World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

BIOTRANSFORMATION OF STEROLS BY ACTINOBACTERIA TO PRODUCE PHARMACEUTICAL PRODUCT

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Article Received on 16/04/2017 **Article Revised on 05/05/2017** **Article Accepted on 26/05/2017**

ABSTRACT

This review consists two parts. In the first part of review we enhance the main aim of the development of pharmaceutical industry by introducing micro biotechnological process on industrial scale and wipeout the many-level chemical coalescence. Also, this article elaborates on the current development of micro sterols biotransformation system. Actinobacteria, which has considerable importance for the production of hormonal drugs (pharmaceutical product) also has main role as a catalyst in the steroid bioconversion and the development of biotechnology. The ability to activate the transformation process of sterol substrate in a broad way, it is feasible to expect the effective use of these microorganism in the advancement of new technologies on the production of pharmaceutical steroids substrate. This article is first attempt to channelize the data on the ability of Actinobacteria to activate the distinct reaction of many biotransformation of steroid such as hydroxylation, reduction and introduction of double bonds, oxidation of steroids, reduction of hydrocarbon (ketones) and degradation, with focus on the importance of biotechnological process and analysis of steroid conversion over the last decade year. The second part of this review precisely emphasis in the location of new enzymatic approaches such as cleavage of steroid side chain.

KEYWORDS: Steroid, Microbial transformation, Side-chain degradation, Hydroxylation Dehydrogenation, Selective cleavage.

INTRODUCTION

Klaus Kieslich elaborate the phenomenon of biotransformation as "chemical reactions by microorganisms or enzymes" (Kieslich, 1985). Later this definition of biotransformation was updated, and primarily focus on knowledge that related with the compounds of microbial transformations of steroid in case of differentiate the phenomenon of microbial transformation from that of bioconversion and also biodegradation (Lilly, 1984).

Previously, for the preparation of compounds many chemical processes are used but now microbial transformation is more significant arsenal for the synthesis of product, which may have been complicated otherwise to produce by ordinary synthetic processes. For the bioconversion of steroids this transformation has been significantly used (Charney and Herzong, 1976). In many past years, Biotransformation is an introductive tool in pharmaceutical industry for the synthesis of many drugs, hormones and antibiotics (Aharnowetz and Choe, 1981). The main benefit of the microbiological conversion is to nullify the considerable chemical process lie in the area of their mild reaction conditions. The main aim of biotransformation is to emphasis on the precise reaction with minimum of side reaction. In order to produce some functional products, the selection of biocatalysts is more significant approach under relatively gentle conditions contrast to its chemical catalyst equivalent to make biocatalysts which are more fascinating and revolutionary (Mark and Flashman, 2016).

These transformations have significant role in the region of steroid and antibiotics. In common contingency, the decrement of cholesterol by microorganism is we defined and famous process. In 1913, Sohngen and *et al* elaborate the process of degradation of cholesterol including microorganism such as *Nocarida* (Turfitt, 1944) and *Mycobacterium* from soil and *Aerobacter aerogenes* and *Pseudomonas jaegeri*. The current study on this process of degradation of cholesterol is very limited and has ability to improve this knowledge. A well-known tracer experiment indicates us that divide the ring might pave the way the cleavage of steroid chain (Stadtman, 1954). The problem of information about the cleavage of cholesterol is still intricate, doubtful and

unsettled. In few years, many research group have been performed experiments to break the selective cleavage of sterol chain by microorganism and to prepare the sterol hormones from steroid but none of them are successful. So, it is great challenging problem for the research to breakdown the cleavage of steroid chain and for this purpose screening of microorganism is carried out (Arima, 1969). This review also explains the potential to break the cholesterol by *Actinobacteria* and the degradation as well.

Steroid

The scope of steroids is expanded in the kingdom of animals and plants. The fundamental architecture of steroid constitutes of seventeen atoms of carbon designed in the form of a perhydrocyclopentanophenanthrene. These compounds alter significantly in architecture and include essential compounds such as cholesterol, insect molting hormones, corticoid hormones, sex hormones, antibiotics, vitamin D, cardiac aglycone and bile acids (Bhatti and Khera, 2012).

Steroid biotransformation is a multimillion dollar industry and inhabited a considerable position among the preparation of pharmaceutical products that are mainly used for curing and averting diseases of different groups in endocrinology, oncology, rheumatology, gynecology, etc. (Fernandes *et al.*, 2003). A distinct type of steroids is majorly utilized as anti-inflammatory, diuretic, anabolic as well as contraceptive and anti-androgenic. Some steroids are used as immunosuppressive while others act as presentational and anticancer agents, and several other applications (Ahmed *et al.*, 1992). These steroids are also considerable to utilized in the cure of breast cancer as an also in prostate gland cancer (Diaz-Chico *et al.*, 2007). Their role in the nursing of hyprcortisolism also known as adrenal insufficiency (Hohnston, 1987) are significant as a replacement agent. Considerably for the prevention of coronary heart diseases (Frye and Leonard, 1987) anti-fungal (Chung *et al.*, 1998) agents are widely used. Their important used in the cure of AIDS, these steroids are used in anti-obesity agent as an active ingredient. Currently, in modern research a glycoside which as steroid executed anti-viral activity on herpes virus (Arthan *et al.*, 2002). The scope of new steroids in business point of view is limited in now days. Whereas their scope can be enhanced to obtain desired metabolites which is active ingredient of novel steroids. In pharmaceutical industry, production of steroids has great importance by biotechnology. According to commerce point of view steroid production is second significant source of antibiotic production (Brown, 1984). Moreover, special microbial transformation steps have been involved for the production of novel hormonal steroids as drugs. The relatively broad nomenclature of efficient steroid drugs is continually expanding. Highly complex structure of steroids molecules renders the use of biocatalysts for the production of pharmacologically important steroid drug intermediates of note, several preparations administered for life-saving indications have no non-steroid analogues. Large scale production of hormonal steroid drugs is based on combining both of biotechnology (i.e. microbial technology) and chemical products (Fernandes, 2003). There is a specific rule in which whole cells are used to develop the biotechnological equipment. The benefit of this biotechnology is that, it is more economical then enzymes (considering isolation, purification and stabilization procedures).

General scheme for the production of steroid drug

Steroid drugs are synthesized mainly by two routes: chemical or microbial routes. However, the scheme of production of both routes involve conversion of steroid precursors to drug intermediates and subsequent conversion of intermediates to steroidal drug as shown in Figure 1.

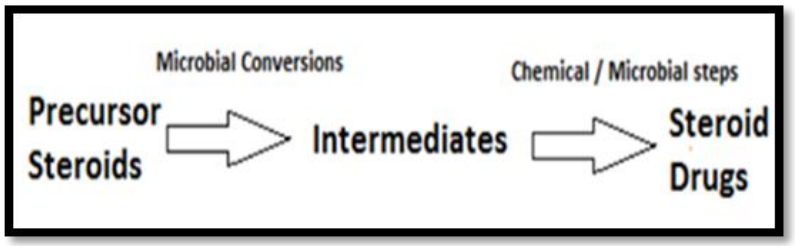

Figure 1: General Steps of Steroid Biotransformation.

Microbial transformations as compared to chemical process degrade the intricate side chains of precursor steroids in only single step and incorporate desirable alterations in steroid nucleus. Biotransformation of steroids and chemical product and entail numerous ways and also required the special reagents that have drawback health hazard and basis of serious ejection issues. The conversion of precursor steroids through microbes as compared to chemical process is less expensive, non-toxic and less time consuming. During bioconversion, microbes provide enzymes which act upon and convert organic compounds or modify it. Microbial transformations are region-specific as well as stereo-specific, however naturally hydrocarbons are transform into required isomers of synthesis product including simple enzymes acted as a catalyst based chemical reactions in the microbial cells. More valuable an important biotechnological use in microbial transformation of active compounds which has capability and application in broader scope of the microorganisms

involving fungi, bacteria, and microalgae in transforming steroid substrate into the pharmacologically or many other fruitful intermediates (Wilson, 1999).

Biotransformation of Sterol

The significance of microbial steroid transformation became evident when in 1950 Upjohn and *et al* discovered a valuable product in which at the location in progesterone 11-alpha hydroxyl group is introduced by a Rhizopus. That's why due to this reaction, a new absurd way which is a path to cortisone was discovered that established a novel and less economical means of producing corticosteroids and their synthetic correspondents and analogues. This discovery takes researchers to elaborate of various other conversion using bacteria and fungi (Mahato, 1989).

One of the major starting materials for steroid industry is the natural steroid sapogenin, diosgenin. However, the route established in its commercial use is chemical conversion of diosgenin to 16-dehydropregnenolone acetate and further synthesis to pharmaceutical product (mainly steroids) (Hanson, 2005). Now many new steroids of useful therapeutic importance can be produced from diosgenin microbial transformation (Wang *et al*., 2011). On the other hand, chemical modification of sapogenins to valuable steroidal products has many disadvantages such as higher costs and low yield, multistep syntheses, wastage of land resources, and enervation of wild plant resources (Wang *et al.* 2011).

