Minireview

Catabolism and biotechnological applications of cholesterol degrading bacteria

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Summary

Cholesterol is a steroid commonly found in nature with a great relevance in biology, medicine and chemistry, playing an essential role as a structural component of animal cell membranes. The ubiquity of cholesterol in the environment has made it a reference biomarker for environmental pollution analysis and a common carbon source for different microorganisms, some of them being important pathogens such as Mycobacterium tuberculosis. This work revises the accumulated biochemical and genetic knowledge on the bacterial pathways that degrade or transform this molecule, given that the characterization of cholesterol metabolism would contribute not only to understand its role in tuberculosis but also to develop new biotechnological processes that use this and other related molecules as starting or target materials.

Introduction

Cholesterol is a steroid, i.e. a class of terpenoid lipids, with a carbon skeleton virtually flat and relatively rigid formed by four fused alicyclic rings (Fig. 1). This polycyclic sterol has a great importance in biology, medicine and chemistry, being one of the most common molecules in nature, as an essential structural component of animal cell membranes (Staytor and Bloch, 1965), although it has been also exceptionally found in some prokaryotes (Hayami et al., 1979).

The metabolism of cholesterol is critical in eukaryotes as precursor of vitamins, steroid hormones and bile acids (Miller, 1988; Björkhem and Eggertsen, 2001) (Fig. 1), but according to its physiological relevance, an imbalance in its blood level causes serious diseases in humans.

Cholesterol is frequently found in the biosphere, not only because of its natural abundance, but also due to its high resistance to microbial degradation. Cholesterol is a recalcitrant molecule to biodegradation because of its low number of functional groups (one CC double bond and a single hydroxyl group), its low solubility in water \( (3 \times 10^{-8} \text{ M}) \) and its complex spatial conformation constituted by four alicyclic rings and two quaternary carbon atoms. The high hydrophobicity and low volatility of cholesterol leads to a high absorption to solid phases. Because of their high rate of persistence, cholesterol and some derived compounds, such as coprostanol, have been used as reference biomarkers for environmental pollution analysis (Veiga et al., 2005).

Steroids, some of them derived from cholesterol, constitute a new class of pollutants discharged into the environment as a result of human activity (Gagné et al., 2006). The potent metabolic activities of these compounds affect a large number of cellular processes and thus, their presence and accumulation in water wastes and in certain ecological niches can affect the endocrine system of animals and humans (Hotchkiss et al., 2008). Moreover, cholesterol is the main component of lanolin, and this and other related sterols are natural contaminants fairly resistant to the anaerobic treatment that is carried out on the effluents from wool-processing industry (Poole and Cord-Ruwisch, 2004).

The ubiquity of cholesterol and related sterols in the environment has made them a common carbon source for many different microorganisms, some of them being important pathogens as Mycobacterium tuberculosis. On the other hand, the bacterial metabolism of steroids has attracted also considerable attention as low-cost natural steroids like cholesterol can be used as starting materials for many bioactive synthetic steroids (Fernandes et al., 2003). Therefore, the characterization of the bacterial metabolism of cholesterol could be useful not only to understand its influence in pathological processes like tuberculosis, but also to develop new organisms by metabolic engineering with potential use as biotechnological tools. In a recent work we reviewed the accumulated...
biochemical and genetic information on the bacterial pathways that degrade or transform biochemically different steroids (García et al., 2011). This review is mainly focused in cholesterol catabolism and its biotechnological applications including the development of cholesterol biosensors for clinical diagnosis and food industry, microbial steroid biotransformations for the production of steroid drugs and hormones, probiotics with cholesterol-lowering effects, and enzymes for insecticide and fungicide purposes.

