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Bioconversion of 16-dehydropregnenolone Acetate to Exclusively 4-androstene-3,17-dione by *Delftia acidovorans* MTCC 3363

PUSHPENDRA AWADHIYA, TUSHAR BANERJEE* and SHRIDHAR PATIL

Applied Microbiology Laboratory, School of Life Sciences, Devi Ahilya University, Indore, India

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Abstract

Delftia acidovorans MTCC 3363 was found to convert 16-dehydropregnenolone acetate (16-DPA) exclusively to 4-androstene-3, 17-dione (AD). Addition of 9α-hydroxylase inhibitors was not required for preventing the accumulation of byproducts. The effect of pH, temperature, substrate concentration, surfactants and carrier solvents on this bioconversion has been studied. 16-DPA was maximally converted in buffered medium at pH 7.0, at temperature 30°C and 0.5 mg ml^{-1} substrate concentration. Detergent addition and temperature above 35°C had deleterious effect on bioconversion. Dioxan was found to be the best carrier solvent for biotransformation of 16-DPA to AD.

Key words: *Delftia acidovorans* MTCC 3363, 4-androstene-3,17-dione, 16-dehydropregnenolone acetate and steroid bioconversion

Introduction

Most of the steroid drugs are derivatives of 4-androstene-3,17-dione (AD) and 1,4 androsta-diene-3,17-dione (ADD) (Kieslich, 1985; Sedlaczek, 1988; Perez *et al*., 2006). Production of AD and related steroids is largely dependent on microbial transformation of natural sterols (Galan *et al*., 2016; Liu and Lo, 1997; Rodriguez-Garcia *et al*., 2016). Soy sterol (from soy oil industry) and wood sterols (from paper pulp industry) are two main sources of natural plant sterols used as raw material in steroid bioconversion (Donova and Egorova, 2012; Perez *et al*., 2006; Yao *et al*., 2013). Owing to the structural similarity, 16-dehydropregnenolone acetate, also known as 3β-(acetyloxy) pregna-5,16-dien-20-one, is used for chemical synthesis of steroid hormones. It can be a potential raw material for steroid bioconversion (Zhang and Guo, 2011). 16-DPA is produced by chemical degradation of solasodine and diosgenin obtained from plant sources (Asolkar and Chadha, 1979; Goswami and kotoky, 2003). Banerjee *et al*. (2003) reported the microbial conversion of 16-DPA to 17-ketosteroids, by mixed culture of *Pseudomonas diminuta* MTCC 3361 and *Commamonas acidovorans* MTCC 3362. Conversion of 16-DPA could be a potential alternative route for production of AD, which is currently dependent mainly on soy sterol bioconversion. Solasodine can be directly converted to AD (Shukla *et al*., 1992) but lower bioconversion yield is a concern. Moreover, availability of solasodine is limited. Microbial conversions offer a single step route to important steroidal intermediates under mild conditions of temperature and pressure.

A soil isolate was found to convert 16-DPA exclusively to AD (Fig. 1). This strain was identified as *Delftia acidovorans* and assigned accession number MTCC 3363.

Optimization of various parameters is required to utilize biotransformation capabilities of *D. acidovorans* MTCC 3363. In the current study, effect of pH, temperature, substrate concentration, some detergents and carriers on bioconversion of 16-DPA to AD by *Delftia acidovorans* MTCC 3363 has been studied.

Experimental

Materials and Methods

Microbial strain. *D. acidovorans* MTCC 3363 was obtained from MTCC, Chandigarh, India. The strain was grown and maintained on nutrient agar slants. For storage the slants were kept at 4–8°C in a refrigerator.

Culture media. Medium used for growth and bioconversion consisted of ($g l^{-1}$) peptone, 5; yeast extract, 2; beef extract, 1; sodium chloride, 5. To study the

* Corresponding author: T. Banerjee, Applied Microbiology Laboratory, School of Life Sciences, Devi Ahilya University, Takshashila campus, Khandwa road, Indore-452 001, India; **e-mail: tushar.banerjee@gmail.com**