Alternatively, natural sterols can be used as starting materials for steroid industry typically steroid 3β-alcohols with the 5(6)- double bond and aliphatic side chain at C-17. Sterols are important constituents of cell membrane playing an important role in membrane fluidity and flexibility, cell differentiation and proliferation (Fernandes and Cabral, 2007). Since the 1980s, microbial transformation of phytosterol remains a focus of research in the field of steroids. Recently, a progressive amount of pharmacologically active steroids is manufactured in large scale through the initial microbial transformation of sterols (Abbott, 1979) such as in pharmaceutical industry the production of steroid hormonal drugs, cholesterol, Beta-sitosterol or campesterol can be selected to degraded by microorganisms, this special type of methodology known as biotransformation has grab elegant concentration that take to the production of many other beneficial phenomenon. Cholesterol is known as animal sterol mainly extract from animal fats and oil, used in the production of hormonal sterols. The primary source of these fat is pig fats (lard), cow, milk fat, tallow, fish oil. Sitosterol, stigmasterol, campesterol, and brassicosterol are rich plant sterols. They are called as phytosterols (plant sterols) mainly of soya origin, or produced from tall oil or pitch; sitosterol are broke up microbially to obtain 17- ketosterols. Ergosterol is a primary sterol of yeasts and fungi. For the manufacturing of teosterone, a male sex hormones (androgen) from cholesterol via a single step transformation process by microorganism was investigated (Lui and Lo, 1997). Incubation of cholesterol with "*Mycobacterium sp.* NRRL B-3805" caused in the development and isolation of testosterone. Sterol-containing wastes of agricultural, food and cellulose manufactures can be used for production of valuable steroid compounds without deep purification of phytosterols, and corresponding publications have grown in number over the last few years. Methods have been described for the manufacturing of androst-4-ene-3,17-dione (AD) and androsta-1,4-diene- 3,17-dione (ADD) from sterol-rich plant-derived sources, edible-oil producing wastes such as soybean oil deodorizer distillate (Olivares and Acevedo, 2011), rice bran oil materials (Sallam *et al*., 2008), sugar can mud (Abd-elsalam *et al.*, 2010), corn flour and soybean flour (Lin *et al.*, 2009a).

Currently, major portion of the steroid bioconversions acted properly to utilize immobilized cell system including special type of reactions such as side chain cleavage. However, comparatively some research work has been canopied out for side chain cleavage in immobilized state, the inferences acquired so far apparent insurable and economical when analogized to free cells (Kieslich, 1985; Mahato, 1989). The rate of reaction of Biocatalysts usually more in aqueous solution of substrate, whereas water is a poor solvent and have ability to slow down the rate of reaction of biocatalyst for nearly all applications in industrial chemistry. Many research and reviews are indulged with the transformation of steroids by microorganisms such as biotransformation of sterols to forerunner of hormonal steroidal drugs have been in process in the past twenty years (Kieslich, 1985). Although, after 1985 particular cleavage of sterols has not been exclusive in spite of bulk to describe research and development of new biotechnologies. "This review dens specially with side chain cleavage of sterols and application of new biotechnologies in steroid biotransformation."

Production of Key Intermediates From (Phyto) Sterols

The key intermediates produced after biotransformation of sterols include some C19 steroids such as AD, ADD, 9α-hydroxy-AD, testosterone, boldenone, as well as C22 steroids of pregnane series such as 20-carboxy-pregna-4-en-3-one, 20-hydroxymethyl pregna-4-en-3-one, and their respective 1-dehydro and 9α-hydroxy analogs. AD and ADD are the most marketed intermediates that are required for the commercial preparation of valuable products such as corticosteroids, mineralocorticoids, oral contraceptives, and other pharmaceutical steroids. The market size of AD/ADD market size is over US$ 1 billion per year. These compounds can be produced in a single microbiological step from phytosterol. Testosterone were also produces by sterol-transforming actinobacteria in a single-step (Liu *et al*. 1997; Egorova *et al.*, 2009). Using *Mycobacteria*, production of

boldenone from phytosterol has been reported to include two steps via intermediate of AD and subsequent 1-dehydrogenation of AD by *Fusarium sp*.

Apart from C19 steroids, valuable 23,24-dinorcholane derivatives were achieved from sterols biotransformation (Andor *et al.*, 2006). These compounds are the significant precursors for corticosteroid synthesis. For example, 9α-hydroxy-C22 steroids can be easily converted to C21 corticosteroids by oxidative decarboxylation (Toro and Ambrus, 1990). Apparent advantages of microbial transformation of valuable precursors i.e. C19 and C22 from phytosterols are shorter process, environmentally friendly and low-cost procedures. However, the relatively low productivity and insufficient selectivity of the strains often remain the bottleneck in their industrial applications. There are many strains of microorganisms that have been described as biocatalysts of sterol biotransformation, e.g., *Arthrobacter spp*. (*Arthrobacter oxydans* 317 AL, *Arthrobacter rubbelus), Brevibacterium spp*., *Pseudomonas spp*., and *Rhodococcus spp*., but their use necessitates the addition of inhibitors to avoid steroid nucleus degradation (Abd-elsalam *et al.*, 2010; Tong and Dong, 2009).

Over the past 100 years, efforts have been made to discover organisms that are capable to convert phytosterols to key steroid precursor efficiently. For example, an AD-producing *Aspergillus oryzae* NCIM 634 strain was selected as an efficient organism (Malaviya and Gomes, 2009); a strain isolated by soil and identified as *Fusarium moniliforme*, converted phytosterol present in corn-flour and soybean flour to AD (Lin *et al.*, 2009a); ADD production from cholesterol was also shown for *Chryseobacterium gleum* ATCC 35910 (Chaudhari *et al.*, 2010). However, Actinobacteria of the genera Mycobacterium and Rhodococcus considered as the most efficient AD/ADD producers (Malaviya and Gomes, 2009).

Biotranformation of Sterols by Actinobacteria

Actinobacteria are prominent among the most efficient biocatalysts of steroid transformation. Actinobacteria are capable of effecting diverse types of steroid transformation, such as dehydrogenation, doubles bond isomerization, oxidation of steroid alcohols, hydrogenation of unsaturated bonds, reduction of steroid ketones, deacetylation and last but not least hydroxylation as well as complete degradation to carbon dioxide and water or partial decrement of the side chain (of sterols, cholinic acids, steroids of the pregnane series) and other reactions. A remarkable number of *Actinobacteria* is able to degrade various sterol (Fernandes *et al*., 2003). A number of strains that are able to transform the side chains of various sterols, either as natural or UV mutants blocked in steroid ring cleavage or requiring inhibitors of steroid ring degradation enzymes are enumerated in Table 1. Some of these strains are or have been used to produce steroid intermediates from cheap sterol sources at industrial- or laboratory scale. At Schering, Germany, the transformation of phytosterols from natural plant resources by *Mycobacterium* sp. mutants has been stated to account for the production of 200 tons of AD and ADD annually (Schmid *et al*., 2001).

Table 1: List of Actinobacterial Strains and/or mutants capable of sterol side chain degradation.

Substrate	Microorganism	Product(s)	References
Cholesterol	*Mycobacterium phlei*	4-androstene-3,17-dione	Stadtman *et al*., 1954
Cholesterol	*Mycobacterium* sp. NRRL B-3805	4-androstene-3,17-dione	Liu & Lee, 1997
Cholesterol	*Arthrobacter simplex and Mycobacterium* sp. NRRL B-3683	1,4-androstene-3,17-dione	Lee *et al*., 1993
Cholesterol	*Rhodococcus equi*	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione	Ahmed *et al*., 1993b
Cholesterol	*Mycobacterium* sp.	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione (iii) 17β-hydroxyandrosta-l,4-dien-3-One	Smith *et al*., 1993
Cholesterol	*Mycobacterium* sp.	17β-hydroxy4-androstene-3-one	Liu *et al*., 1997
Cholesterol	*Rhodococcus equi*	(i) 1,4-androstadiene-3,17-dione (ii) 4-androstene-3,17-dione	Ahmed *et al*., 1993b
2α,3α- dihydroxy-5α-cholestan- 6-one	*Mycobacterium vaccae*	(i) 2α,3α,6α-trihydroxy-5α-androstane-17-one (ii) 2α-hydroxyandrost-4-ene-3,17-Dione	Vorbrodt *et al*., 1991
ergosterol ergosterol-3-acetate	*Mycobacterium* sp. VKM Ac-1815D	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione	Dovbnya *et al*., 2010

