available on the conversion process and products proportion, e.g., sterol side chain cleavage, carried out using multi-catabolic enzymes promoting many consecutive reactions [23].

Mycobacterium sp. is the most widely used microorganism for sterol biotransformation to produce ADD and AD [6, 17]. AD and ADD are produced simultaneously with biotransformation. It is difficult to separate these substances in downstream processing because of their high structural similarity, thereby reducing product yield and affecting product quality [8, 28]. Considerable efforts have been made to generate high-purity AD(D) using medium engineering, process optimization, and strain improvement through mutagenesis and selective genetic engineering [8, 14, 28]. Recent work by Wang et al. found that lecithin behaves like surfactants and increases final product yield, productivity, and ADD/AD ratio [27]. In addition to increasing solubility of cholesterol in the media, lecithin interferes with the fatty acid profile of the cell envelope and enhances sterol penetration through the cell envelope [19, 27]. Similar to lecithin, CDs can also extract lipophilic components from biomembranes [5, 21] and increase cell membrane permeability, which make them a useful agent for improving transformation productivity and ADD/AD ratio. Garai et al. [9] studied the effects of β-CD on progesterone biotransformation by acetone-dried *Aspergillus fumigatus* and demonstrated that the activity of the enzyme system responsible for side chain degradation of progesterone was inhibited in the presence of β-CD, with pregna-1,4-diene-3,20-dione being the only isolable metabolite. Compared with β-CD, hydroxypropyl-β-cyclodextrin (HP-β-CD) and its complexes have high water solubility, strong complexing ability, and good biocompatibility, suggesting a broader practical application [10, 24]. In the present work, three strains with different ADD/AD ratios, *Mycobacterium neoaurum* TCCC 11028 (*MNR*) and two mutant *M. neoaurum* TCCC 11028 strains, namely *M. neoaurum* TCCC 11028 M1 (*MNR* M1) and *M. neoaurum* TCCC 11028 M3 (*MNR* M3), were used to evaluate the influences of HP-β-CD on PS side chain degradation (Fig. 1). High-performance liquid chromatography (HPLC) analysis revealed that *MNR* accumulated ADD as the main product, whereas *MNR* M1 and *MNR* M3 accumulated AD. ADD/AD molar yields with *MNR*, *MNR* M1, and *MNR* M3 were about 1.2:1, 1:3.0, and 1:30.3, respectively. The influences of HP-β-CD on molar conversion, reaction rate, and particularly on the alteration of product ADD/AD ratio were investigated systematically. Our data provide a theoretical foundation and basic data for the application of CD in steroid biotransformation.

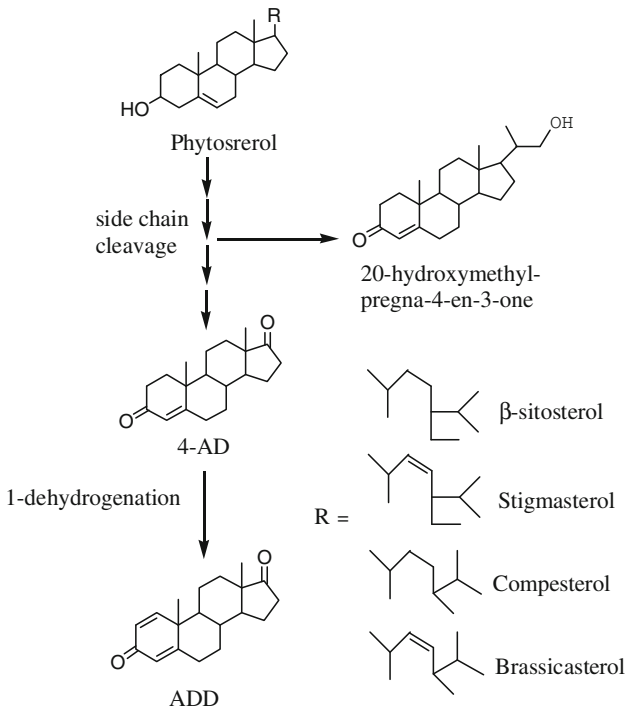

Fig. 1 Side chain degradation of phytosterols by *M. neoaurum*

Materials and methods

Materials

Substrate phytosterol (PS; 98.4 % purity) was obtained from Wuhan Kaidi Fine Chemical Industrial Co. Ltd. Standard 4-androstene-3,17-dione (AD) and 1,4-androstdiene-3,17-dione (ADD) were obtained from Sigma-Aldrich Co. (USA), and HP-β-CD (31.7 % degree of substitution, 1,523 average relative molecular mass) from Xi'an Deli Biology & Chemical Industry Co. Ltd. All chemical solvents and salts used were of analytical grade or higher.