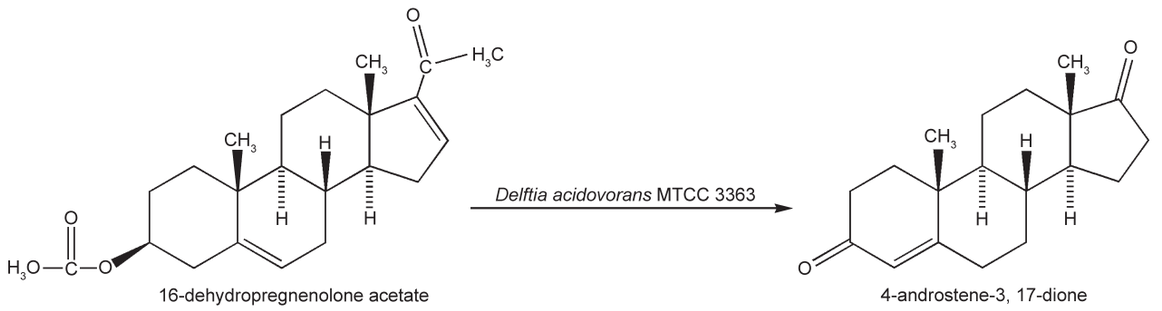

Fig. 1. Biotransformation of 16-dehydropregnenolone acetate (16-DPA) to 4-androstene-3,17-dione (AD) by *D. acidovorans* MTCC 3363.

effect of pH, medium was supplemented with 1%, 1 M sodium phosphate buffer of suitable pH in place of sodium chloride.

Sterols and steroids. 16-DPA, AD and ADD were obtained from Sigma Aldrich, USA.

Other chemicals. Peptone, yeast extract, beef extract and sodium chloride, were obtained from Himedia laboratories, India. Tween 40, Tween 80 and Triton X-100 was purchased from SD Fine-chem Ltd., India. DMF, dioxan and glycerol were procured from Merck specialties Pvt. Ltd. India. Refined soy oil was obtained from local market. The chemicals used were of laboratory reagent grade.

Biotransformation. All bioconversion experiments were conducted in triplicates in 100 ml Erlenmeyer flasks containing 20 ml bioconversion medium. The media was supplemented with 0.01 mg ml^{-1} 16-DPA as an inducer. After adjusting to desired pH, the medium was dispensed in flasks and autoclaved at 15 psi for 20 min. The medium was inoculated by 2 ml of exponentially growing seed culture of *D. acidovorans* MTCC 3363. Seed was grown in nutrient broth medium for 24 hours at 30°C at 200 rpm in gyratory shaker. Inoculated flasks were incubated on a gyratory shaker (200 rpm, 1.5 cm eccentric throw) at desired temperature. After 12 h of incubation, 16-DPA (0.5 mg ml^{-1}) dissolved in dimethylformamide/other substrate carriers (1% of culture volume) was aseptically added to the culture medium. Mol% conversion was calculated as per the formula:

$$\text{Mol\% conversion} = \frac{(\text{concentration of AD in broth})}{(0.804 \times \text{substrate concentration})} \times 100$$

The factor 0.8 in denominator of the formula represents the ratio of molecular weights of AD and 16-DPA.

Analysis of products. At regular intervals, 1 ml sample of bioconversion medium was aseptically drawn from bioconversion flasks. The sample was extracted twice by 2 ml ethyl acetate. The solvent layers were separated and pooled in a fresh tube. The residual water was removed by addition of anhydrous sodium sulfate. Aliquot (0.2 ml) of ethyl acetate extract was taken for quantitative analysis of bioconversion product and rest of the extract was concentrated under vacuum for qualitative analysis by thin layer chromatography. Qualitative analysis of the bioconversion products and residual substrate was carried by thin layer chromatography as described by Shah *et al.* (1980). Identification was done by colour of the spot and R*f* of co-chromatographed authentic samples. AD was estimated by modified Zimmermann reaction as described by Ahmad and Johri (1991). Briefly; The samples (as such or diluted as required) were dried over vacuum at room temperature. The residue was re-dissolved in 0.1 ml of 10% m-dinitrobenzene in pyridine. To each tube 0.05 ml 2.5 N aqueous KOH was added. The tubes were incubated at 45°C for 30 min in a water bath. After completion of reaction, the reaction mixture was cooled to room temperature and diluted with freshly prepared mixture of ethyl acetate and pyridine (1:1). The absorbance was recorded immediately at 572.5 nm on Shimadzu 2401-PC spectrophotometer. Concentration of AD was calculated using a calibration graph.

For preparation of calibration graph, 5 mg AD dissolved in 100 ml ethyl acetate was used as stock solution. 0.1 ml to 1.0 ml aliquots form stock solution was pipette out into separate test tubes and estimated as described above.