		(iii) hydroxymethyl-4-pregnene-3-one	
ergosterol	*Mycobacterium* sp. NRRL B-3805	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione	Ambrus *et al*., 1995
19-hydroxycholesterol	*Rhodococcus* mutant k-3	(i) estra-l,3,5(10)-triene-3-ol (ii) 2(3-hydroxy-1,3,5(10)-estra-triene-17-yl)-propionic acid (iii) 2-methyl-6(3-hydroxy-1,3,5(10)-estratriene-17-yl)-heptanoic acid (iv)2(3-hydroxy-1,3,5(10),17-estratetraene-17-yl)-propionic acid	Murohisa & Iida, 1993a
19-hydroxycampesterol	*Rhodococcus* mutant k-3	(i)2(3-hydroxy-1,3,5(10),17-estratetraene-17-yl)-propionic acid (ii) 2,3-dimethyl-6-(3-hydroxy-1,3,5(10)-estratriene-17-yl)-heptanoic Acid	Murohisa & Iida, 1993a
lanosta-7,9(11)-dien-3β-ol	*Mycobacterium* sp. NRRL B-3805	4,8(14)-androstadiene-3,17-dione	Weber *et al*., 1992
lithocholic acid	*Mycobacterium* sp.	20α-hydroxy-4-methylpregnene-3-one	Weber *et al*., 1992
3β-methoxyergosta-5,7,22-triene	*Mycobacterium*	3β-methoxymethoxy-21-hydroxy-20-methyl-5,7-pregnadiene	Weber *et al*., 1992
β-sitosterol	*Arthrobacter oxydans*	(i) 3-oxo-4-cholene-24-oic acid (ii) 27-nor-4-cholestene-3,24-dione'	Dutta *et al*., 1992
β-sitosterol	*Rhodococcus equi* k-3	(i) 3-oxo-1,4-ergostadiene-26-oic acid (ii) 3-oxo-4-ergostene-26-oic acid (iii) 20-carboxy-4-pregnene-3-one (iv)20-carboxy-1,4-pregnadiene-3-one (v) 4-androstene-3,17-dione (vi) 1,4-androstadiene-3,17-dione	Murohisa & Iida, 1993a
β-sitosterol	*Mycobacterium* sp.	9α-hydroxy-4-androstene-3,17-dione	Borman *et al*., 1992
β-sitosterol	*Mycobacterium* NRRL B-3683	1,4-androstadiene-3,17-dione	Roy *et al*., 1992
β-sitosterol	*Nocardia* sp. M 29	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione	Martin & Wagner, 1976
solasodiene	*Mycobacterium sp.* NRRL B-3805	4-androstene-3,17-dione	Shukla *et al*., 1992
sterol	*Mycobacterium fortuitum*	9α-hydroxy-4-androstene-3,17-dione	Seidel and Hoerhold, 1992
sterol	*Mycobacterium NRRL B-3805*	4-androstene-3,17-dione	Lee, 1990
sterol	*Mycobacterium fortuitum*	9α-hydroxy-4-androstene-3,17-dione	Atrat *et al*., 1991
sterol	*Mycobacterium sp.*	(i) 4-androstene-3,17-dione (ii) 1,4-androstadiene-3,17-dione	Zhang *et al*., 1992

Sterols that exist naturally, such as cholesterol and Phyto-sterols, are utilized as hydrocarbon and energy sources. They have common physiological habitats of some actinomycetes, such as Actinobacteria, *Mycobacteria* and *Rhodococci* (Wipperman, 2014). In *M. tuberculosis* disease, which is pathogenic strains are catabolism of steroids is highly concerned due to its close relevance to pathogenesis and persistence. Interestingly, sterol metabolism in nonpathogenic microorganisms generates metabolites that can be used as ideal precursors to synthesize steroids as pharmaceutical products (Figure. 2; Donova *et al.*, 2012). Commonly, C19 steroids and C22 steroids (22-hydroxyl 23, 24- bisnoocholenic steroids, HBC), are the two major significant intermediates, that can be synthesis from the metabolism of sterols. The only main difference in these intermediates steroids is the side chain carbon located 17 (Figure 2). In pharmaceutical industrial manufacturing of C19 steroids as well as androst-4-ene-3,17-dione (AD), androst- 1,4-dien-3,17-dione (ADD) and 9α-hydroxy-androst-4-ene-3,17-dione (9-OHAD) , all of them are used to produce sex and adrenocortical hormones which may have been largely manufactured. The main reason of this steroid production is due to the

successful development of pharmaceutical strains by metabolic engineering or mutant breeding (Figure 2). The optimization value of the sterol C22 is very less, is the case of ideal industrial strains that have yet to be organized. However, few C22 steroids, including 22-hydroxy-23,24-bisnorchol-4-ene-3-one (4-HBC), 22-hydroxy-23,24-bisnorchol-1,4-dien-3-one (1,4-HBC) and 9,22-dihydroxy-23,24-bisnorchol-4-ene-3-one (9-OHHBC), have very high significant precursors in synthesizing progestational and adrenocortical hormones (Figure 2). (Donova *et al*., 2009).

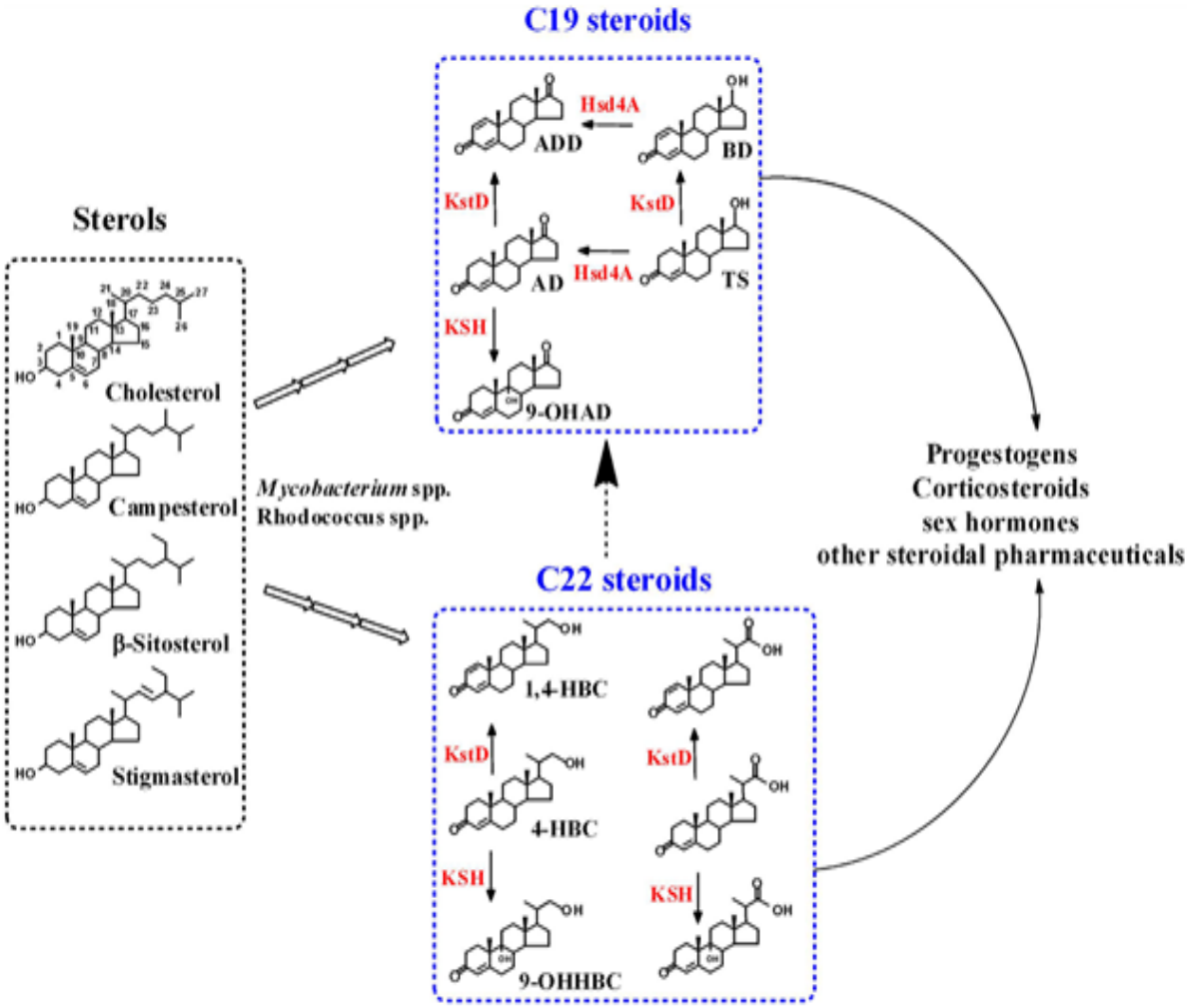

Figure 2: An overview of Biotransformation of Sterols into Steroidal Pharmaceuticals.