Microorganism cultivation and bioconversion

M. neoaurum TCCC 11028 (*MNR*) used in this study was obtained from Tianjin University of Science & Technology Culture Collection Center (TCCC), Tianjin, China. *M. neoaurum* TCCC 11028 M1 (*MNR* M1) and *M. neoaurum* TCCC 11028 M3 (*MNR* M3) were spontaneous mutant *M. neoaurum* TCCC 11028 strains. The strains cultivated in shake flasks in two consecutive cultivation steps: 36 h for seed culture and 168 h for transformation culture. Seed medium was prepared as described by Shen et al. [21]. The transformation culture medium contained (g/L): glucose 10, $MgSO_4$ 0.5, K_2HPO_4 0.5, $(NH_4)_2HPO_4$ 3.5, citric acid 2, Tween 80 7, PS 5, ammonium iron citrate 0.05, pH 7.2.

Whole cells were grown in 500-mL shake flasks containing 100 mL culture medium with HP-β-CD (0 or 25 mM) on a rotary shaker (200 rpm) at 29 °C using 7 % (v/v) of the seed culture as inoculum. During biotransformation, 1-mL samples were withdrawn at various time intervals, and then analyzed by HPLC. The microorganism growth and PS transformation were completed simultaneously during transformation culture [26]. In order to estimate each strain's ability to catalyze the conversion of AD to ADD, the cells were incubated with PS-free liquid supplemented with 1 g/L AD with or without HP-β-CD 24 h after the growth of *M. neoaurum*.

For the susceptibility test, 1 g/L AD(D) was added to the Tween 80/PS-free liquid medium with or without HP-β-CD 24 h after the growth of *M. neoaurum*, which was then incubated at 29 °C, 200 rpm for 96 h. During incubation, optical density at 600 nm was used to measure growth [17]. All experiments were performed in triplicate and the data were statistically analyzed by one-way ANOVA.

Quantification of the bioconversion product

The samples were extracted using ethyl acetate and then dried in vacuum. Subsequently, the solid extracts were redissolved in the HPLC mobile phase and filtered through a 0.22-μm filter before HPLC analysis. HPLC analysis was performed on a Kromasil C18 column (250 × 4.6 mm) with methanol/water (4:1, v/v) as mobile phase at 30 °C with UV absorbance detection at 254 nm. The concentrations of AD and ADD were determined from the calibration curves of eluent solutions of standard AD and ADD.

Results and discussion

Effect of HP-β-CD on PS side chain degradation

The effect of HP-β-CD on PS bioconversion was investigated using different strains of *M. neoaurum* in the presence of 25 mM HP-β-CD. As shown in Table 1, HP-β-CD significantly enhanced molar conversion for all three PS-biotransforming strains. At 0.5 g/L of PS in the systems containing *MNR*, *MNR* M1, and *MNR* M3, the molar conversion reached 87.3, 86.7, and 82.3 % in the presence of 25 mM HP-β-CD, respectively. In comparison, the control experiments displayed the highest molar conversion of 37.1, 36.5, and 35.2 % at 168 h, respectively. This indicated an increase of 2.4, 2.4, and 2.3 times, respectively, in AD(D) production from the HP-β-CD conversion system in comparison to the control. The increased conversion on PS when using HP-β-CD can be attributed to the increased solubility of PS in the media, allowing for a more efficient utilization of PS by the cells. This observation is in general accord with previous findings, which indicated that CDs enhance the biotransformation of steroid compounds [2, 23]. On other hand, the increased conversion on PS when using HP-β-CD might be associated with the reduction of the inhibitory effect of the products [17]. Smith et al. [22] reported that ADD toxicity might account for the low ADD accumulation in Tween 80 medium. As shown in Table 2, HP-β-CD considerably alleviated the inhibitory toxic effects of product on growth of *M. neoaurum* by sequestering AD and ADD, which might improve the performance of *M. neoaurum* on the bioconversion of PS. The *M. neoaurum* strains were unable to utilize HP-β-CD as a carbon source, similarly to other *Mycobacterium* sp. AD(D) has a certain inhibiting effect and HP-β-CD has a promoting effect on cell growth, but both these effects were much weaker on *MNR* M3 than on *MNR* and *MNR* M1. This may be an interesting finding to study further, and may help to explain the lower bioconversion showed by *MNR* M3.

