Bacterial Metabolism of Steroids

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Abstract

Steroids are naturally occurring hydrophobic molecules frequently found in the biosphere. Currently, a considerable amount of steroid hormones are released into the environment as a result of human activity being now considered a new class of pollutants. This fact is generating an increasing concern about its effects in the environment, because in spite of its ubiquity in nature, most of the steroidal compounds are highly recalcitrant to microbial degradation. Bacterial transformation of steroid compounds has attracted increasing interest due to the biotechnological applications since sterol-degrading microorganisms have already been used for industrial production of steroidal drugs from low-cost natural sterols such as phytosterols. In these bacteria, a large set of catabolic genes has been identified based on gene annotation and biochemical and transcriptomic analyses. The recent knowledge on the microbial metabolism of steroids is reviewed by

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describing the steps involved in the catabolic pathways under both aerobic and anaerobic conditions. This background information will be helpful for metabolic engineering of steroid-transforming bacteria for biotechnological applications.

1 Introduction

Steroids are naturally occurring hydrophobic molecules that have the perhydro-1,2-cyclopentanophenanthrene ring system in common (Fig. 1). This chemical structure can present several modifications being sterols, which consist of the aforementioned steroid ring system with a β-hydroxyl group at C-3, one of the most important steroids because of the essential roles they play in the physiology of eukaryotic organisms. Sterols are frequently found in the biosphere (e.g., cholesterol, ergosterol, and phytosterols) and are considered to be one of the most abundant compounds in nature (Fig. 1). Among them, the most relevant sterol is cholesterol, an essential structural component of animal cell membrane and the precursor to fat-soluble vitamins, bile acids, and steroid hormones (Fig. 1).

On the other hand, a considerable amount of bile salts and steroid hormones are released into the environment with feces (Ridlon et al. 2006) and urine (Hayakawa 1982) or as a result of human activity (Gagné et al. 2006). As a consequence, steroids are now considered to constitute a new class of pollutants, generating an increasing concern about its effects in the environment as some of them act as endocrine disruptors (Galli and Braun 2008; Fahrbach 2006). In spite of their ubiquity in nature, steroids are highly recalcitrant to microbial degradation because of the low number of functional groups present in their structure and their extremely low solubility in water.

The study of the bacterial metabolism of cholesterol has also become especially relevant because of its role in the pathogenicity of *Mycobacterium tuberculosis*. The presence of this set of catabolic genes allows the utilization of host cholesterol by the pathogen, a characteristic proved to be crucial for the maintenance of the bacterial infection and its persistence in macrophages (Pandey and Sassetti 2008).

Beyond pathogenesis, bacterial transformation of steroid compounds has attracted increasing interest due to the biotechnological applications of the sterol-transforming enzymes that usually have a high regio- and stereospecificity, an important advantage with respect to the chemical synthesis. In this sense, whole cells of cholesterol-degrading microorganisms have already been used for industrial production of steroidal drugs from low-cost natural sterols such as phytosterols (Fernandes et al. 2003; Donova et al. 2005a, b; Andor et al. 2006; Donova and Egorova 2012; García et al. 2012; Galán et al. 2016). This review is mainly focused on the current knowledge about the bacterial metabolism of steroids, especially of cholesterol, which is relevant not only to understand its influence in pathological processes but also to develop new organisms with potential use as biotechnological tools.