The data obtained for replicates was averaged and standard deviation determined. The inter sample variance was analysed by one-way ANOVA and t-test.

Results

The present work brings to foreground the suitability of *D. acidovorans* MTCC 3363 for the bioconversion of 16-DPA to AD. A single intermediate was accumulated during this bioconversion, which was utilized completely as the fermentation progressed. AD is the most important steroidal derivative as it can be transformed to multiple products including ADD by different microorganisms (Zhang *et al.*, 2013).

Effect of temperature. Incubation temperature of 30°C was found suitable for bioconversion of 16-DPA to AD. At this temperature, maximum ~71.8 mol% conversion was recorded after 120 h incubation (Table I). AD was the only bioconversion product at 30°C. Mol% yield of AD was reduced at 35°C, yielding 55.6 mol%. Appreciable quantities of ADD (as evidenced by TLC) were recorded at 35°C. No accumulation of AD was found when the flasks were incubated at 40°C.

Table I
Effect of temperature on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

Temperature (°C)	Mol% conversion after incubation period (h)			
	48	72	96	120
30	62.25 ± 3.18	59.59 ± 2.24	65.86 ± 1.59	71.85 ± 1.48
35	53.60* ± 3.03	54.21* ± 2.08	55.60* ± 4.28	53.03* ± 4.18
40	ND	ND	ND	ND

Data are mean of three replicates ± standard deviation.
ND = not detected.
* significance at α = 0.05; # represents significance at α = 0.1

Effect of pH. The effect of pH was studied using buffered biotransformation medium at 30°C. The effect of pH on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363 in buffered media is presented in Table II. A maximum of 82.88 mole% bioconversion was recorded at neutral pH. Slightly acidic or alkaline pH resulted in less AD accumulation. However, AD accumulation was reduced more in acidic pH (6.5), as compared with alkaline pH (7.5) resulting in ~69 and 77 Mol% bioconversion respectively.

Table III
Effect of substrate concentration on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

16-DPA (mg/ml)	AD (mg/ml) accumulated at incubation period (h)			
	48	72	96	120
0.5	0.20 ± 0.027	0.23 ± 0.038	0.25 ± 0.022	0.21 ± 0.037
1[a]	0.31* ± 0.046	0.32* ± 0.024	0.34* ± 0.047	0.26# ± 0.012
1[b]	0.33* ± 0.022	0.35* ± 0.024	0.31# ± 0.047	0.28* ± 0.009
1.5	0.47* ± 0.038	0.50* ± 0.043	0.50* ± 0.009	0.41* ± 0.032

Data are means of three replicates ± standard deviation;
[a] substrate added as a single dose; [b] substrate added in two split doses at 12 h (I) and 24 h (II).
* significance at α = 0.05; # represents significance at α = 0.1

Effect of substrate concentration. The amount of AD accumulated at different substrate loadings is presented in Table III. At 0.5, 1.0 and 1.5 mg ml^{-1} substrate, highest mol% conversion was recorded at 96 hours. A maximum of 61.63, 42.81 and 41.42 mol% bioconversion was recorded at 0.5, 1.0 and 1.5 mg ml^{-1} 16-DPA loading respectively. The Mol% conversion reduced correspondingly with the increase in substrate concentration. No significant difference in AD accumulation was observed between the two modes of substrate addition. However, split dosing favored achieving highest mol% conversion (43.50 mol%) within 72 hours. TLC analysis revealed differences in 16-DPA utilization pattern by *D. acidovorans* MTCC 3363 at different substrate loadings. The substrate was completely utilized within 72 hours at 0.5 mg ml^{-1} of 16-DPA, whereas, it persisted for 96 and 120 h at 1and 1.5 mg ml^{-1} 16-DPA loading, respectively.

Table II
Effect of pH on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

pH	Mol% conversion after incubation period (h)			
	48	72	96	120
6.5	59.25 ± 4.91	64.63 ± 1.69	69.15 ± 4.16	63.15 ± 2.08
7.0	73.36* ± 1.91	76.53* ± 3.77	82.88* ± 1.35	74.73* ± 1.48
7.5	69.32* ± 3.73	76.40* ± 0.47	77.26# ± 3.83	73.27* ± 1.56

Data are mean of three replicates ± standard deviation.
* significance at α = 0.05; # represents significance at α = 0.1

Effect of surfactants. Steroidal precursors like 16-DPA are hydrophobic in nature and form aggregates, limiting the mass transfer. Surfactants improve dispersion of hydrophobic aggregates. Effect of some nonionic surfactants (Tween 40, Tween 80, and triton X-100) was tested at 0.1 and 0.5% concentrations on biotransformation of 16-DPA (0.5 mg ml^{-1}) to AD by *D. acidovorans* MTCC 3363 (Fig. 2). In this study, all the concentrations of tested surfactants negatively influenced AD accumulation.