Effect of HP-β-CD on product proportion

The influence of HP-β-CD on product proportion is shown in Table 1. HP-β-CD increased the proportion of ADD and the by-product 20-hydroxymethylpregna-4-en-3-one (HMP) significantly for all three PS-biotransforming strains. The ADD proportion reached 82.5 % in the presence of 25 mM HP-β-CD for *MNR*, whereas that of the control only 53.8 %. Interestingly, the PS biotransformation

Table 1 PS bioconversion by different strains of *M. neoaurum* at 29 °C in the presence of 25 mM HP-β-CD

Corresponding parameters	Strains								
	MNR			*MNR* M1			*MNR* M3		
	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)
Conversion ratio (%)	37.1	87.3	2.4	36.5	86.7	2.4	35.2	82.3	2.3
AD proportion (%)	46.2	1.9	–	75.3	4.4	–	96.8	80.7	–
ADD proportion (%)	53.8	82.5	1.5	24.7	79.4	3.2	3.2	9.8	3.1
HMP proportion (%)	Trace	15.6	–	Trace	16.2	–	Trace	9.5	–

Table 2 *M. neoaurum* growth on AD(D) with or without 25 mM HP-β-CD

Strains	Initial A_{600}[a]	ΔA_{600}[b]					
		−HP-β-CD[c]			+HP-β-CD[c]		
		Control	AD	ADD	Control	AD	ADD
NMR	0.154	0.589	0.163	0.004	0.783	0.642	0.604
NMR M1	0.118	0.609	0.246	0.028	0.773	0.631	0.631
NMR M3	0.055	0.672	0.509	0.351	0.738	0.667	0.682

[a] After 24 h of incubation

[b] ΔA_{600} is defined as the final A_{600} minus the initial A_{600}

[c] Strain growth in PS-free liquid medium without (−) or with (+) 25 mM HP-β-CD

induced by HP-β-CD for *MNR* M1 led to a drastic shift in the AD/ADD ratio toward ADD, with the main product changing from AD (75.3 %) to ADD (79.4 %). For *MNR M3*, the main product was not changed, but the ADD proportion increased by 3.1-fold compared with the control experiment.

The proportion of ADD and HMP increased significantly with the addition of HP-β-CD. This might be explained either by the higher rate of substrate diffusion from the liquid medium into the cells in the presence of HP-β-CD compared with the control and a resulting enhancement of substrate supplement [13], or by the intrinsically higher combination and catalytic abilities of the enzyme [23]. In contrast, the effect seems to be correlated to the lowering of AD formation and might suggest some kind of regulating mechanism in the Δ^1-dehydrogenase mediated by HP-β-CD. To support this assumption, an additional experiment was carried out. All three PS-biotransforming strains (*MNR*, *MNR* M1, and *MNR* M3) were pregrown in a transformation culture medium with PS-free liquid and tested for bioconversion of 1 g/L AD with or without HP-β-CD 24 h after the growth of *M. neoaurum*. As shown in Table 3, the conversion of AD was clearly intensified with HP-β-CD. For *MNR*, *MNR* M1, and *MNR* M3, the AD molar conversion increased by 1.2, 2.6, and 3.9 times, respectively, compared with the control experiment. *MNR* showed high activity of Δ^1-dehydrogenase which resulted in high AD conversion; in contrast, the Δ^1-dehydrogenase activity of *MNR* M3 was very low. This could explain the high ADD proportion of *MNR*, whereas *MNR* M3 had a high AD proportion (Table 1).