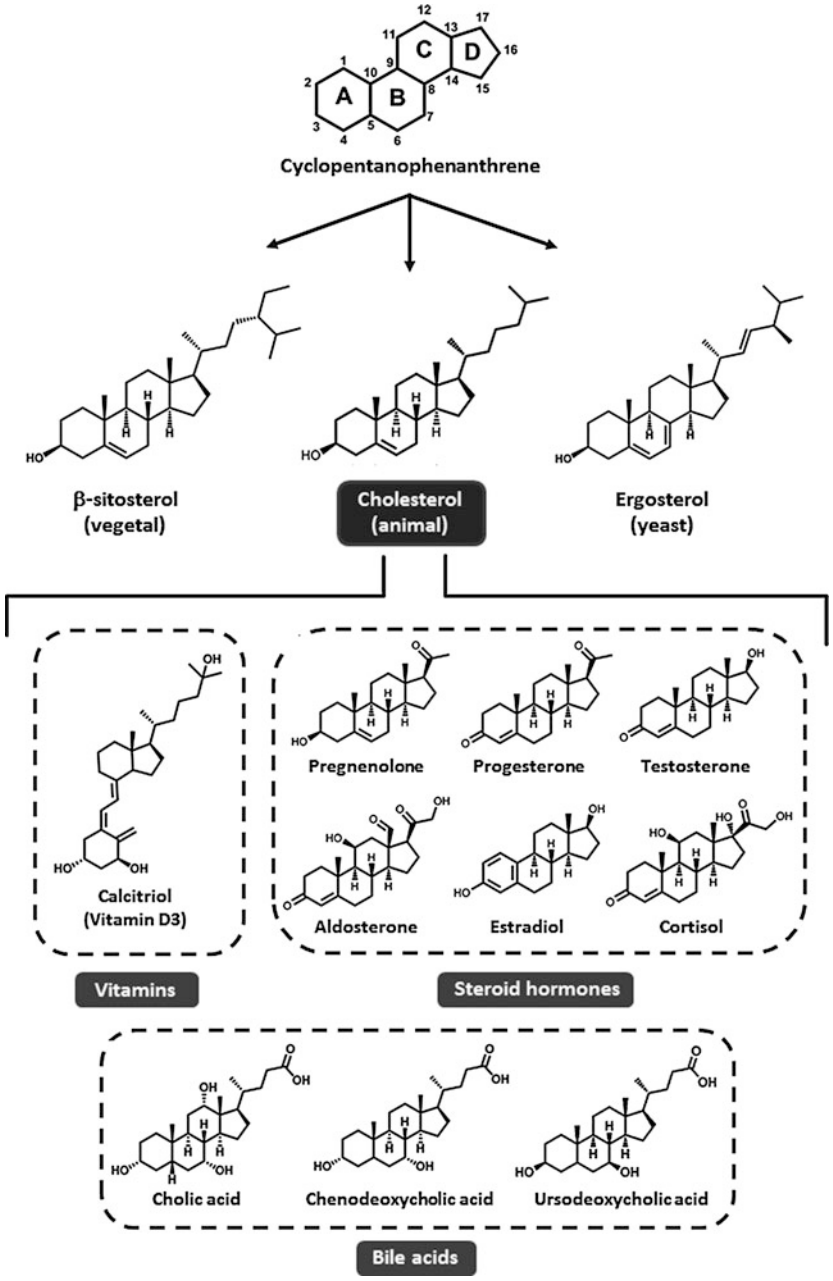

Fig. 1 Chemical structures containing the cyclopentanophenanthrene. Structures of prevalent sterols occurring in plants, animals, and fungus. Structures of cholesterol derivatives (hormones, vitamins, and bile acids)

2 Bacterial Catabolism of Cholesterol

2.1 Aerobic Degradation

The aerobic degradation pathway of cholesterol has not been completely elucidated yet and has been postulated based on different biochemical and genetic studies in diverse steroid-degrading bacteria. The MetaCyc curated interactive database provides an excellent graphical overview of the enzymes and metabolite structures involved in some of the sterol-degrading microorganisms like *M. tuberculosis* and *Rhodococcus jostii* (http://biocyc.com/META/NEW-IMAGE?object=Cholesterol-Degradation) (Caspi et al. 2014).

2.1.1 Transformation of Cholesterol into Cholest-5-en-3-one

In *Actinobacteria*, one of the first reactions for ring modification consists in the oxidation and isomerization of cholesterol into cholest-4-en-3-one (Fig. 2, compound III). This biochemical step is catalyzed either by a cholesterol oxidase (ChOx) (Li et al. 1993; Navas et al. 2001; Fernández de las Heras et al. 2011) or by a NAD (P)-dependent 3-β-hydroxy-Δ(5)-steroid dehydrogenase (3β-HSD) (Horinouchi et al. 1991; Yang et al. 2007; Uhía et al. 2011b; Brzostek et al. 2013) (Fig. 2). Bacterial ChOx is a member of the glucose-methanol-choline oxidoreductase family. It is an extracellular enzyme that binds flavin adenine dinucleotide (FAD) as a cofactor and uses O_2 as electron acceptor which is finally reduced to hydrogen peroxide to regenerate FAD. 3β-HSD is a member of the short-chain dehydrogenase superfamily and uses NAD+ or NADP+ as electron acceptor. *Nocardia* sp. (Horinouchi et al. 1991), *C. testosteroni* (Horinouchi et al. 2012), *R. jostii* (Rosloniec et al. 2009), and *M. smegmatis* (Uhia et al. 2011b) utilize 3β-HSD, while *Streptomyces* spp. (Ishizaki et al. 1989), *Rhodococcus equi* (Machang'u and Prescott 1991), and *Gordonia cholesterolivorans* (Drzyzga et al. 2011) utilize ChOx (Drzyzga et al. 2009).

2.1.2 Cholesterol Side-Chain Metabolism

The first step for the removal of the long alkyl side chain of cholesterol is performed by two P450 cytochromes named CYP125 and CYP142 that catalyze the C-27 hydroxylation of cholesterol and subsequent oxidation of the hydroxylation product to (25S)-3-oxocholest-4-en-26-oate (compound VI) via an aldehyde intermediate (compound V) (Fig. 2) (Rosloniec et al. 2009; Capyk et al. 2009; McLean et al. 2009; Ouellet et al. 2010; Garcia-Fernandez et al. 2013). In this sense, some attention has turned out to a third cytochrome CYP125A4 (*MSMEG_3524*) that shares approximately 65% sequence identity with CYP125A3 (Frank et al. 2015a, b) because, unlike the relative *M. tuberculosis*, the *M. smegmatis Δcyp125a3/Δcyp142a2* double mutant retains its ability to utilize cholesterol as the only carbon source for growth (Garcia-Fernandez et al. 2013). Although in vitro studies showed a weak activity of this cytochrome toward cholesterol and 4-cholest-3-one, it had robust activity against 7α-hydroxy-4-cholest-3-one rendering 7α-26-dihydroxy-4-cholest-3-one, an oxysterol involved in immune cell migration and signaling in