Effect of carrier solvents. Dimethyformamide (DMF), dioxan, soy oil and glycerol were tested as carrier solvents for 16-DPA biotransformation at 0.5 mg ml^{-1} (Table IV). All the carrier solvents were used at 0.1% concentration (with respect to the medium). Dioxan was found best amongst tested carriers, 77.46 mol%

Table IV
Effect of carrier solvents on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

Carrier	Mol% conversion after incubation period (h)			
	48	72	96	120
DMF	50.33 ± 5.80	70.78 ± 5.48	70.94 ± 4.83	40.33 ± 5.86
Dioxan	61.82* ± 5.85	74.73 ± 0.97	77.46 ± 5.22	53.84* ± 4.88
Soy oil	ND	ND	ND	ND
Glycerol	48.09 ± 2.67	40.16* ± 0.79	31.80* ± 0.78	30.72# ± 3.94

Data are mean of three triplicates ± standard deviation.
Volume of carrier solvents used was 1% of incubation medium.
ND = not detected.
* significance at α = 0.05; # represents significance at α = 0.1

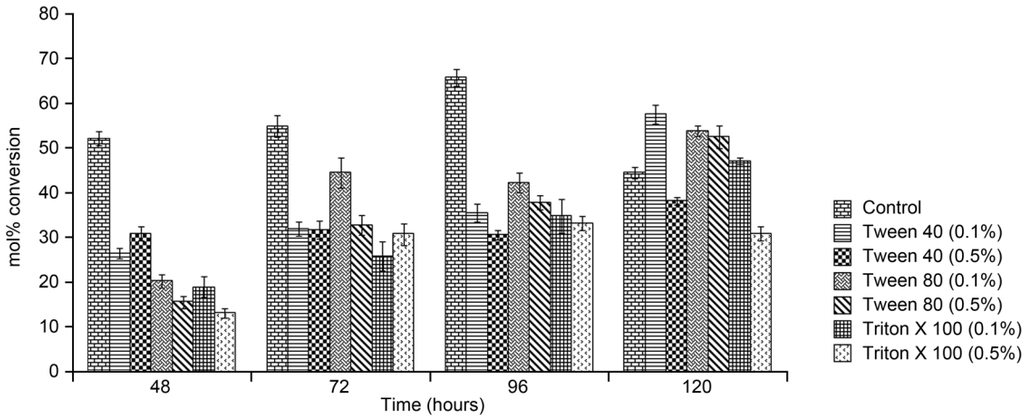

Fig. 2. Effect of some nonionic surfactants on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

biotransformation was recorded (as maximum) at 96 h. DMF yielded similar trend of AD accumulation as dioxan with slightly lower AD accumulation. Interestingly, with soy oil as carrier solvent, no biotransformation was recorded. Using glycerol as a carrier solvent resulted in peak conversion within 48 hours (48.09 mol%) which reduced on further incubation.

The effect of various concentrations of dioxan (1, 1.5 and 2%) was studied for accumulation of AD on biotransformation of 16-DPA by *Delftia acidovorans* MTCC 3363 (Fig. 3). Maximum biotransformation was recorded with 1% dioxan. Increasing the dioxan quantity resulted in decrease in AD accumulation.

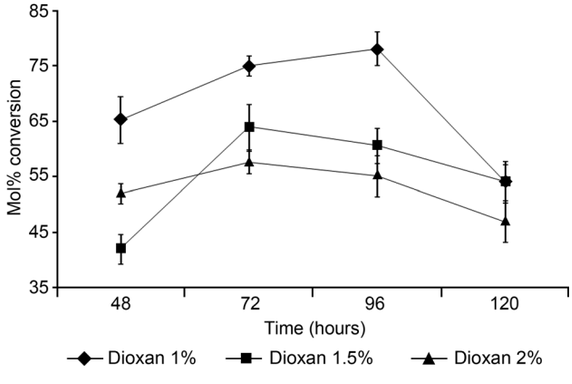

Fig. 3. Effect of concentration of dioxan on bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363.