Effect of HP-β-CD on each reaction stage of PS bioconversion

The overall PS bioconversion process by *M. neoaurum* is complicated. Many consecutive reactions are required to form the product ADD. The conversion can be divided into two major reactions: the conversion of the PS to AD and the conversion of the AD moiety to ADD, which involve the side chain degradation reaction and 1-dehydrogenation reaction, respectively (Fig. 1). Hence, the AD conversion ratio in the degradation reaction is defined as the mole ratio of produced ADD and AD to the original PS (reaction 1), and the ADD conversion ratio in the 1-dehydrogenation reaction is defined as the mole ratio of produced ADD to AD and ADD (reaction 2).

The effect of HP-β-CD on each reaction stage of PS bioconversion by different strains of *M. neoaurum* is shown in Table 4. HP-β-CD significantly increased the molar conversion of both reactions for all PS-biotransforming strains. For *MNR*, *MNR* M1, and *MNR* M3, the percentage yield of AD increased 2.0, 2.0, and 2.1 times, respectively, compared with the control experiment. The similar percentage yield of AD and enhancement factor with or without HP-β-CD together with the overall PS conversion studies (Table 1) implied that the efficiency of the PS side chain degradation for all PS-biotransforming strains and PS

Table 3 Bioconversion of AD (1 g/L) to ADD in shake flasks by different strains of *M. neoaurum* at 29 °C in the absence (control) and presence of HP-β-CD at 25 mM

Corresponding parameters	Strains								
	NMR			*NMR* M1			*NMR* M3		
	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)
Conversion ratio (%)	67.0	81.7	1.2	29.1	74.5	2.6	0.7	2.5	3.6
ADD/AD	2.0:1	4.5:1	–	1:2.4	2.9:1	–	141.9:1	39.0:1	–

Table 4 PS biotransformation process by different strains of *M. neoaurum* at 29 °C in the presence of 25 mM HP-β-CD

Corresponding parameters	Strains								
	NMR			*NMR* M1			*NMR* M3		
	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)	Control	HP-β-CD	Enhancement (times)
Percentage yield of AD[a]	37.1	73.7	2.0	36.5	72.6	2.0	35.2	74.5	2.1
Percentage yield of ADD[b]	53.8	97.8	1.8	24.7	94.8	3.8	3.2	10.8	3.4
ADD/AD	1.2:1	44.5:1	–	1:3.0	18.2:1	–	1:30.3	1:8.3	–

[a] Percentage yield of AD in degradation reaction (reaction 1) is defined as the mole ratio of produced ADD and AD to the original PS

[b] Percentage yield of ADD in 1-dehydrogenation reaction (reaction 2) is defined as the mole ratio of produced ADD to AD and ADD