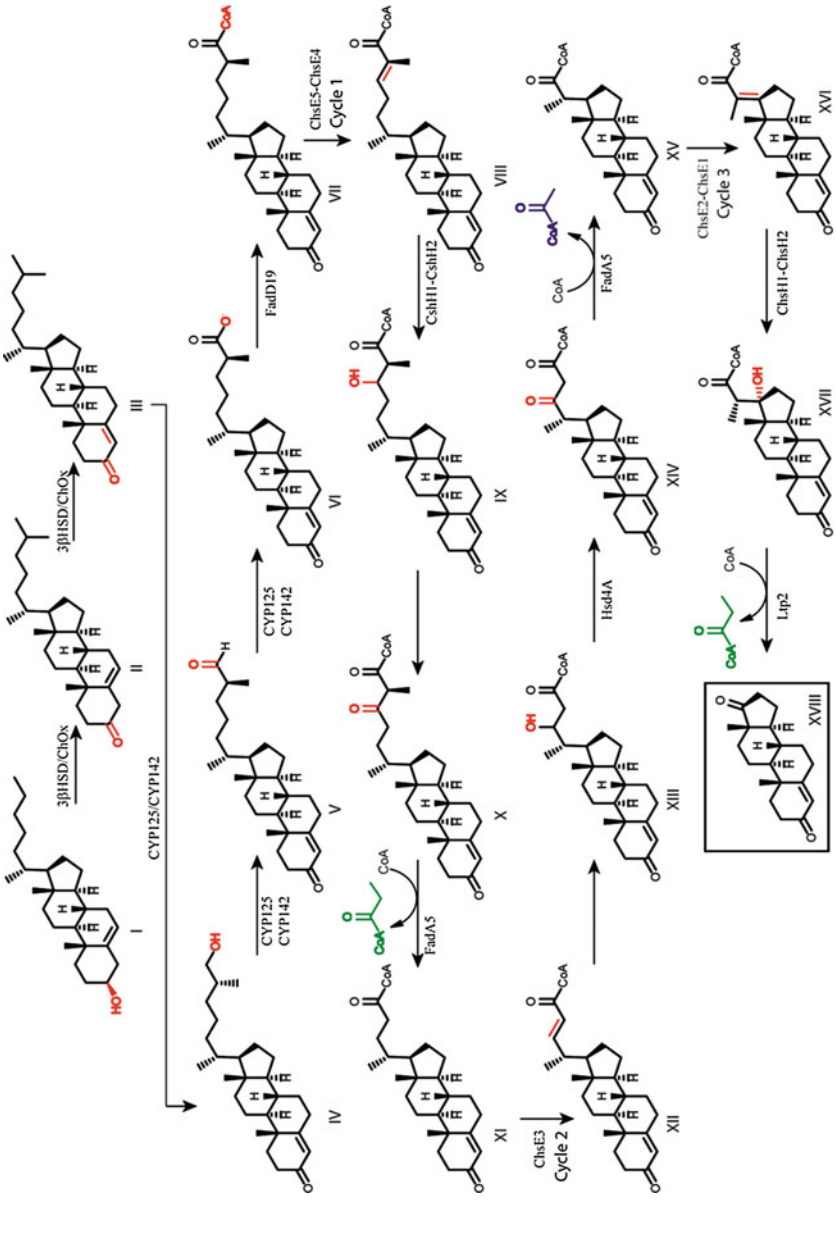

Fig. 2 Activation of cholesterol ring A and side-chain metabolism in *Actinobacteria*. Ring A is oxidized either by a 3-βHSD or a ChOx, and the cytochromes P450 CYP125 and CYP142 initiate cholesterol side degradation. FadA19 acyl-CoA ligase activates the resultant steroid carboxylic acid through esterification

humans (Liu et al. 2011; Hannedouche et al. 2011). Therefore, the discovery of CYP125A4 has broadened the ability of *M. smegmatis* as an environmental mycobacterium to utilize diverse sterol substrates as carbon sources.

The complete metabolism of the cholesterol side chain proceeds via three cycles of a β-oxidative-like type process resulting finally in a 17-ketosteroid intermediate, one acetyl-CoA, and two propionyl-CoA molecules (Fig. 2). The first step has been described in *Rhodococcus rhodochrous* DSM 43269 and consists in the activation of the side-chain carboxylate by CoA mediated by the FadD19 steroid-CoA ligase (compound VII) (Wilbrink et al. 2011).