Identification of bioconversion product. The isolated and purified bioconversion product was identified by UV, IR spectra and co-TLC with authentic sample. UV λ_{max} 241 nm, IR (KBr) cm^{-1}, 1736 (17 – C = O), 1661.1 and 1614.8 (Δ^4 – 3 – C = O); co-TLC with authentic sample, R*f* = 0.54 ± 0.03, colour with 2% ceric ammonium sulphate in 60% sulphuric acid – Greenish blue confirmed the product to be AD (Fig. 4).

Discussion

Active hydrogen ion concentration *i.e.* pH is considered as an important factor for biological reactions. Biotransformation of 16-DPA into AD by *D. acidovorans* MTCC 3363 requires neutral pH for optimal bioconversion while slightly acidic or alkaline pH resulted in reduced biotransformation. Slightly acidic pH proved more detrimental as compared to alkaline pH as less AD was accumulated on a comparative basis. Previous reports suggest that the optimum biotransformation requires neutral to slightly alkaline pH for bacteria mediated biotransformation of steroid substrates. Shiwei and Youhua (1982) observed the maximum bioconversion of 16-dehydro-allopregnane-3-acetoxy-

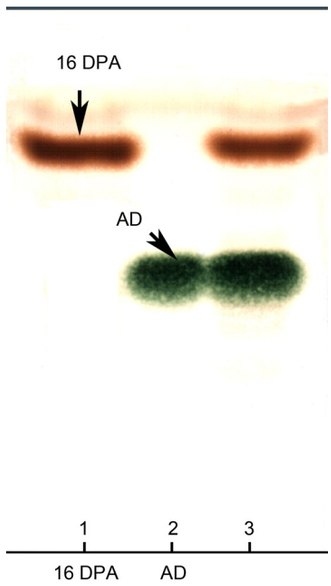

Fig. 4. Thin layer chromatography (TLC) showing authentic 16-DPA (Lane-1), AD (Lane-2) and bioconversion of 16-DPA to AD by *D. acidovorans* MTCC 3363 (Lane-3).

20-one in *p*H range of 7.0 to 8.0. Nagasawa *et al.* (1970) found that 16-dehydroprogesterone was converted only 17.5 mole% up to 48 h at pH 7.0. Viola *et al.* (1983) reported 20% conversion of pregnenolone acetate to AD along with minor quantities of testosterone, testosterone acetate and testololactone at pH 7.5.

Alongside to the pH, temperature affects the physiology of steroid biotransformation. Changes in temperature can enhance/suppress the activity of enzymes. In the present study accumulation of ADD along with AD at 35°C was recorded while at 30°C only AD was a major product. The accumulation of ADD along with AD indicates appreciable steroid 1(2)-dehydrogenase activity at 35°C. The results are in agreement with Shukla (1994), who obtained maximum accumulation of ADD at 35°C. The absence of biotransformation products at 40°C is suggestive of inactivation of steroid degrading enzymes responsible for AD and ADD bioconversion. Roy *et al.* (1991) reported rapid loss of activity of *Mycobacterium* cells on elevating the temperature from 28 to 35°C and total cessation of activity at 41°C during sitosterol side chain cleavage.

Reduced bioconversion of steroids (in terms of mol%) at high substrate concentrations is a frequently reported phenomenon attributed to the substrate toxicity (Sallam *et al.*, 1977; Roy *et al.*, 1991; Shukla 1994). In the present study, splitting the steroid substrate addition in two equal doses did not reveal higher bioconversion of 16-DPA. However, addition of substrate in two equal doses shortened the time of maximum product accumulation. When substrate is added into two equal doses, maximum product accumulation was achieved within 72 hours, while adding substrate in single bolus, has taken 96 hours for maximum accumulation of the AD. No significant change in accumulation of ADD was recorded. Increasing the substrate loading resulted in increased product accumulation but it does not reflected in linear increase in mol% conversion. The effect can possibly be attributed to increased influx of substrate (higher diffusion pressure) leading to a higher conversion, while causing the toxicity to restrict the mol% conversion to a lower value.