Hydroxylation of Some Sterols by Actinobacteria

Actinobacteria has tendency to move hydroxyl groups at different location of the nucleus of steroid as shown in Figure.3. The 9α location of hydroxyl group is carried out by mentioning the matching in *Nocardia, Rhodococcus,* and *Mycobacterium.* A ternary 9-KSH complex comprising a flavoprotein reductase and two ferredoxin proteins was isolated from *Nocardia sp.* M117 (van der Gieze *et al.*, 2007). In *Arthrobacter oxydans* 317, the "9α-hydroxylase and 3-ketosteroid-1-dehydrogenase (3-KSD)" are both plasmid-encoded (Dutta *et al.*, 1992). The 9-KSH of *Rhodococcus erythropolis* SQ1 includes a dimeric [2Fe–2S] monooxygenase type IA component; the genes encoding 9-KSH have been identified (kshA and kshB). When any of the genes was deleted, the resulting mutants lost the ability to grow on androst-4-ene-3,17-dione (AD) or androsta-1,4-diene-3,17-dione (ADD), but retained the capacity for using 9α-hydroxyandrost-4-ene-3,17-dione (9-OH-AD) as a substrate. Of interest, deletion of kshA did not affect the process of degradation of sterol. The process of degradation continues and held complete with AD or without ADD (intermediates), which shows that the event of 9-KSH was preserved. Contra positively the removal of kshB inference in a whole loss of capability to cleave the steroid side chain; the mutants retained only the capacity for oxidizing sitosterol to sitost-4-ene-3-one. It has been pointed that the gene kshB is either involved in 9α-hydroxylation of sterols as a constituent of the tentative enzyme, 9-KSH, or represents a part of the C-26- hydroxylating system, which initiates side chain degradation of sitosterol (Donova *et al.*, 2007).

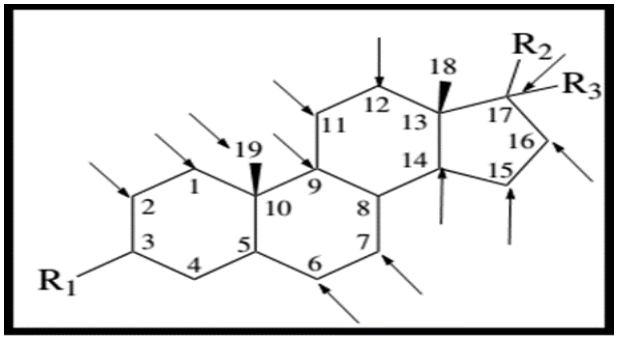

Figure 3: Position within steroid molecule at which Actinobacteria introduce Hydroxyl Group.

Identification of novel biological activities of 7α-hydroxysteroids-which serve as antiglucocorticoid agents or means of diagnosing and treating neoplastic, neurological/mental, and immune disorders, as well as Alzheimer's disease—justifies increasing interest in and search for strains of Actinobacteria with high 7α-hydroxylase activity. *Proactymonyces* sp. is capable of catalyzing 7-hydroxylation of cholesterol (whether the hydroxyl is at position α or β has not been determined).The search for microbial biocatalysts capable of hydroxylating sterols (both known and newly synthesized) is a continual process driven by the high physiological activity of hydroxy derivatives of sterols. However, recent progress in selecting new organisms with unusual region and stereospecific activity has not been significant (Donova *et al.*, 2007)

Dehydrogenation of Sterols by Actinobacteria

Actinobacteria has a distinct feature i.e the ability to dehydrogenate C-C bonds within steroid nucleus. *Corynebacterium, Micromonospora, Mycobacterium, Nocardia, Nocardioides, Rhodococcus,* and *Streptomyces* having this feature. Strains capable of introducing Δ1(2), Δ4(5),Δ7(8) , Δ8(9), Δ9(11), and Δ16(17) double bonds (Atrat *et al.,* 1991; van der Gieze *et al.,* 2007).

Data from the literature indicate that Actinobacteria contain several isoforms of 3-KSD. Selective deletion of the gene kstD, which encodes 3-KSD 1 of R. erythropolis SQ1, did not affect the ability of the resulting mutant to grow on AD, ADD, and 9-OH-AD. Biochemical data demonstrated the presence of another 3-KSD isoform, designated 3-KSD 2. This enzyme was inactivated in *R. erythropolis* RG1-UV29, which was obtained by UV-mutagenesis of the strain *R. erythropolis* RG1. Deletion of both genes, i.e., those encoding 3-KSD 1 and 3-KSD 2, blocked the growth on AD and 9-OH-AD, without affecting the growth on ADD. Thus, both isoenzymes were demonstrated to be involved in sterol degradation in R. erythropolis SQ1 in such a way that the presence of at least one of them was sufficient for the degradation of the sterol nucleus. The ability of some Actinobacteria (*N. restrictus*, etc.) to introduce a Δ17(20) bond in the course of sterol transformation is also reported in this work (Mahato *et al.,* 1989; van der Gieze *et al.,* 2007; Donova *et al,* 2007).

Oxidation of Sterols by Actinobacteria

The oxidation of sterols involved in sterol degradation have been studied at the biochemical as well as genomic level using Actinobacteria. An accepted metabolic pathway for sterol degradation by actinobacteria was recommended based on the documentation of their intermediates. To date, however, not all enzymes involved in oxidation of sterols have been fully identified. Considerable progress in sterol bioconversion studies was achieved due to the excellent works by van der Geize's team on the disclosure of cluster of genes involved in sterol degradation in Rhodococcus and Mycobacterium (Petrusma *et al.,* 2000). Those works initiated intensive research into cholesterol catabolism by *Mycobacterium tuberculosis* H37Rv and other Actinobacterial pathogens and are of importance at the discovery of new targets for the therapy of tuberculosis and other pulmonary diseases (Ouellet *et al.,* 2011). The acute role of cholesterol catabolism for these infections has been also confirmed by other authors (Nesbitt *et al.,* 2010). On the other hand, these data are significant for the future generation of strains capable of producing important steroid precursors, and for the regulation of process selectivity. In general, degradation of sterols by Actinobacteria involves three main routes: (i) uptake of sterol, (ii) removal of aliphatic side chain at C17 (Atrat *et al.,* 1991; Donova, 2009) and (iii) steroid nucleus oxidation.

The mechanism of sterol uptake by actinobacteria includes a direct contact of the cell surface with hydrophobic sterol particles. Cells adhere to sterol particles and gradually imbed into them. Bio-surfactants or bio-emulsifiers produced by actinobacteria extracellularly can increase the bioavailability of the substrate. A flexible mesophase formation in the contact zone, composed of extracellular components where sterols are partially solubilized, was hypothesized earlier (Atrat *et al.,* 1991). A confirmation was obtained that destruction of the cell outermost leaflet of the lipid bilayer, full or partial removal of non-covalent bound lipids at the preserved intactness of the basal cell wall skeleton can facilitate steroidal substrate influx and metabolite efflux and result in the enhancement of steroid Participation of the Mce4 steroid transporter system in active transport of sterols into the cell was evidenced for *Rhodococcus jostii* RHA1, whose transcription was upregulated during growth of strain RHA1 on cholesterol on contrast to pyruvate grown cells (van der Geize *et al.,* 2007). The cluster consists of an operon of 11 genes that encodes two permeases (*supA* and *supB*) and the Mce4A-Mce4I proteins that penetrate the outer layer of the mycolic acids and possibly are involved in substrate binding, together constituting a complex ATP-binding cassette (ABC) transporter system. The ATPase domain, however, was neither encoded by the *mce4* locus, nor was it found elsewhere in the strain RHA1 cholesterol catabolic gene cluster (van der Geize *et al.,* 2007; Mohn *et al.,* 2008). The *mceG* gene of '*M. tuberculosis'* encodes an ATPase

domain which has been shown to interact with proteins determined by the *mce1* and *mce4* loci, but is not located proximal to either of the two loci (Joshi *et al*., 2006). In strain RHA1, *ro01974* and *ro02744* encode MceG orthologs, therefore, either one of them or both may be involved in the Mce4 transport system (Mohn *et al*., 2008). The Mce4 transport system mediates sterol uptake specifically, since mutagenesis studies with genes from the *mce* cluster revealed that they are vital for growth of strain "RHA1" on sterols, including cholesterol, but not other steroids (Mohn *et al*., 2008**)**. It cannot be ruled out that the Mce4 system also transports various steroids, but most likely other mechanism(s) for uptake of steroids exist that are either specific for steroids or that can complement steroid uptake in strain RHA1 *mce4* gene deletion mutants (Mohn *et al*., 2008).It was proposed that extracellular cholesterol oxidase may contribute in sterol transportation into the actinobacterial cells.

Initial steps of sterol core oxidation

Biotransformation of sterols is initiated by alteration of the 3β-ol-5-ene- to 3-oxo-4-ene moiety. The role of cholesterol oxidase (or 3β-hydroxysteroid oxidase) in this process has been elucidated. This enzyme is able to oxidize the β-hydroxyl group at C3 and further isomerization of the Δ5 double bond to a Δ4 (Figure 3). Cholesterol oxidase (CHO) enzymes are flavoproteins in nature that can either contain a covalently or non-covalently bound FAD (Vrielink and Ghisla, 2009**)**. Moreover, they may function outside or inside the cell, depending on the type of organism and enzyme used**.** Cholesterol oxidases transfer the hydrogen atoms to molecular oxygen from various steroid or sterol substrates, thus forming hydrogen peroxide. Also, some cholesterol oxidases also perform hydroxylation reaction of cholesterol, eventually forming 4-cholesten-6β-ol-3-one from cholesterol. Many *Actinobacteria* produce cholesterol oxidase enzymes, including members of *Rhodococcus* **(**Navas *et al*., 2001**;** Fernández de Las Heras *et al*., 2011), *Brevibacterium* and *Streptomyces* (Ishizaki *et al*., 1989). Also 3β-hydroxysteroid dehydrogenases (3β-HSD) are known to catalyze 3- keto-4-ene formation (Figure 6), in *Nocardia* sp. (Horinouchi *et al*., 1991) and *M. tuberculosis* (Yang *et al*., 2007). This enzyme uses NAD(P)+ as electron acceptor as a replacement for molecular oxygen and function intracellularly. The 3β-HSD enzyme Rv1106c of *M. tuberculosis* was shown to be active on cholesterol, pregnenolone and dehydroepiandrosterone, while the highest activities were found for the latter two compounds (Yang *et al*., 2007) which are C21 and C19 steroids, respectively, and are expected pathway intermediates of cholesterol degradation. Therefore, it is likely that in *M. tuberculosis* cholesterol side chain degradation occurs prior to ring oxidation. Recent mutational and biochemical studies have shown that in *R. jostii* RHA1 C26 hydroxylation is the obligate first step in cholesterol degradation, prior to the action of CHO or 3β-HSD, while in *R. rhodochrous* DSM43269 there was no clear preference for either of these two reactions (Rosłoniec *et al*., 2009).