### Catabolism of cholesterol

#### Historical issues

The search for microorganisms capable of degrading cholesterol was started over 70 years ago. In the early 20th century, it was observed that several species of Mycobacterium could use cholesterol as the sole source of carbon and energy (Söhngen, 1913; Tak, 1942). Later on, it was reported that microorganisms belonging to the genus Proactinomyces, like Rhodococcus erythropolis (formerly Nocardia erythropolis), have the ability to degrade cholesterol and a potential capacity for the synthesis of new steroid derivatives (Turfitt, 1944; 1948). Moreover, some species of Azotobacter transform cholesterol in cholest-4-en-3-one (cholestenone) or 7-dehydrocholesterol, and have the ability to hydrolyse the side-chain of cholesterol generating methylheptanone (Horvath and Kramli, 1947). Thereafter, other bacteria belonging to the genera Nocardia, Arthrobacter, Bacillus, Brevibacterium, Corynebacterium, Streptomyces, Microbacterium, Serratia, Achromobacter, Pseudomonas or Protaminobacter,
among others, were reported to accomplish partial or complete cholesterol degradation (Whitmarsh, 1964; Brown and Peterson, 1966; Arima et al., 1969; Chipley et al., 1975; Martin, 1977; Ferreira and Tracey, 1984; Drzyzga et al., 2009; 2011; Fernández de Las Heras et al., 2009; Ge et al., 2011). Nevertheless, in spite of the large number of described bacteria able to use cholesterol as a sole source of carbon and energy only the genomes of few of them have been completely sequenced (Table 1). When we analysed in the genome of these microorganisms the presence of some of the most representative gene loci involved in cholesterol metabolism [e.g. igr (side-chain degradation), has (central pathway) and mec (transport)], we have observed that some of them do not contain these genes. This suggests that although these bacteria could transform cholesterol they may not be able to completely mineralize this compound. The relevance of these genes in cholesterol catabolism has been also confirmed recently by high-density mutagenesis and deep-sequencing analysis in M. tuberculosis (Griffin et al., 2011). Finally, it is worth to mention that most of these genes are randomly located in different clusters in the bacterial chromosome with the exception of M. tuberculosis where it has been assumed that the catabolic predicted genes are mainly located in a single cluster (80 genes in region of about 50 kb) (Van der Geize et al., 2007). However, the recent work of Griffin and colleagues (2011) have shown that while the genes found to be required for growth in cholesterol were clustered in this region, the majority (60%) were distributed elsewhere in the chromosome.

### Aerobic degradation of cholesterol

Although the bacterial catabolism of cholesterol has not been fully elucidated in any of the bacterial strains degrading sterols, the metabolic pathway can be postulated by combining the biochemical and genetic studies carried out in different organisms (Martin, 1977; Schoemer and Martin, 1980; Owen et al., 1983; Kieslich, 1985; Sedlaczek and Smith, 1988; Szentirmai, 1990; Yam et al., 2010; García et al., 2011; Griffin et al., 2011; Ouellet et al., 2011) (Fig. 2). Experiments performed with 14C-radiolabelled cholesterol showed that carbon from the sterol ring system was preferentially converted to CO2 and used for energy generation via tricarboxylic acid cycle, whereas the side-chain is assimilated into the mycobacterial lipids (Cox et al., 1999). To facilitate the comprehension of the catabolic process it can be divided into four major steps as presented below.

### Transformation of cholesterol into cholestenone

It is generally assumed that the first reaction in the aerobic metabolism of cholesterol is its oxidation to cholestenone through two sequential reactions. That is, the oxidation of cholesterol to cholest-5-en-3-one [Fig. 2 (2)] followed by its isomerization to cholestenone [Fig. 2 (3)]. The enzymes responsible for this activity are cholesterol oxidases (ChOx), or 3β-hydroxysteroid dehydrogenase/isomerases (HSDs).

ChOx is a monomeric flavoenzyme that catalyses the oxidation and isomerization of cholesterol to cholestenone with a concomitant reduction of FAD. Regeneration of the oxidized cofactor is achieved by the reduction of O2 to hydrogen peroxide. The enzyme is extracellular and occurs as secreted and/or cell-surface-associated forms, depending on the microorganism and growth conditions (Kreit and Sampson, 2009). The ChOx from Streptomyces sp. SA-COO (Yue et al., 1999; Lario et al., 2003) and the BOC1 form of ChOx from Brevibacterium sterolicum (Vrielink et al., 1991; Li et al., 1993) have been classified as type I oxidases and belong to the glucose-methanol-choline (GMC) oxidoreductase family of flavoenzymes where the FAD cofactor is non-covalently bound. However, the BOC2 form of ChOx from B. sterolicum (Coulombe et al., 2001) has been classified as a type II oxidase belonging to the family of vanillyl-alcohol oxidase.

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### Table 1. Sequenced genomes of described organisms able to grow in cholesterol as a sole source of carbon and energy.

| Organismsa | GenBank | Chromosome (nt) | Igrr | Hasa | Mecb |
|------------|---------|----------------|------|------|------|
| Gordonia neofelisa | NZ_AEUD00000000 | 4 257 286 | Yes* | Yes | Yes |
| Mycobacterium avium | NC_008595 | 5 475 491 | Yes | Yes | Yes |
| Mycobacterium bovis | NC_008769 | 4 374 522 | Yes | Yes | Yes |
| Mycobacterium smegmatis | NC_008596 | 6 988 209 | Yes | Yes | Yes |
| Mycobacterium tuberculosis | NC_000962 | 4 411 532 | Yes | Yes | Yes |
| Rhodococcus equi | NC_014659 | 5 043 170 | Yes* | Yes | Yes |
| Rhodococcus erythropolys | NC_012490 | 6 516 310 | Yes* | Yes | Yes |
| Rhodococcus jostii | NC_008268 | 7 804 765 | Yes* | Yes | Yes |
| Streptomyces venezuelae | FR845719.1 | 8 226 158 | Yes* | No | Yes |
| Streptomyces viridochromogenes | NZ_ACEZ00000000 | 8 548 109 | Uncomplete* | No | Yes |