In *M. tuberculosis,* the first β-oxidation cycle is catalyzed by ChsE4-ChsE5 (an acyl-CoA dehydrogenase) and consists in the dehydrogenation of 3-oxocholest-4-en-26-oyl-CoA (compound VII) to render 3-oxocholest-4,24-dien-26-oyl-CoA (compound VIII) (Thomas et al. 2011; Thomas and Sampson 2013; Yang et al. 2015). ChsH1–ChsH2 encoded by the *Rv3541c* and *Rv3542c* genes form a MaoC-like enoyl-CoA hydratase that catalyzes the hydratation of compound VIII to 24-hydroxy-3-oxocholest-4-en-26-oyl-CoA (compound IX) (Yang et al. 2014). Hsd4A protein from *M. tuberculosis* encoded by the *Rv3502c* gene has been proposed to be the β-hydroxy acyl-CoA dehydrogenase involved in the next step in side-chain β-oxidation although there is no experimental evidence yet (Griffin et al. 2012; Wipperman et al. 2013). The next biochemical step is performed by a thiolase (steroid acyl-CoA-acyltransferase) named FadA5 that catalyzes the cleavage of 3,24-dioxocholest-4-en-26-oyl-CoA (compound X) into 3-oxochol-4-en-24-oyl-CoA (compound XI) and propanoyl-CoA (Nesbitt et al. 2010; Schaefer et al. 2015). Yang et al. 2015 have demonstrated that the second β-oxidation cycle is started by dehydrogenase ChsE3, followed by enoyl-CoA hydration to produce a quaternary alcohol. The resulting compound is then hydrated by an unknown enzyme and dehydrogenated by HsdA4 rendering 3,22-dioxochol-4-en-24-oyl-CoA (compound XIV) (Xu et al. 2016), which is substrate of the FadA5 thiolase rendering 3-oxo-pregne-20-carboxyl-CoA (compound XV) and one molecule of acetyl-CoA (Nesbitt et al. 2010; Griffin et al. 2012; Schaefer et al. 2015). The degradation of the side chain is completed by a third β-oxidation cycle that starts with the dehydrogenation of compound XV by the ChsE2-ChsE1 proteins generating 3-oxo-4,17-pregne-20-carboxyl-CoA (compound XVI) (Thomas et al. 2011; Yang et al. 2015).

Fig. 2 (continued) with CoA. The steroid side chain is degraded via three cycles of β-oxidation to yield one acetyl-CoA (highlighted in *red*) and two propionyl CoA molecules (highlighted in *blue*) and androstenedione (compound XVIII). The first step in each β-oxidation cycle is indicated. The bonds undergoing modifications are highlighted in *red*. Compound I, cholesterol; compound II, cholest-5-en-3-one; compound III, cholest-4-en-3-one; compound IV, 3-oxocholest-4-en-26-ol; compound V, (25S)-3-oxocholest-4-en-26-al; compound VI, (25S)-3-oxocholest-4-en-26-oate; compound VII, (25S)-3-oxocholest-4-en-26-oyl-CoA; compound VIII, 3-oxocholest-4,24-dien-26-oyl-CoA; compound IX, 24-hydroxy-3-oxocholest-4-en-26-oyl-CoA; compound X, 3,24-dioxocholest-4-en-26-oyl-CoA; compound XI, 3-oxochol-4-en-24-oyl-CoA; compound XII, 3-oxochol-4,22-dien-24-oyl-CoA; compound XIII, 22-hydroxy-3-oxochol-4-en-24-oyl-CoA; compound XIV, 3,22-dioxochol-4-en-24-oyl-CoA; compound XV, 3-oxo-4-pregnene-20-carboxyl-CoA; compound XVI, 3-oxo-4,17-pregnadiene-20-carboxyl-CoA; compound XVII, 17-hydroxy-3-oxo-4-pregnane-20-carboxyl-CoA

The side-chain oxidation finishes by an enoyl-CoA hydration caused by the ChsH2–ChsH1 proteins (Thomas et al. 2011) which then undergoes a retroaldol C1-C2' cleavage reaction catalyzed by a third enzyme, Ltp2, producing androst-4-ene-3,17-dione (AD) (compound XVIII) and liberating another propanoyl-CoA molecule (Thomas et al. 2011).

2.1.3 Central and Lower Cholesterol Degradation Pathways

Thereafter, the catabolism of cholesterol in most aerobic bacteria appears to proceed through a common catabolic pathway for C-19 steroids (Fig. 3). The first steroid intermediate in this route, named androstenedione (4-androstene-3,17-dione; AD), has been postulated to be the result of cholesterol side-chain degradation. The enzymatic reactions of the 9,10-seco pathway that metabolize AD are described below.