Zhang and Guo (2011) reported, 16-DPA being insoluble in water, hence is not highly amenable to microbial bioconversion. Although the carrier solvents may exert toxic effects on the organisms, they are routinely employed to dissolve the steroid substrate to improve its mass transfer (Fernandes *et al.*, 2003). The use of natural carrier solvents is assumed to be advantageous in terms of viability (by acting as reservoirs of substrate, restricting the exposure of cells to the toxic effects) and thus biotransformation. In this study, soy oil and glycerol were included as less toxic carrier solvents. The soy oil inhibited accumulation of AD from 16-DPA. On the other hand using glycerol as a carrier solvent resulted in less AD accumulation which further degraded. The inhibition of 16-DPA conversion in case of soy oil could be attributed to a preference of substrate utilization *D. acidovorans* MTCC 3363. However, the degradation of product with glycerol indicates towards a co-metabolism of product with the carrier.

Maximum amount of AD was accumulated with dioxan as a carrier solvent. However, at higher concentrations, dioxan adversely affected the biotransformation. The reduction in biotransformation can be attributed to toxic effects of the solvent. On the comparative scale, DMF performed better than soy oil and glycerol.

Surfactant reduces the aggregation of hydrophobic molecules and facilitates better dispersion of the substrate in the medium (Wang *et al.*, 2004; Malaviya and Gomes, 2008). In the present study reduction/delay in the biotransformation in presence of non ionic surfactants was recorded. The reduction in AD accumulation can be contributed to the toxic effects of the surfactants, primarily exerted by cell wall lipid dissolution (Wang *et al.*, 2005).

Greater amounts of detergents are needed in order to completely dissolve the sterol substrate, although it is shown by Atrat *et al.* (1992) that there is no direct correlation between detergent mediated dissociation of steroidal aggregates and the transformation activity of the bacterial cells.

The present study clearly demonstrates the unique potential of single product accumulation (AD) by *D. acidovorans* MTCC 3363. Moreover, the applicability of 16-DPA as an alternate steroid substrate to soy sterols is shown. Under optimized conditions, fairly high conversion of 16-DPA to AD have been obtained with this strain in shake flasks. Further scale up at laboratory and pilot scale fermentation levels is necessary to use the potential of the organism for commercial production of AD from 16-DPA. Moreover, faster growth rate of the organism as compared to the routinely employed *Mycobacterium* sp. for the conversion of steroids to AD might prove advantageous at industrial scale.

Literature

Ahmed S. and B.N. Johri. 1991. A cholesterol degrading bacteria: isolation, characterization and bioconversion. *Indian J. Exptl. Biol.* 29: 76–77.

Asolkar L.V. and Y.R. Chadha. 1979. Diosgenin and Other Steroid Drug Precursors. Publications and Information Directorate, CSIR, New Delhi.

Atrat P.G., B. Koch, B. Szekalla and C. Horhold-Schubert. 1992. Application of newly synthesized detergents in the side chain degradation of plant sterols by *Mycobacterium fortuitum*. *J. Basic. Microbiol.* 32: 147–157.

Banerjee T., A. Shukla, K. Shinde and S. Patil. 2003. Mixed culture bioconversion of 16-dehydropregnenolone acetate to androsta-1,4-diene-3,17-dione: optimization of parameters. *Biotechnol. Prog.* 19: 662–664.

Donova M.V. and O.V. Egorova. 2012. Microbial steroid transformations: current state and prospects. *Appl. Microbiol. Biotechnol.* 94: 1423–1447.

Fernandes P., A. Cruz, B. Angelova, H. Pinherio and J.M. Cabral. 2003. Microbial conversion of steroid compounds: recent developments. *Enzyme. Microb. Technol.* 32: 688–705.

Galán B., I. Uhía, E. García-Fernández, I. Martínez, E. Bahíllo, J.L. de la Fuente, J.L. Barredo, L. Fernández-Cabezón and J.L. García. 2016. *Mycobacterium smegmatis* is a suitable cell factory for the production of steroidic synthons. *Microb. Biotechnol*. 10(1): 138–150.

Kieslich K. 1985. Microbial side-chain degradation of sterols. *J. Basic. Microbiol.* 25: 461–474.

Korycka-Machala M., A. Rumijowska-Galewicz and J. Dziadek. 2005. The effect of ethambutol on mycobacterial cell wall permeability to hydrophobic compounds. *Pol. J. Microbiol.* 54: 5–11

Liu W.H. and C.K. Lo. 1997. Production of testosterone from cholesterol using a single-step microbial transformation of *Mycobacterium* sp. *J. Ind. Microbiol. Biotechnol.* 19: 269–272.