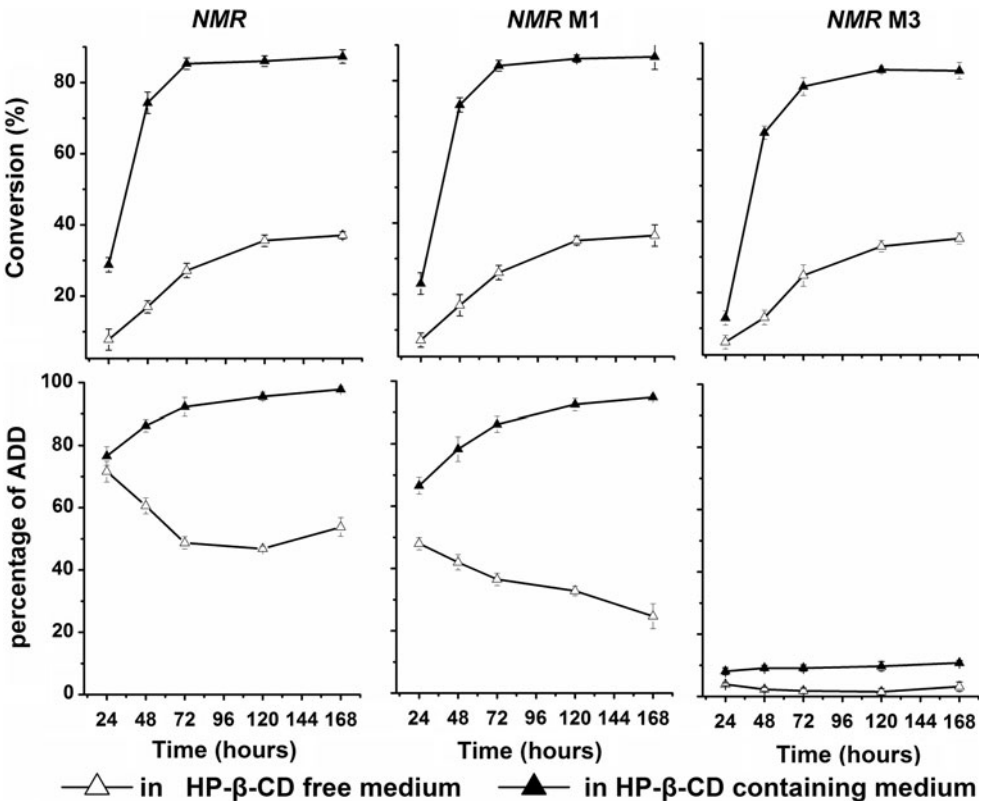

Fig. 2 Time courses of PS conversion to AD(D) by *Mycobacterium neoaurum* TCCC 11028 (*MNR*), *M. neoaurum* TCCC 11028 M1 (*MNR* M1), and *M. neoaurum* TCCC 11028 M3 (*MNR* M3) with or without 25 mM HP-β-CD. The *error bars* represent mean $\pm$ SD ($n = 3$)

solubilizing effect played the main roles in the biotransformation in the presence of HP-β-CD [13]. For *MNR*, *MNR* M1, and *MNR* M3, the percentage yield of ADD increased 1.8, 3.8, and 3.4 times, respectively, compared with the control experiment in reaction stage 2. The increased enhancement factors were in general accord with those of the ADD production by 1-dehydrogenation reaction from AD (Table 3). The solubilizing effect of HP-β-CD and the Δ^1-dehydrogenase activity were the main factors influencing the percentage yield of ADD. The molar ratios of ADD to AD in the products of strains *MNR*, *MNR* M1, and *MNR* M3 were 1.2:1, 1:3.0, and 1:30.3 in the absence of HP-β-CD, respectively, whereas the ratios reached 44.5:1, 18.2:1, and 1:8.3 in the presence of HP-β-CD. These studies indicated that a similar solubilizing effect (similar concentration of AD) may not have a prominent influence on the ADD/AD ratio. However, the different activities of Δ^1-dehydrogenase would restrict the bioconversion of AD, which results in the different ADD production [17]. ADD was the main product (higher than

97 %) for *MNR* in the presence of HP-β-CD, with a trace amount of AD produced. The ADD/AD ratio is an indication of the relative biotransformation activity of the cells toward one product (ADD) over the other (AD). A higher ratio is desirable because the product is purer and easier to separate in downstream processing [14, 27].

Time course of microbial transformation reaction

The time course of microbial transformation was studied at a substrate concentration of 5 g/L and an HP-β-CD concentration of 25 mM. The addition of HP-β-CD significantly enhanced the bioconversion rates, degree of conversion, and product ratio of ADD/(AD + ADD). As shown in Fig. 2, the conversion reached a maximum on day 3 for *MNR* and *MNR* M1, and day 4 for *MNR* M3. For *MNR*, *MNR* M1, and *MNR* M3, the bioconversion rates (mg L^{-1} h^{-1}) reached 94.8, 104.8, and 108.3 from 24 to 48 h in the presence of 25 mM HP-β-CD, respectively, whereas the control experiment (without HP-β-CD) displayed bioconversion rates (mg L^{-1} h^{-1}) of 19.2, 20.4, and 14.4, respectively. Figure 2 indicates that the conversion for *MNR* M3 was lower than for the other two strains at the first transformation stage because the cell concentration for *MNR* M3 was 2- to 3-fold lower at 24 h (Table 2). The product ratios of ADD/(AD + ADD) for the tested strains increased with the lapse of time for conversion in the presence of HP-β-CD. This was in agreement with previous findings that HP-β-CD enhances the catalytic activity of Δ^1-dehydrogenase to different degrees.