First, a 3-ketosteroid-Δ1-dehydrogenase of low specificity as KsdD in *M. smegmatis* (Brzostek et al. 2005), KstD in *Rhodococcus erythropolis* (van der Geize et al. 2000, 2001, 2002a) and *M. tuberculosis* (Brzostek et al. 2009), or TesH in *Comamonas testosteroni* (Horinouchi et al. 2003a) transforms the 4-androstadiene-3,17-dione (AD) into 1,4-androstadiene-3,17-dione (ADD). Then, a 9α-hydroxylation catalyzed by a 3-ketosteroid 9α-hydroxylase, KstH in *M. smegmatis* (Andor et al. 2006) and KshAB in *R. erythropolis* (van der Geize et al. 2002b; 2008) and in *M. tuberculosis* (Capyk et al. 2009), is followed by the nonenzymatic transformation of the 9α-hydroxy-1,4-androstadiene-3,17-dione into the 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (3-HSA). The subsequent hydroxylation of 3-HSA by a two-component oxygenase (TesA1A2 in *C. testosteroni* (Horinouchi et al. 2004), HsaAB in *R. jostii* RHA1 and

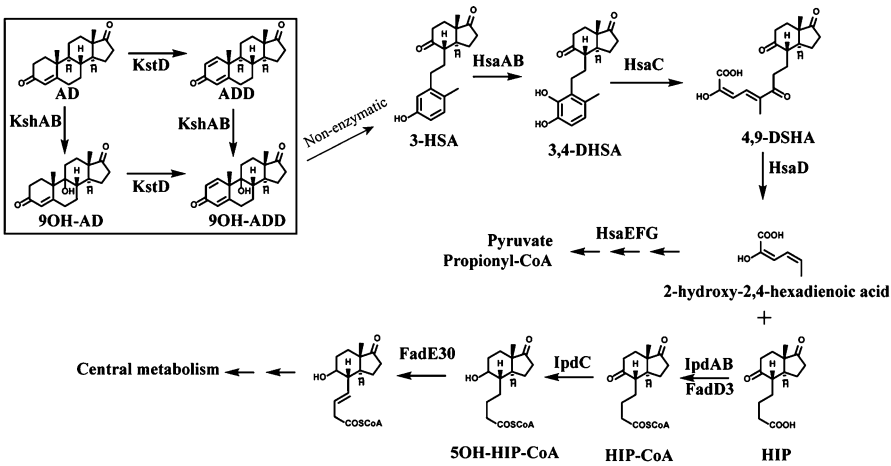

Fig. 3 Central and lower pathways for cholesterol metabolism in *Actinobacteria*. The enzymes accounting for the opening of steroid ring B are 3-ketosteroid-9α-hydroxylase (*KshAB*) and 3-ketosteroid-1-dehydrogenase (*KstD*)

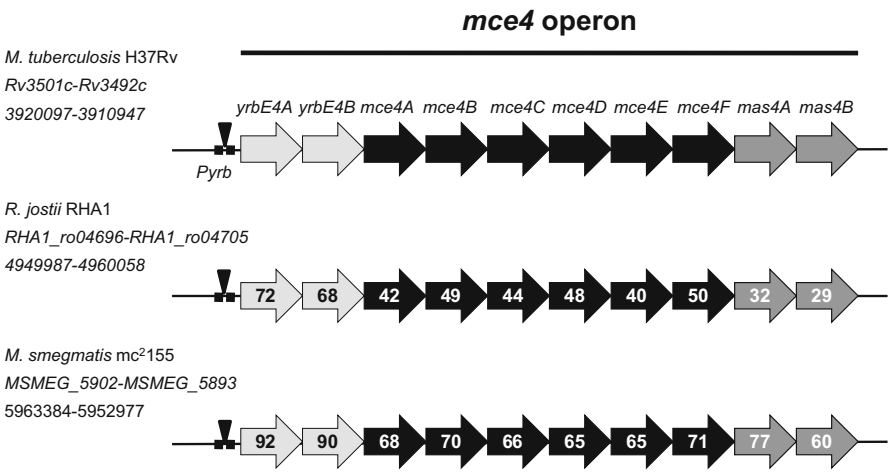

Fig. 4 Schematic representation of the genomic region corresponding to the Mce4 operon in *M. tuberculosis* H37Rv, *R. jostii* RHAI, and *M. smegmatis* mc^{2}155. The percentage of identity in amino acids of each gene with respect to the ones in *M. tuberculosis* H37Rv is shown. The triangle located in the *Pyrb* area represents the operator sequence recognized for the KstR repressor, and the squares represent the putative −10 and −35 boxes

M. tuberculosis (Dresen et al. 2010), leads to 3,4-dihydroxy-9,10-secoandrosta-1,3,5 (10)-triene-9,17-dione (3,4-DHSA), a catecholic derivative, which is then opened by *meta*-cleavage by an extradiol dioxygenase (TesB in *C. testosteroni* (Horinouchi et al. 2001), HsaC in *R. jostii* RHA1 (van der Geize et al. 2007) and in *M. tuberculosis* (Yam et al. 2009)) yielding 4,5,9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10), 2-diene-4-oic acid (4,9-DSHA). This compound is hydrolyzed by TesD in *C. testosteroni* (Horinouchi et al. 2003a) and HsaD in *M. tuberculosis* (Lack et al. 2008, 2010) or *R. jostii* RHA1 (van der Geize et al. 2007) to 2-hydroxyhexa-2, 4-dienoic acid and 3aα-H-4α(3′-propanoate)-7a-β-methylhexahydro-1,5-indanedione (HIP). The catabolism of the 2-hydroxyhexa-2,4-dienoic acid in *Actinobacteria* most probably involves catabolic genes similar to the *tesE*, *tesF*, and *tesG* genes of *C. testosteroni* (Horinouchi et al. 2005), leading to metabolites that finally enter the central pathways (Kieslich 1985). Briefly, the 2-hydroxyhexa-2,4-dienoic acid can be transformed by a TesE-like hydratase to 4-hydroxy-2-oxohexanoic acid that can be metabolized into pyruvic acid and propionaldehyde by the action of a TesG-like aldolase. The propionaldehyde will be later transformed into propionic acid by the action of a TesF-like aldehyde dehydrogenase (Horinouchi et al. 2005).