Malaviya A. and J. Gomes. 2008. Nutrient broth/PEG200/TritonX114/Tween80/Chloroform microemulsion as a reservoir of solubilized sitosterol for biotransformation to androstenedione. *J. Ind. Microbiol. Biotechnol.* 35: 1435–1140.

Nagasawa M., N. Watanabe, H. Hashiba, G. Tamura and K. Arima. 1970. Microbial transformation of sterols. III. Substrate specificity for cleaving steroid side chain by *Arthrobacter simplex*. *Agr. Biol. Chem*. 34: 798–800.

Perez C., A. Falero, H.L. Duc, Y. Balcinde and B.R. Hung. 2006. A very efficient bioconversion of soybean phytosterols mixtures to androstanes by mycobacteria. *J. Ind. Microbiol. Biotechnol.* 33: 719–723.

Rodríguez-García A., E. Fernández-Alegre, A. Morales, A. Sola-Landa, J. Lorraine, S. Macdonald, D. Dovbnya, M.C.M. Smith, M. Donova and C. Barreiro. 2016. Complete genome sequence of *Mycobacterium neoaurum* NRRL B-3805, an androstenedione (AD) producer for industrial biotransformation of sterols. *J. Biotechnol.* 224: 64–65.

Roy P.K., A.W. Khan, and S.K. Basu. 1991. Transformation of sitosterol to androsta-1, 4-diene-3,17-dione by immobilized *Mycobacterium cells*. *Indian J. Biochem. Biophy*. 28: 150–154.

Sallam L.A.R., N. Naim, M. H. El-Kinawy, and A. H. El-Refai. 1977. Effect of different factors on progesterone side chain degrading enzymes of *Penicillium liliacinum*. *Indian J. Exptl. Biol.* 15: 949–951.

Sedlaczek L. 1988. Biotransformations of steroids. *Crit. Rev. Biotechnol.* 7: 187–236.

Shah K., I. Mehdi, A.W. Khan and V.C. Vora. 1980. Microbial transformation of sterols I – Microbial transformation of phytosterol to androsta-1, 4-diene-3,17-dione by *Arthrobacter simplex*. *Eur. J. Appl. Microbiol. Biotechnol.* 10: 167–169.

Shiwei X. and F.A. Youhua. 1982. The microbial transformation of ring A saturated steroid: 2. Degradation of Δ^{16}-allopregnen-3β-acetoxy-20-one to $\Delta^{1,4}$-androstadiene-3,17-dione. *Acta Microbiol. Sin*. 22: 361–364.

Shukla A., S. Patil and S. Bharti. 1992. Microbial conversion of solasodine to 1-androstene-3,17-dione (AD), a key intermediate for androgen synthesis. *Lett. Appl. Microbiol.* 15: 86–88.

Shukla A. 1994. Ph.D. Thesis, Biotransformation of some steroids to industrially important 17-keto intermediates. Devi Ahilya University, Indore, India.

Viola F., O. Caputo, G. Balliano, L. Delprino and L. Cattel. 1983. Side chain degradation and microbial reduction of different steroids by *Aspergillus aureofulgens. J. Steroid Biochem*. 19: 1451–1458.

Wang Z., F. Zhao, D. Chen and D. Li. 2005. Cloud point system as a tool to improve efficiency of biotransformation. *Enzyme Microb. Technol*. 36: 589–594.

Wang Z., F. Zhao, X. Hao, D. Chen and D. Li. 2004. Microbial transformation of hydrophobic compound in cloud point system. *J. Mol. Catal. B Enzym*. 27: 147–153.

Yao K., F.Q. Wang, H.C. Zhang and D.Z. Wei. 2013. Identification and engineering of cholesterol oxidases involved in the initial step of sterols catabolism in *Mycobacterium neoaurum*. *Metab. Eng*. 15: 75–87.

Zhang Y. and S. Guo. 2011. Measurement and correlation for solubilities of 16-dehydropregnenolone acetate in different solvents. *J. Chem. Eng. Data* 56: 2403–2406.

Zhang W., M. Shao, Z. Rao, M. Xu, X. Zhang, T. Yang, H. Li and Z. Xu. 2013. Bioconversion of 4-androstene-3,17-dione to androst-1,4-diene-3,17-dione by recombinant *Bacillus subtilis* expressing *ksdd* gene encoding 3-ketosteroid-Δ^1-dehydrogenase from *Mycobacterium neoaurum* JC-12. *J. Steroid Biochem. Mol. Biol.* 135: 36–42.