Conclusions

This study has demonstrated that HP-β-CD significantly increased the reaction rate, molar conversion, and product ADD/AD ratio. Our results implied that the efficiency of the PS side chain degradation for all PS-biotransforming strains and the PS solubilizing effect play main roles in the biotransformation in the presence of HP-β-CD. The similar solubilizing effect of AD may not induce a prominent influence on the ADD/AD ratio. However, the different activities of Δ^1-dehydrogenase result in different ADD production and ADD/AD ratios. Our results showed that HP-β-CD has multiple effects on the PS biotransformation abilities of *M. neoaurum* strains, including increased solubilization, reduced product inhibition on growth of *M. neoaurum*, and enhancement of the catalytic activity of Δ^1-dehydrogenase.

Acknowledgments This work was supported by the National Natural Science Foundation of China (no. 21076158), the Program for New Century Excellent Talents in University (no. NCET-08-0911), the National High Technology Research and Development of China (2011AA02A211), and the Foundation for Excellent Doctoral Dissertations of TianJin University of Science and Technology in 2010 (no. B201001).

References

1. Bie ST, Lu FP, Du LX, Qiu Q, Zhang Y (2008) Effect of phase composition on the bioconversion of methyltestosterone in a biphasic system. J Mol Catal B Enzym 55:1–5
2. Carvalho F, Marques MPC, de Carvalho CCCR, Cabral JMS, Fernandes P (2009) Sitosterol bioconversion with resting cells in liquid polymer based systems. Bioresource Technol 100:4050–4053
3. Chincholkar SB, Sukhodol'skaya GV, Baklashova TG (1992) Peculiaritie of the 11β-hydroxylation of steroid compounds by the mycelium of *Gurvularia lunata* VKMF 644 in the presence of β-cyclodextrin. Prikl Biokhim Microbiol 28(7):685–693
4. Cruz A, Fernandes P, Cabral JMS (2002) Effect of phase composition on the whole-cell bioconversion of β-sitosterol in biphasic media. J Mol Catal B Enzym 19(2):371–375
5. Donova MV, Nikolayeva VM, Dovbnya DV, Gulevskaya SA, Suzina NE (2007) Methyl-β-cyclodextrin alters growth, activity and cell envelope features of sterol-transforming mycobacteria. Microbiology 153:1981–1992
6. Egorova OV, Gulevskaya SA, Puntus IF, Filonov AE, Donova MV (2002) Production of androstenedione using mutants of *Mycobacterium* sp. J Chem Technol Biotechnol 77:141–147
7. Egorova OV, Nikolayeva VM, Sukhodolskaya GV, Donova MV (2009) Transformation of C19-steroids and testosterone production by sterol-transforming strains of *Mycobacterium* spp. J Mol Catal B Enzym 57:198–203
8. Fernandes P, Cruz A, Angelova B, Pinheiro HM, Cabral JMS (2003) Microbial conversion of steroid compounds: recent developments. Enzyme Microb Technol 32:688–705
9. Garai S, Banerjee S, Mahato SB (1995) Selective 1-dehydrogenation of progesterone by *Aspergillus fumigatus*. J Chem Res Synop (10):408–409
10. Gould S, Scott RC (2005) 2-Hydroxypropyl-beta-cyclodextrin (HP-beta-CD): a toxicology review. Food Chem Toxicol 43(10):1451–1459
11. Hesselink PGM, Vliet SV, Vries HO (1989) Optimication of steroid side chain cleavage by cyclodextrins. Enzyme Microb Technol 11:398–404
12. Lu W, Du L, Wang M, Guo Y, Lu F, Sun B, Wen J, Jia X (2007) A novel substrate addition mode in the 11β-hydroxylation of steroids by *Curvularia lunata*. Food Bioprod Process 85(C1):1–11
13. Ma YH, Wang M, Fan Z, Shen YB, Zhang LT (2009) The influence of host-guest inclusion complex formation on the biotransformation of cortisone acetate Δ1-dehydrogenation. J Steroid Biochem 117(4–5):146–151
14. Malaviya A, Gomes J (2008) Enhanced biotransformation of sitosterol to androstenedione by *Mycobacterium* sp. using cell wall permeabilizing antibiotics. J Ind Microbiol Biotechnol 35:1235–1239
15. Manosroi A, Saowakhon S, Manosroi J (2008) Enhancement of 17α-hydroxyprogesterone production from progesterone by biotransformation using hydroxypropyl-β-cyclodextrin complexation technique. J Steroid Biochem 108:132–136
16. Manosroi A, Saowakhon S, Manosroi J (2007) Enhancement of and rostadienedione production from progesterone by biotransformation using the hydroxypropyl-β-cyclodextrin complexation technique. J Steroid Biochem 30(1):1–5