The catabolism of HIP has not been completely elucidated yet. In *R. jostii* RHA1, an acyl-CoA synthetase as FadD3 carries out the transformation of HIP to 3aα-H-4α (3′-propanoyl-CoA)-7aβ-methylhexahydro-1,5-indanedione (HIP-CoA) (Casabon et al. 2013). Other genes such as the *fadE30* and *ipdAB* genes encoding an acyl-CoA dehydrogenase and a heterodimeric CoA transferase, respectively, also appear to be involved in HIP degradation in *R. equi* (van der Geize et al. 2011).

In the recent years, several studies have demonstrated that some of the A/B ring-modifying enzymes have higher affinity for acyl-CoA intermediates that still hold at least three carbons of the cholesterol side chain (Capyk et al. 2011; Penfield et al. 2014). In addition, the interruption of the cholesterol catabolic pathway at the level of the 9α-hydroxylation and/or Δ1,2-dehydrogenation also leads to the accumulation of C-22 or C-24 intermediates as well as C-19 steroids (AD, ADD, and/or 9OH-AD) in different actinobacterial mutant strains (e.g., Marsheck et al. 1972; Donova et al. 2005b; Yeh et al. 2014; Xu et al. 2016; Galán et al. 2016). Moreover, the deletion of the *igr* locus encoding some side-chain-degrading enzymes in *M. tuberculosis* yielded a mutant that accumulates a HIP derivative containing a partially degraded side chain (Thomas et al. 2011). All these facts strongly suggest that the modifications of the A/B rings can occur simultaneously with side-chain degradation in cholesterol catabolism, so the postulated pathway described above somehow might have to be reformulated.

2.1.4 Anaerobic Degradation

The oxic degradation pathways of cholesterol are relatively well characterized; however, much less is known about the anoxic degradation on this compound (Ismail and Chiang 2011). The best studied anoxic reactions so far involve the incomplete transformation of cholesterol, in which the double bond in cholesterol is reduced by intestinal bacteria to form coprostanol (Li et al. 1995; Freier et al. 1994). However, to our knowledge, none of these bacteria are capable to completely mineralize cholesterol or coprostanol.

So far, only two denitrifying bacterial strain members of the β-proteobacteria, 72Chol and *Sterolibacterium denitrificans*, have been described as capable to mineralize cholesterol to carbon dioxide under anoxic conditions, being *S. denitrificans* the current model for the study of the anaerobic metabolism of cholesterol (Harder and Probian 1997; Talera and Denner 2003). This bacterium can grow on cholesterol as sole carbon and energy source, both under oxic and under strictly anoxic conditions when nitrate is supplied as an electron acceptor (Talera and Denner 2003).

The anoxic biochemical pathway involves unprecedented hydroxylations that use water as an oxygen donor. This novel pathway can operate in the presence or absence of oxygen (Chiang et al. 2007, 2008a, b; Dermer and Fuchs 2012, Wang et al. 2013) and differs from the classical aerobic degradation pathway in some important steps. The first step is catalyzed by the bifunctional dehydrogenase AcmA that is similar to 3β-HSD enzymes playing a role in the aerobic pathway in *Actinobacteria* and therefore produces the oxidation of cholesterol to cholest-5-en-3-one followed by its isomerization to cholest-4-en-3-one (Chiang et al. 2008a). The second enzyme of the proposed pathway is the cholest-4-en-3-one-Δ1-dehydrogenase (AcmB) that catalyzes the oxidation of cholest-4-en-3-one to cholesta-1,4-dien-3-one (Chiang et al. 2008b). The subsequent substrate activation proceeds through C-25 hydroxylation in which the cholest-4-en-3-one or cholesta-1,4-dien-3-one is oxidized to 25-hydroxycholest-4-en-3-one and 25-hydroxycholesta-1,4-dien-3-one, respectively, by an oxygen-independent molybdoenzyme (Dermer and Fuchs 2012). These enzymes are heterotrimeric and membrane associated, and they use water as

source of the oxygen atom incorporated into the product and required an electron acceptor (Dermer and Fuchs 2012). Once the side chain is degraded, the resulting androgen intermediate is activated by adding water to the C1-C2 double bond (Wang et al. 2013). Finally, the cleavage of the core ring system of cholesterol starts at the A ring by a hydrolytic reaction (Wang et al. 2013, 2014).

2.2 The Steroid Uptake System

The importance of steroids and their transformation by microorganisms have stimulated a deep study of the mechanisms developed for their degradation during the last years. However, our knowledge about the selective transport of steroid in bacteria, one of the key elements in the process, is still limited.