Cholesterol oxidase ChoD is not crucial for sterol catabolism in the fast-growing AD producing *Mycobacterium sp*. VKM Ac-1815D strain, and the knock-out of choD gene does not abrogate sterol ring-A oxidation (Ivashina *et al*., 2012). Similar conclusions were made earlier for *Mycobacterium smegmatis* mc2 155 (Uhía *et al*., 2011a). In *Rhodococcus erythropolis* CECT3014, cholesterol oxidase gene ChoG was shown to be a major inducible extracellular cholesterol oxidase, but its disruption did not alter cell growth on cholesterol (Fernández *et al*., 2011). However, in *Streptomyces virginiae* IB L-14, inactivation of cholesterol oxidase ChoL led to abrogate the oxidation of diosgenin to diosgenone and other 3-oxosteroids.Two cholesterol oxidases genes, ChoM1 and ChoM2, were identified in *Mycobacterium neoaurum* NwIB and described to be essential for consumption of phytosterol as a carbon source (Wei *et al*., 2010). In addition to its proposed function in sterol transport and A-ring oxidation, cholesterol oxidase can play a title role in the pathogenicity of '*M. tuberculosis* H37Rv' (Brzostek *et al*., 2009) and *Rhodococcus equi* (Navas *et al*. 2001), and along with other sterol-modifying enzymes can regulate the exceptional ability of pathogenic mycobacteria to stay alive in macrophages (van der Gieze *et al*., 2007).

It was reported that utilization of cholesterol in mycobacteria is controlled by two TetR-type transcriptional regulator genes: kstR and. KstR controls the expression of 83 cholesterolic catabolism genes. These results were generally confirmed by recent results of Uhía with co-authors (2012) on the comparative transcriptome studies of *M. smegmatis* mc2 155 cells growing on cholesterol and glycerol as the only carbon sources. The microarray analyses publicized that total 89 genes were upregulated three times during the growth of strain on cholesterol with 39 catabolic genes organized in three specific clusters. The function of KstR and KstR2 as auto-regulated repressors of cholesterol degradation was supported.

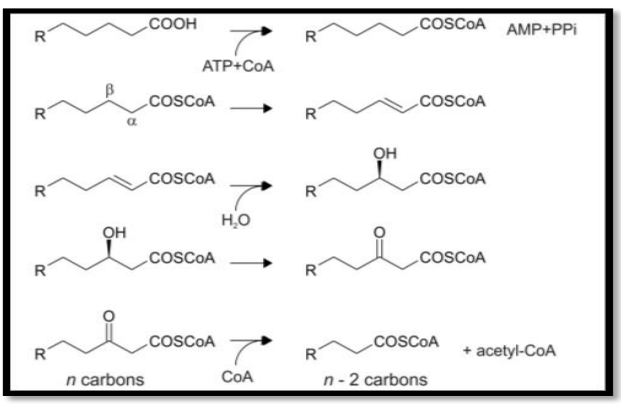

Figure 4: Schematic overview of steps involved in β-oxidation reaction.

Side-chain degradation

Side-chain degradation and steroid nucleus oxidation were confirmed to be independent processes, at least in actinobacteria, and the order of these processes can vary even in one genus (Rosłoniec *et al.*, 2009). The initial step of the side-chain oxidation of sterols (and other C27 steroids) is hydroxylation at C-26 (or C-27). The reaction is catalyzed by cytochrome P450 monooxygenase CYP 125 as shown for *R. jostii* RHA1, *Mycobacterium bovis* BCG, and *M. tuberculosis* H37Rv (Rosłoniec *et al.*, 2009). Further cleavage of the alkyl sterol side chain at C-17 was shown to proceed via the fatty acid β-oxidation process, the mechanism of which has been elucidated several decades ago, by identifying reaction intermediates that accumulated in natural or UV-mutagenized strains of *Nocardia* and *Mycobacterium* that were blocked in various steroid nucleus degradation steps. The genes responsible for this process in actinobacteria constitute part of the sterol catabolic gene. It was deduced that sterol side chain degradation proceeds via a process similar to β-oxidation (Fujimoto *et al*., 1982a). The general mechanism of β-oxidation and the enzymatic steps involved are shown below (Figure 4).

Although the mechanism of sterol side chain degradation is well understood, very limited information is available on the genes and purified enzymes involved in the process: virtually all experiments were done using crude extracts from strains whose genomic sequence data is unavailable. The subject of microbial sterol side chain degradation is relevant for applications in the pharmaceutical industry and has been reviewed regularly (Kieslich, 1985; Fernandes *et al*., 2003). Recent studies elucidated the essential role of steroid coenzyme A ligase encoded by gene fadD19 in side-chain cleavage of C24 branched sterols with RG32 mutant of *R. rhodochrous* DSM43269. AD/ADD is unable to produce from cholesterol after deletion of fadA5 gene (Nesbitt *et al.*, 2010).

Sterol Side Chain Activation

The cleavage of side chains of sterols like cholesterol is a stepwise process same as that of β-oxidation of fatty acids (Figure 5A). The first reaction of β-oxidation is activation of the sterol carboxylic acid moiety with CoA, catalyzed by steroid-CoA ligase. CoA ligase catalyzed reactions are driven by ATP hydrolysis and require Mg2+ as a cofactor. CoA activation of acyl substrates is a two-step process involving an enzyme-bound adenylated intermediate and thioester formation, where the AMP is replaced by CoA (Chang *et al*., 1997). A 65 kDa CoA ligase from *Mycobacterium* sp. NRRL B-3805 was purified to near homogeneity and is highly specific towards C26-carboxylic acid steroids (Chen, 1985). The CoA ligase was shown to be present as a single enzyme, while the rest of the β- oxidation enzymes were aggregated, forming a loosely bound complex (Chen, 1985). The gene encoding the steroid-CoA ligase, or any of the other β-oxidation enzymes involved, remained unidentified.

β-Oxidation Enzyme Complex

Further degradation of the CoA-activated sterol side chain has been demonstrated in crude extracts, involving a multi-enzyme complex consisting of an acyl-CoA dehydrogenase or oxidase, enoyl-CoA hydratase, 3β-hydroxyacyl-CoA dehydrogenase and thiolase (Chen, 1985). In *Mycobacterium* sp. NRRL B-3805, the first dehydrogenation step is performed by an FMN dependent acyl-CoA oxidase (Chen, 1985).

The next step in side chain degradation is hydration of the double bond by an enoyl-CoA hydratase. However, no enzymes or genes for this activity are presently known. The strain RHA1, gene cluster for cholesterol catabolism contains *echA19*, a gene encoding a putative enoyl-CoA hydratase involved in side chain degradation, was suggested to be responsible for the double bond hydration of sterol side chains (van der Geize *et al*., 2007). Though, this remains to be confirmed by experimental studies.

After the hydration step, another dehydrogenation reaction is performed, catalyzed by 3β-hydroxyacyl-CoA dehydrogenase. In *Mycobacterium* sp. NRRL-B3805, this reaction was shown to require NAD+ as a cofactor, but the enzyme has not been characterized in more detail (Chen, 1985).

The final reaction of a β-oxidation cycle is thiolytic cleavage of the substrate. In the case of cholesterol side chain degradation, thiolase mediated C-C bond cleavage of carbons 24-25 and 22-23 results in formation of C24 and C22 steroids and release of propionyl-CoA and acetyl-CoA, respectively (Fujimoto *et al*., 1982a). The remaining 3-carbon side chain of the C22 steroid intermediate is then catalyzed by a mechanism different from β-oxidation, most likely involving a reverse aldol-lyase reaction (Fujimoto *et al*., 1982a).

C24-Branched Chain Sterol Side Chain Degradation

Degradation of C24-branched chain sterols, like the phytosterols β-sitosterol, campesterol and the fungal ergosterol, requires several additional enzymatic steps compared to cholesterol. Fujimoto *et al*. (1982b) used a cell-free system derived from *Mycobacterium* sp. NRRL B-3805 to demonstrate that the C24-branched side chains of β-sitosterol and campesterol are carboxylated at the C28 position, following C26 oxidation and CoA activation (Figure 5B). The carboxylase responsible for incorporation of HCO3- at the C28 position was not inhibited by avidin, an effective inhibitor of biotin dependent enzymes. This carboxylase thus appears to lack biotin or this prosthetic group is deeply buried inside the enzyme (Chen, 1985).