17. Perez C, Falero A, Llanes N, Hung BR, Hervé ME, Palmero A, Martí E (2003) Resistance to androstanes as an approach for androstandienedione yield enhancement in industrial mycobacteria. J Ind Microbiol Biotechnol 30:623–626
18. Roglic U, Plazl P, Plazl I (2005) The influence of β-cyclodextrin on the kinetics of progesterone transformation by *Rhizopus nigricans*. Biocatal Biotransfor 9(5):299–305
19. Rumijowska A, Lisowska K, Ziolkowski A, Sedlaczek L (1997) Transformation of sterols by *Mycobacterium vaccae*: effects of lecithin on the permeability of cell envelopes to sterols. World J Microbiol Biotechnol 13:89–95
20. Schlosser D, Irrgang S, Schmauder HP (1993) Steroid hydroxylation with free and immobilized cells of *Penicillium raistrickii* in the presence of β-cyclodextrin. Appl Microbiol Biotechnol 39(1):16–20
21. Shen YB, Wang M, Zhang LT, Ma YH, Ma B, Zheng Y, Liu H, Luo JM (2011) Effects of hydroxypropyl-β-cyclodextrin on cell growth, activity, and integrity of steroid-transforming *Arthrobacter simplex* and *Mycobacterium neoaurum*. Appl Microbiol Biotechnol 90:1995–2003
22. Smith M, Zahnley J, Pfeifer D, Goff D (1993) Growth and cholesterol oxidation by *Mycobacterium* species in Tween 80 medium. Appl Environ Microbiol 59:1425–1429
23. Szentirrnai A (1990) Microbial physiology of side-chain degradation of sterols. J Ind Microbiol 6:101–116
24. Wang M, Zhang LT, Shen YB, Ma YH, Zheng Y, Luo JM (2009) Effects of hydroxypropyl-β-cyclodextrin on steroids 1-en-dehydrogenation biotransformation by *Arthrobacter simplex* TCCC 11037. J Mol Catal B Enzym 59:58–63
25. Wang Z, Xu JH, Chen D (2008) Whole cell microbial transformation in cloud point system. J Ind Microbiol Biotechnol 35:645–656
26. Wang Z, Zhao F, Chen D, Dao D (2006) Biotransformation of phytosterol to produce androsta-diene-dione by resting cells of *Mycobacterium* in cloud point system. Process Biochem 41:557–561
27. Wang ZF, Huang YL, Rathman JF, Yang ST (2002) Lecithin-enhanced biotransformation of cholesterol to androsta-1,4-diene-3,17-dione and androsta-4-ene-3,17-dione. J Chem Technol Biotechnol 77:1349–1357
28. Wei W, Wang FQ, Fan SY, Wei DZ (2010) Inactivation and augmentation of the primary 3-ketosteroid-delta(1)-dehydrogenase in *Mycobacterium neoaurum* NwIB-01: biotransformation of soybean phytosterols to 4-androstene-3,17-dione or 1,4-androstadiene-3,17-dione. Appl Environ Microbiol 76(13):4578–4582
29. Zhang LT, Wang M, Shen YB, Ma YH, Luo JM (2009) Improvement of steroid biotransformation with hydroxypropyl-β-cyclodextrin induced complexation. Appl Biochem Biotech 159(3):642–654