This lack of knowledge is especially evident in Gram-negative bacteria, where the presence of an outer membrane impairs the passive diffusion of steroids (Plésiat and Nikaido 1992) and the lack of ATP in the periplasmic space (Wülfing and Plückthun 1994) excludes the possibility of finding active transporters in the outer membrane. One of the few available studies regarding the steroid uptake in Gram-negative bacteria was carried out by Mallonee and Hylemon (1996) who characterized the BaiG transporter involved in the biliar acid uptake in *Eubacterium* sp. strain VPI 12708. In the case of the most hydrophobic steroids as cholesterol, the only Gram-negative bacteria known to be able to degrade this compound are *S. denitrificans*, which seems to possess a FadL-like transport system able to specifically uptake different C-27 steroids into the periplasm (Lin et al. 2015).

The steroid uptake process in Gram-positive bacteria is better described, but mainly focused in *Actinobacteria*. Several studies carried out in this phylum suggest that different uptake mechanisms are employed for the most hydrophobic steroids as cholesterol versus the more hydrophilic ones as bile acids. In this sense, it has been described in *R. jostii* RHAI that porins appear essential for the uptake of bile acids by mycolic acid bacteria (Somalinga and Mohn 2013). On the contrary, Gram-positive bacteria proved to be able to degrade cholesterol as *M. tuberculosis* and *R. jostii* RHAI, and *M. smegmatis* possess an operon called *mce4* that encodes a complex ABC system responsible for its uptake into the cell (Pandey and Sassetti 2008; Mohn et al. 2008; Klepp et al. 2012). Genome sequence analysis revealed that this operon is exclusively found in *Actinobacteria* and contains ten different genes named *yrbE4ABmce4ABCDEFmas4AB*. The two first ones encode the permeases of the system, and the rest, of unknown function, are postulated to encode substrate-binding proteins (Casali and Riley 2007). Additionally, the ATPase activity of this ABC system is provided by the *mceG* gene encoding an Mkl-like enzyme that is located away from the *mce4* operon in *M. tuberculosis* and whose function is thought to be shared with other Mce systems present in the same cell (Joshi et al. 2006; Sassetti et al. 2012). The reason why these Mce systems require many more proteins than do classical ABC transporters remains unclear, but it has been suggested that these proteins might form a large complex necessary for the movement of high hydrophobic substrates across the complex cell wall of *Actinobacteria* (Song et al. 2008). The Mce4 system seems to be exclusively

involved in the uptake of steroid compounds with long side chains as cholesterol, while compounds having shorter polar side chains as androstenedione (AD) are transported through a Mce-independent mechanism (Mohn et al. 2008).

2.3 Transcriptional Regulation of Steroid Catabolism

It has been shown that cholesterol utilization in Mycobacteria is controlled by two TetR-type transcriptional repressors named KstR and KstR2 (Kendall et al. 2007, 2010; Uhia et al. 2011a, 2012). KstR is encoded by the *MSMEG_6042* gene in *M. smegmatis* and the *Rv3574* gene in *M. tuberculosis* and controls the expression of 83 catabolic genes (*kstR* regulon) responsible for activating the upper and central degradation pathway (cholesterol uptake system, β-oxidation of the cholesterol aliphatic side chain, and opening and removal of steroidal rings A and B) (Kendall et al. 2007; Uhia et al. 2011a, 2012). KstR2 is encoded by the *MSMEG_6009* gene in *M. smegmatis* and the *Rv3557* gene in *M. tuberculosis* and controls the expression of 15 cholesterol catabolic genes (*kstR2* regulon) responsible for the lower pathway that involves the steroid C and D ring degradation. Both KstR1 and KstR2 negatively regulate their own expression. The highest sequence similarity lies in their N-terminal DNA-binding domain, whereas their C-terminal ligand-binding domains are rather different suggesting that they respond to different effectors. García-Fernández et al. (2014) established the 3-oxocholest-4-en-26-oic (3OChA) as a ligand for *M. smegmatis* KstR, but more recently Ho et al. (2016) broaden the range of KstR1 effectors to cholesterol CoA-derivatives with four intact steroid rings (3OChA-CoA and 4-BCN-CoA). The KstR ligand free and in complex with these two CoA-metabolite crystal structures was determined allowing the identification of the residues involved in ligand specificity (Ho et al. 2016). Footprint analyses demonstrated that KstR specifically binds to the KstR-dependent promoter of the *MSMEG_5228* gene of *Mycobacterium smegmatis*, which encodes the 3-βHSD, to an operator region of 31 nt containing the quasi-palindromic sequence AACTGGAACGTGTTTCAGTT (Uhia et al. 2011a).

The DNA operator site of KstR2 was experimentally determined in *M. smegmatis* by García-Fernández et al. (2015), being a region of 29 nucleotides showing the palindromic sequence AAGCAAGNNCTTGCTT. Casabon et al. (2013) demonstrated experimentally that the inducer molecule of KstR2 is HIP-CoA. The crystal structure of KstR2 from *M. tuberculosis* has been determined in complex with HIP-CoA revealing that each one of the subunits of the KstR2 dimer accommodates one molecule of HIP-CoA (Crowe et al. 2015).