Upon formation of the carboxylate intermediate, the action of enoyl-CoA hydratase is followed by breakage

of 'C24-C25' by an aldol lyase, resulting in release of propionyl-CoA from β-sitosterol and campesterol (Figure 5B, Chen, 1985; Fujimoto *et al*., 1982a). Interestingly, deletion of *ltp3* or *ltp4* in *R. rhodochrous* strain RG32 resulted in complete blockage of β-sitosterol side chain degradation, but not of that of cholesterol. Both *ltp3* and *ltp4* encode proteins with similarities to the thiolase SCPx. Therefore, *ltp3* and *ltp4* were suggested to encode aldol lyase type enzymes with a specific and essential role in C-C cleavage of C24-branched side chain sterols in *Rhodococcus* strains.

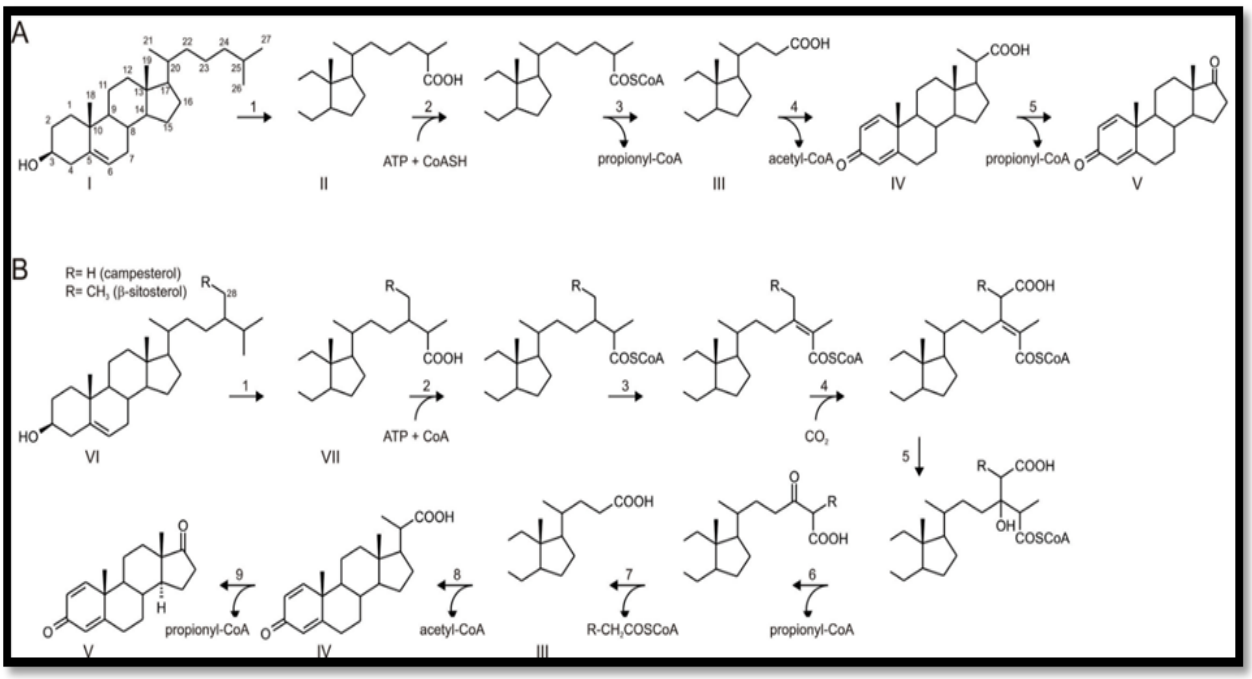

Figure 5: Overview of degradation pathway of side chain of Cholesterol (A) and branched side chain of sitosterol and campsterol (B) in Actinobacteria.

Steroid Nucleus Oxidation

Steroid nucleus degradation is still among the most intricate problems in production of androstenones from sterols by whole-cell microbial catalysis. The enzymes responsible for the opening of steroid 'ring-B' are 3-ketosteroid-9α-hydroxylase (Ksh) and 3-ketosteroid-1-dehydrogenase (KstD; Figure 6). Due to the action of these two enzymes, chemically unstable 1,4-dien-9α-hydroxy steroid intermediates are formed to spontaneously break the 9(10) bond in "ring-B" followed by further core disruption. In order to obtain AD as the only product, both enzymes should be inactivated in the sterol side-chain cleaving strain, while the inactivation of either Kshs or KstDs can result in the formation of ADD or 9-OH-AD, respectively. But, in practice, a mixture of AD, ADD, testosterone, unconverted substrate, and products of incomplete side-chain degradation (mostly, C22 steroids) is often obtained (Wang *et al*., 2011).

Random mutations or mutations induced by UV or chemical treatment can result in an improvement of certain strain (Brzostek *et al.*, 2009). However, selective interference of genes that encode for either Ksh or KstD seems to be the most promising way to generate highly selective strains (van dar Gieze *et al.*, 2007). For example, inactivation or augmentation of kstD gene in *Mycobacterium neoaurum* NwIB-01 resulted in efficient production of AD or ADD, respectively (Wei *et al.*, 2010).

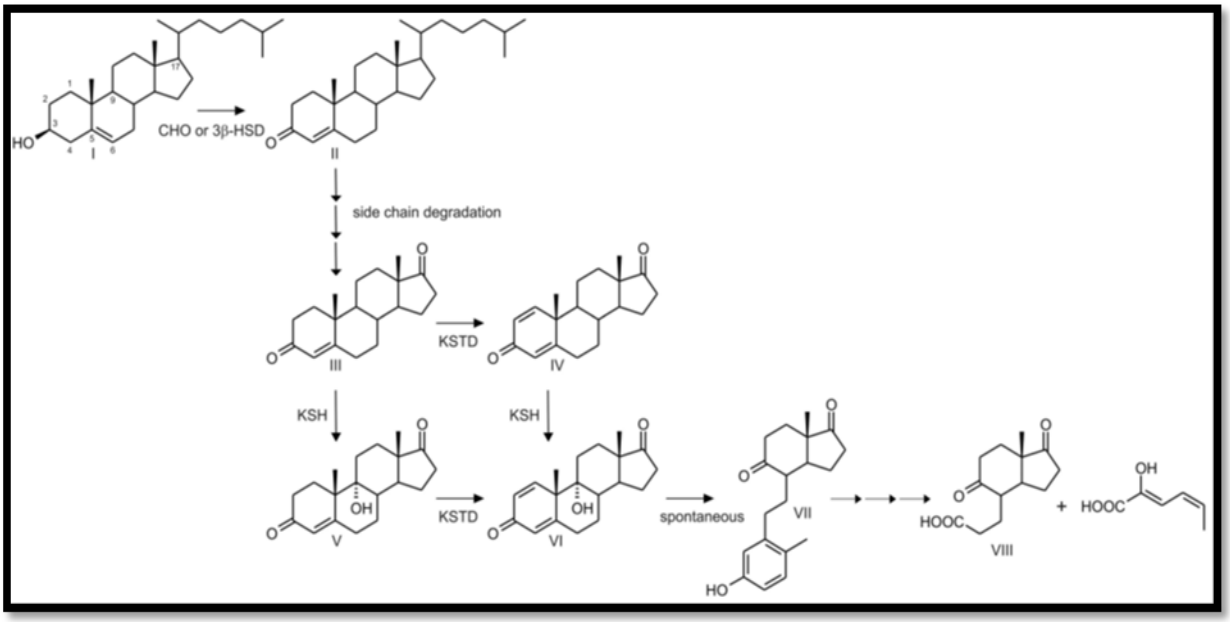

Figure 6: Deduced pathway of microbial cholesterol nucleus opening.

Generation of highly efficient strains capable of selective production of desired C19 steroids from phytosterol is complicated by the multiplicity of ksh and kstD genes in actinobacteria (such as *Mycobacteria* or *rhodococci*) and their integration into sterol catabolism when targeted destruction of one gene influences other key reactions (Petrusma *et al.*, 2009). For example, six kstD homologs were revealed in *M. smegmatis* mc2 155 genome. Targeted disruption of one of them (kstD-1) resulted in partial inactivation of the cholesterol degradation pathway and accumulation of AD (Brzostek *et al*., 2009). Several homologous genes encoding KshA and KshB, which are the two gears terminal oxygenase and ferredoxin reductase, respectively) of 3-ketosteroid-9α-hydroxylase in *R. rhodochrous* DSM43269, were identified (van dar Gieize *et al.*, 2007). The recognition of five KshA homologs, each of them displayi a unique pattern of steroid induction and also substrate range thus confirmed that 9α-hydroxylation can take place at different steps of steroid oxidation (Petrusma *et al.*, 2009).The new fundamental findings of sterol catabolism by actinobacteria enable a prediction that novel efficient strains selectively producing valuable C19 or C22 steroids from sterols could be constructed in the very near future. The new fundamental findings of sterol catabolism by actinobacteria enable a prediction that novel efficient strains selectively producing valuable C19 or C22 steroids from sterols could be constructed in the very near future.

CONCLUSION

However, the data set is used in this review article are not complete and everything. They just supply a signal of width of the biocatalytic capability of actinobacteria in deep phenomenon of transformation of sterol. In most of the key reaction their application is getting preference in case of another microorganism or chemical product. In future research for the biotechnical capability of actinobacterial the search for new steroid-transforming mutants thereof will definitely offer much new promise for steroid biotechnology. For the growth of pharmaceutical industry, diosgenin drugs in the modern era has been scarcity and the demand of sterol drugs is come into account. Transformation of sterols such as cholesterols and sitosterol gain fascinating importance for the researcher to fulfil the pharmaceutical industry requirements and improve the capital. Additional optimization and remarkable improvement of sterol transformation is possible in engineering aspect whereas methods to improve strain is possible by chemostat mutation and DNA technology. The economical feasible process of sterol conversion is side chain cleavage at industrial scale it would be significantly important for researchers in biotransformation of sterols.so our scheme to biotransformation of sterol is highly suitable for economic development of pharmaceutical industry as well as gateway for novel and innovative future work in microbiology filed.

REFERENCES

1. Abbott B. Some new approaches to biotransformation. *Dev. Ind. Microbiol.*, 1979; 20: 345-365.
2. Abd-elsalam I.S., Salam L.A. and Abd-Elhady S.A. Optimization of sugar cane phytosterols bioconversion using *Arthrobacter rubellus*. *J Appl Sci Res.*, 2010; 6(9): 1334–1339.
3. Aharnowetz V. and Choe G. The microbiological production of pharmaceuticals. *Scientific American.*, 1981; 240: 140-153.
4. Ahmad S., Garg S.K. and Johri B.N. Biotransformation of sterols: selective cleavage of the side chain. *Biotechnol Adv*., 1992; 10: 1–67.

5. Ahmed S., Roy P.K. and Basu S.K. Cholesterol side-chain cleavage by immobilized cells of *Rhodococcus equi* DSM 89-133. *Indian J. Exp. Biol*., 1993; 31: 319-322.
6. Ambrus G., Jekkel A., Ilkoy E., Horvath G. and Bocksei Z.. Novel 26-oxygenated products in microbial degradation of ergosterol. *Steroids*, 1995; 60: 626-629.
7. Andor A., Jekkel A., Hopwood D.A., Jeanplong F., Ilkoy E., Konya A. and Ambrus G. *Appl Envir Microbiol.*, 2006; 72(10): 6554–6559.
8. Arima K. Microbial Transformation of Sterols. *Agr. Biol. Chern.*, 1969; 33: 1636-1643.
9. Arthan D., Svasti J., Kittakoop J., Pittayakhachonwut D., Tanticharoen M. and Thebtaranonth Y. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of Solanum torvum. *Phytochemistry*, 2002; 59: 459–63.
10. Atrat P., Hösel P., Richter W., Meyer H.W. and Hörhold C. Interactions of *Mycobacterium fortuitum* with solid sterol substrate particles. *J Basic Microbiol.*, 1991; 31: 413–422.
11. Bhatti H.N. and Khera. R.A. Biological transformations of steroidal compounds: a review. *Elsevier Inc,*. 2012; 77: 1267-1290.
12. Borman E.A., Redikulifsev Y.V., Koshcheenko K.A., Turuta A.M. and Kamernitskii A.V. Transformation of sitosterol to 9alpha-hydroxy-androstene-dione by *Mycobacterium sp*. 207 cells in the presence of an adsorption resin. *Prikl. Biokhim. Mikrobiol*., 1992; 28: 551-556.
13. Brown W.E. The impact of biotechnology to health care industry. *Ann Rep Fermen Proc.*, 1984; 7: 135.
14. Brzostek A., Pawelczyk J., Rumijowska-Galewicz D.B. and Dziadek J. *Mycobacterium tuberculosis* is able to accumulate and utilize cholesterol. *J Bacteriol.*, 2009; 191: 6584–6591.
15. Chang K.H., Xiang H. and Dunaway-Mariano D. Acyl-adenylate motif of the acyladenylate/thioester-forming enzyme superfamily: a site-directed mutagenesis study with the *Pseudomonas sp.* strain CBS3 4-chlorobenzoate:coenzyme A ligase. *Biochemistry.*, 1997; 36: 15650-15659.
16. Charney W. and Herzong L.H. Microbial transformations of steroids. *New York: Academic Press.*, 1976; 5–73.
17. Chaudhari P.N., Chaudhari B.L. and Chincholkar S.B. Cholesterol biotransformation to androsta-1,4-diene-3,17-dione by growing cells of Chryseobacterium gleum. *Biotechnol Lett.*, 2010; 32: 695–699.
18. Chen C.S. The mechanism of degradation of side chains of phytosterols by microorganisms. Ph.D. thesis. *University of Wisconsin-Madison, Ann Arbor, M.I.* 1985.
19. Chung S.K., Ryoo C.H.., Yang H.W., Shim J.Y., Kang M.G., Lee K.W. and Kang H.I. Synthesis and bioactivities of steroid derivatives as antifungal agents. *Tetrahedron.*, 1998; 52: 15899–914.
20. Diaz-Chico B.N., Rodrıguez F.G., Gonzalez A., Ramırez R., Bilbao C., Leon A.C., Jaime A.A., Chirino R.and Navarro D. Androgens and androgen receptors in breast cancer. *J Steroid Biochem Mol Biol*., 2007; 105: 1–15.
21. Donova M.V. Transformation steroid compounds by actinobacteria. *Appl Biochem Microbiol.*, 2007; 43: 1–14.
22. Donova M.V. and Egorova O.V. Microbial steroid transformations: current state and prospects. *Appl. Microbiol. Biotechnol.*, 2012; 94: 1423–1447.
23. Dovbnya D.V., Egorova O.V. and Donova M.V. Microbial side-chain degradation of ergosterol and its 3-substituted derivatives: a new route for obtaining of deltanoids. *Steroids.*, 2010; 75: 653-658.
24. Dutta R.K., Roy M. and Singh H.D.S. Metabolic blocks in the degradation of beta-sitosterol by a plasmid-cured strain of *Arthrobacter oxydans*. *J. Basic Microbiol*., 1992; 32: 167-176.
25. Egorova O.V., Nikolayeva V.M, Sukhodolskaya G., Donova M.V. Transformation of C19-steroids and testosterone production by sterol-transforming strains of *Mycobacterium spp*. *J Mol Cat B: Enzym.*, 2009; 5: 198–203.
26. Fujimoto Y., Chen C.S, Szeleczky Z., Di Tullio D. and Sih C.J. Microbial degradation of the phytosterol side chain. I. Enzymatic conversion of 3-oxo-24-ethylcholest-4-en-26-oic acid into 3-oxochol-4-en-24-oic acid and androst-4-ene-3, 17-dione. *J. Am. Chem. Soc.*, 1982; 104: 4718-4720.
27. Fernandes P., Cruz A., Angelova B., Pinheiro H.M., and Cabral J.M.S. Microbial conversion of steroid compounds: recent developments. *Enzyme Microb Tec.*, 2003; 32: 688–705.
28. Fernandes P. and Cabral J.M.S. Phytosterols: applications and recovery methods. *Biores Technol.*, 2007; 98: 2335–2350.
29. Fernández de las Heras L., Mascaraque L., Fernández E.G., Navarro- Llorens J.M, Perera J. and Drzyzga O. ChoG is the main inducible extracellular cholesterol oxidase of *Rhodococcus sp.* strain CECT3014. *Microb Research.*, 2011; 166(5): 403–418.
30. Frye L.L. and Leonard D.A. Lanosterol analog: dual action inhibitors of cholesterol biosynthesis. In: Parish EJ, Nes WD, editors. Biochemistry and function of sterols. *Boca Raton, FL: CRC Press.*, 1987; 23–53.
31. Hanson J.R. Steroids: reactions and partial synthesis. *Nat Prod Rep*., 2005; 22: 104–110.
32. Hohnston J.O. Aromatase inhibitors. In: Parish EJ, Nes WD, editors. Biochemistry and function of sterols. *CRC Press*., 1987; 23–53.
33. Horinouchi S., Ishizuka H. and Beppu T. Cloning, nucleotide sequence, and transcriptional analysis of the NAD(P)-dependent cholesterol dehydrogenase gene from a *Nocardia sp.* and its hyperexpression in