3 Bacterial Degradation of Other Steroids

Apart from natural sterols and their metabolic intermediates described above, bacteria can mineralize other steroids. In this sense, a bioinformatic analysis has identified 265 putative steroid degraders within *Actinobacteria* and *Proteobacteria* (Bergstrand et al. 2016).

One of the best studied steroid catabolic pathways is that involved in the aerobic degradation of testosterone (TES) that has been mainly described in *Comamonas testosteroni* by the group of Horinouchi et al. (2001, 2003a, b, 2004a, b, 2005, 2006, 2010) as well as by others groups (Oppermann and Maser 1996; Möbus and Maser 1998; Maser et al. 2001; Skowasch et al. 2002; Gong et al. 2012a, b; Ji et al. 2014; Yu et al. 2015; Zhang et al. 2015). The catabolism of TES is very similar to that of AD, and the enzymes involved in the metabolic steps are homologous to those described above for the degradation of sterols in *Actinobacteria*. The regulation of the genes involved in the degradation of steroids in *C. testosteroni* has been studied in some detail (Möbus et al. 1997; Cabrera et al. 2000; Xiong and Maser 2001; Xiong et al. 2001, 2003a, b, 2009; Pruneda-Paz et al. 2004a, b; Göhler et al. 2008; Linares et al. 2008; Gong et al. 2012b; Li et al. 2013; Pan et al. 2015; Wu et al. 2015). Remarkably, TES can be also degraded under anaerobic conditions by *Steroidobacter denitrificans* (Fahrbach et al. 2010; Chiang et al. 2010; Leu et al. 2011) and by *S. denitrificans* DSMZ 13999 (Wang et al. 2014).

Bile salts are very abundant in nature, and as expected they can be catabolized by many bacteria both Gram-positive (Mohn et al. 2012; Swain et al. 2012; Somalinga and Mohn 2013) and Gram-negative (Birkenmaier et al. 2007; Horinouchi et al. 2008; Rösch et al. 2008; Holert et al. 2013a, b, c, 2014, 2016; Merino et al. 2013; Barrientos et al. 2015; Chen et al. 2015; Philipp 2011; Philipp et al. 2006; Yücel et al. 2016). Bile salts are metabolized by pathways very similar to those used to degrade sterols. The regulation of these pathways has been scarcely studied and remains to be elucidated.

Steroidal endocrine disruptors such as 17β-estradiol, estrone, estriol, or ethinylestradiol are abundant in municipal wastewaters, and their biodegradation has been extensively studied for environmental reasons. A number of bacteria able to degrade these compounds have been isolated or studied in consortia (Fujii et al. 2002, 2003; Shi et al. 2004, 2010; Yoshimoto et al. 2004; Weber et al. 2005; Fahrbach et al. 2006; Ke et al. 2007; Yu et al. 2007; Pauwels et al. 2008; Zang et al. 2008, 2011, 2013; Klepp et al. 2010, 2015; Muller et al. 2010; Roh and Chu 2010; Jiang et al. 2010; Ribeiro et al. 2010; Hu et al. 2011; Isabelle et al. 2011; Li et al. 2012; Liang et al. 2012; Villemur et al. 2013; Chen et al. 2016; Ma et al. 2016). Although it is assumed that the catabolism of these compounds is similar to that of sterols, it has not been studied in depth.

4 Future Research Needs

Despite the large number of works that have been carried out, mainly in *Actinobacteria*, the characterization of the bacterial catabolism of sterols is still far from being completely understood. There are several steps that require further studies such as i) the degradation of the steroid side chain, ii) the last steps of the catabolic pathway controlled by the *kstR2* regulon, or iii) the sterol uptake systems. The redundancy of catabolic enzymes with similar functions and relaxed specificities present in the sterol-degrading pathways usually adds further complexity to analyze

the genes involved by using conventional genetic knockout approaches. This problem has been partially overcome by using omic techniques that have facilitated the analyses of the pathways at genomic scale. However, the implementation in *Actinobacteria* of modern high-throughput site-directed mutagenic techniques or multiple gene silencing tools using antisense RNA to allow the targeting of multiple genes/sites at the same time is still required. The metabolic knowledge is fundamental to rationally apply genetic engineering and systems biology tools for upgrading the steroid-transforming microorganisms currently used at industrial scale.

On the other hand, compared to the oxic metabolism of sterols, the anoxic catabolism has still been very poorly investigated. In the same sense, bacteria able to degrade steroidal endocrine disruptors (e.g., estradiol, estrone, etc.) have not been studied in depth, and the catabolic pathways for these molecules have not been precisely elucidated yet.

The metabolism of cholesterol and bile acids by gut microbiota has been extensively studied mainly using classical microbiological approaches. Modern omic techniques, particularly metagenomic analyses of these microbiomes, will allow the discovery of novel genes involved in steroid metabolism.

Although cholesterol has been reported to play an important role during active and latent infection of *M. tuberculosis*, there are still many molecular aspects of bacterial response to this substrate that are not fully understood.

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