Streptomyces spp. Appl. Environ. Microbiol., 1991; 57: 1386-1393.
34. Ishizaki T.,Hirayama N., Shinkawa H., Nimi O. and Murooka Y. Nucleotide sequence of the gene for cholesterol oxidase from a *Streptomyces sp*. *J. Bacteriol*., 1989; 171: 596-601.
35. Ivashina T.V., Nikolayeva V.M, Dovbnya D.V. and Donova M.V. Cholesterol oxidase ChoD is not a critical enzyme accounting for oxidation of sterols to 3-keto-4-ene steroids in fast-growing *Mycobacterium sp*. VKM Ac-1815D. *J Steroid Biochem Mol Biol*., 2012; 129: 47–53.
36. Joshi S.M., Pandey A.K., Capite N., Rubin E.J. and Sassetti C.M. Characterization of mycobacterial virulence genes through genetic interaction mapping. *Proc. Natl. Acad. Sci. USA.,* 2006; 103: 11760-11765.
37. Kieslich K. Microbial side chain degradation of sterols. *J. Basic Microbiol.,* 1985; 25: 461-475.
38. Lee C.Y., Chen C.D. and Liu W.H. Production of androsta-l,4-diene-3,17-dione from cholesterol using two-step microbial transformation. *Appl. Microbiol. Biotechnol*., 1993; 38: 447-452.
39. Lilly M.D. Advances in biotransformation processes. *Trans Inst Chem Eng.,* 1984; 72: 27-34.
40. Lin Y., Song X, Fu J, Lin J. and Qu Y.. Microbial transformation of phytosterol in corn flour and soybean flour to 4-androstene-3, 17-dione by Fusarium moniliforme Sheld. *Biores Technol.,* 2009; 10: 1864–1867.
41. Liu W.H. and Lo C.K. Production of testosterone from cholesterol using a single step microbial transformation of *Mycobacterium sp*.. *J Ind Microbiol Biotechnol.,* 1997; 19: 269–72.
42. Mahato S.B. Steroid transformation by microorganism III. *Phytochemistry.,* 1989; 28: 7-40.
43. Malaviya A. and Gomes J. Rapid screening and isolation of a fungus for sitosterol to androstenedione biotransformation. *Appl Biochem Biotechnol*., 2009; 158: 374–386.
44. Martin C.K.A. and Wagner F. Microbial transformation of beta-sitosterol by *Nocardia sp.* M29. *Eur. J. Appl. Microbiol*., 1976; 2: 243-255.
45. Mark D. W. and Flashman E. Catalytic strategies of the non-heme iron dependent oxygenases and their roles in plant biology. *Elsevier. Inc.,* 2016; 31: 126-135.
46. Mohn W.W., van der Geize R., Stewart G.R., Okamoto S., Liu J., Dijkhuizen L. and Eltis L.D. The actinobacterial *mce4* locus encodes a steroid transporter. *J. Biol. Chem.,* 2008; 283: 35368-35374.
47. Murohisa T. and Iida M. Studies on microbial transformation (XXVI). Microbial degradation of 19-hydroxysterol side-chains. *J. Ferment. Bioeng*., 1993; 75: 13-17.
48. Nesbitt N.M., Yang X., Fontan P., Kolesnikova I., Smith I., Sampson N.S. and Dubnau E. A thiolase of Mycobacterium tuberculosis is required for virulence and production of androstenedione and androstanedione from cholesterol. *Infect Immun.,* 2010; 78: 275–282.
49. Navas J., Gonzalez-Zorn B., Ladron N., Garrido P. and Vazquez-Boland J.A. Identification and mutagenesis by allelic exchange of choE, encoding a cholesterol oxidase from the intracellular pathogen *Rhodococcus equi*. *J. Bacteriol.,* 2001; 183(16): 4796–4805.
50. Roy P.K., Khan A.W., Kumar J., Chopra S.D.K and Basu S.K. Steroid transformation in a laboratory-scale glass airlift fermentor. *World J. Microbiol. Biotechnol*., 1992; 8: 399-401.
51. Olivares A. and Acevedo F. Effect of inoculation strategies, substrate to biomass ratio and nitrogen sources on the bioconversion of wood sterols by *Mycobacterium sp. World J Microbiol Biotechnol.,* 2011; 27(11): 2513–2520.
52. Ouellet H., Johnston J.B. and de Montellano P.R.O. Cholesterol catabolism as a therapeutic target in *Mycobacterium tuberculosis. Trends Microbiol.,* 2011; 19: 530–553
53. Petrusma M., Dijkhuizen L. and van der Geize R. *Rhodococcus rhodochrous* DSM 43269 3-ketosteroid-9α-hydroxylase, a two-component iron-sulfur-containing monooxygenase with subtle steroid substrate specificity. *Appl Environ Microbiol.,* 2009; 75: 5300–5307.
54. Rosłoniec K.Z., Wilbrink M. H., Capyk J.K., Mohn W.W., Ostendorf M.,Van der Geize R., Dijkhuizen L. and Eltis L.D. Cytochrome P450 125 (CYP125) catalyses C26-hydroxylation to initiate sterol side-chain degradation in *Rhodococcus jostii* RHA1. *Mol. Microbiol*., 2009; 74: 1031-43.
55. Sallam L.A.R., Osman M.E.S, Hamdy A.A. and Zaghlol G.M. Microbial transformation of phytosterols mixture from rice bran oil unsaponifiable matter by selected bacteria. *World J Microbiol Biotechnol.,* 2008; 24: 1643–1656.
56. Schaefer C.M. *et al.* FadA5 a thiolase from *Mycobacterium tuberculosis*: a steroid-binding pocket reveals the potential for drug development against tuberculosis. *Structure*., 2015; 23: 21–33.
57. Schmid A., Dordick J.S., Hauer B., Kiener A., Wubbolts M. and Witholt B. Industrial biocatalysis today and tomorrow. *Nature*., 2001; 409: 258-268.
58. Seidel L. and Hoerhold C. Selection and characterization of new microorganisms for the manufacture of 9-OH-AD from sterols. *J. Basic Microbiol.,* 1992; 32: 49-55.
59. Shukla A, Patil S. and Bharti S. Microbial conversion of solasodine to 4-androstene-3, 17-dione (AD), a key intermediate for androgen synthesis. *Lett. Appl. Microbiol.,* 1992; 15: 86-88.
60. Smith M., Zahnley J., Pfeifer D. and Goff. Growth and cholesterol oxidation by *Mycobacterium species* in Tween 80 medium. *Appl. Environ. Microbiol.,* 1993; 59: 1425-1429.
61. Stadtman T.C. Steroidal Alcohol. *J. Biol.chem.,* 1954; 206: 511.

62. Tong W.Y. and Dong X. Microbial biotransformation: recent developments on steroid drugs. *Recent Patents on Biotechnol.*, 1940; 3: 141–153.
63. Toro A. and Ambrus G. Oxidative Decarboxylation of 17(20)-Dehydro-23, 24-dinorcholanoic acids. *Tetrahed Let.*, 1990; 31:3475–3476.
64. Turfitt G.E. The Microbial Degradation of Steroids. *Biochem J.*, 1944; 38: 492-496.
65. Van der Geize, Yam R.K, Heuser T, Wilbrink M.H, Hara H., Anderton M.C. and Eltis L.D. A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into *Mycobacterium tuberculosis* survival in macrophages. *Proc Nat Acad Sci USA.*, 2007; 104: 1947–1952.
66. Uhía I., Galán B., Morales V. and García J.L. Initial step in the catabolism of cholesterol by *Mycobacterium smegmatis* mc2 155. *Environ Microbiol.*, 2011; 13(4): 943.
67. Vorbrodt H.M., Adam G., Porzel A., Hoerhold C., Daenhardt S. and Boehme K.H. Microbial degradation of 2alpha,3alpha-dihydroxy-5alpha-cholestan-6-one by *Mycobacterium vaccae. Steroids.*, 1991; 56: 586-588.
68. Vrielink A. and Ghisla S. Cholesterol oxidase: biochemistry and structural features. *FEBS J.*, 2009; 276: 6826-6843.
69. Wang F.Q., Yao K. and Wei D.Z, From soybean phytosterols to steroid hormones. In: El-Shemy H(ed) Soybean and health. *InTech—Open Access Publisher, Rijeka.*, 2011; 232–252.
70. Weber A, Kennecke M. and Neef G. 20-Methyl-5,7-pregnadiene-3beta,2l-diol derivatives and their manufacture with *Mycobacterium. World Patent 0903.*, 1992; 465. (Schering A-G).
71. Wei W., Wang F.Q, Fan S.Y. and Wei D. Z. 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.*, 2010; 76: 4578–4582.
72. Wilson M.R., Gallimore W.A and Reese P.D. Steroid transformations with Fusari-um oxysporum var. cubense and Colleto-trichum musae. *Steroids.*, 2014; 64: 834-843.
73. Wipperman M.F., Sampson N.S. and Thomas S.T. Pathogen roid rage: cholesterol utilization by *Mycobacterium tuberculosis. Crit. Rev. Biochem. Mol. Biol*., 2014; 49: 269–293.
74. Yang X. Dubnau E., Smith I. and Sampson N.H. Rv1106c from *Mycobacterium tuberculosis* is a 3β-hydroxysteroid dehydrogenase. *Biochemistry.*, 2007; 46: 9058-9067.
75. Zhang L.Q., Bian E.P. and Wang Y. Side-chain cleavage of sterols by *Mycobacterium sp*. MI2. *Yao Xue Xue Bao.*, 1992; 27: 903-907.