**a.** Only one representative organism has been included in the table.

**b.** The presence of the igr, has and mec genes has been analysed. The asterisk (*) means that the Cyp125 encoding gene is not present in the locus.
enzymes, which contain a covalently bound FAD molecule (Motteran et al., 2001). Both classes of ChOxs share the same catalytic activity but show significant differences in their redox and kinetic properties. In general, they exhibit a broad range of steroid specificities and can oxidize a number of hydroxysterols, including sterols, steroid hormones and bile acids, but the presence of a 3β-hydroxyl group is an important requirement for activity (Pollegioni et al., 1999). Remarkably, some ChOxs, like those from *Burkholderia cepacia* ST-200, *Pseudomonas* spp. and *Chromobacterium* sp. DS-1, oxidize cholesterol mainly to 3β-hydroperoxy-cholest-4-en-3-one and hydrogen peroxide instead of cholestenone (Doukyu and Aono, 1999; Doukyu et al., 2008; Doukyu, 2009) but this intermediate has not been associated so far to any cholesterol degradation pathway. Recently, a cholesterol inducible ChoX encoding gene (*choG*) has been identified in *Rhodococcus* sp. strain CECT3014 within a gene cluster involved in the metabolism of cholesterol (Fernández de Las Heras et al., 2011).

The 3β-hydroxy-Δ5-steroid HSD belongs to a family of enzymes that catalyse the conversion of 3β-hydroxy-Δ5-ene-steroids to 3-oxo-4-ene-steroids through consecutive oxidation and isomerization reactions, using NAD or NADP as cofactors. Gene database analysis revealed the existence of a protein superfamily that in addition to the bacterial HSDs includes mammalian 3β-hydroxysteroid dehydrogenases, plant dihydroflavonol reductases, bacterial UDP-galactose-4-epimerases and viral 3β-hydroxysteroid dehydrogenase (Baker and Blasco, 1992). All of them present a glycine-rich sequence (Gly-X-X-Gly-X-X-Gly) similar to the Rossman fold sequence and an atypical active site motif (Tyr-X-X-X-Lys). The NAD(P)-dependent HSD from *Nocardia* sp. has been well characterized and appears to be responsible for the transformation of cholesterol to cholestenone in this cholesterol degrader organism (Horinouchi et al., 1991). Recent genetic and biochemical studies have also demonstrated the direct involvement of a NAD-dependent HSD in mycobacterial cholesterol catabolism (Yang et al.,
Cholesterol side-chain degradation

It has been assumed that simultaneously and independently of the first oxidative process, the long aliphatic chain of cholesterol is removed via a process similar to the \( \beta \)-oxidation of fatty acids, leading to the formation of a steroid C22-oic acid intermediate with the concomitant release of two molecules of propionyl-CoA and one acetyl-CoA (Griffin et al., 2011). This process has been elucidated at the biochemical level in strains of Mycobacterium and Nocardia and proceeds differently for cholesterol and \( \beta \)-sitosterol (Si, 1968; Martin, 1976; Wilbrink et al., 2011).

Recent studies have pointed out that the Cyp125 cytochrome from Rhodococcus jostii RHA1 (ro04679 coding gene) and M. tuberculosis (Rv3545c coding gene) initiates the side-chain degradation of cholesterol (Capyk et al., 2009a; Rosloniec et al., 2009). This cytochrome belongs to the P450 family and catalyses the hydroxylation at carbon C-26 yielding 26-hydroxycholest-4-en-3-one. Cyp125 from M. tuberculosis CDC1551 was proved to be involved not only in the hydroxylation of cholesterol but also in its further oxidation to cholest-4-en-3-one-27 oic acid (Ouellet et al., 2010). The crystallographic structure of Cyp125 from M. tuberculosis has been recently determined (McLean et al., 2009).

Driscoll and colleagues (2010) and Johnston and colleagues (2010) have demonstrated that Cyp142 is a cytochrome that is able to replace the activity of Cyp125 in M. tuberculosis H37Rv, explaining why a Cyp125 deletion mutant of this strain is still able to grow using cholesterol as a carbon source. Furthermore, \( \Delta \)cyp125 mutant strains of Mycobacterium bovis and M. bovis BCG (Driscoll et al., 2010) and M. tuberculosis CDC1551 (Johnston et al., 2010) could not grow on cholesterol because they have a defective cyp142 gene. In addition, its crystal structure was very similar to that of Cyp125 (Driscoll et al., 2010). Although Griffin and colleagues (2011) have identified Cyp125 as the sole monoxygenase significantly required for growth on cholesterol the involvement of Cyp142 cannot be completely discarded.

After the formation of the carboxylic acid intermediate, the cholesterol side-chain is shortened by a \( \beta \)-oxidation-like process initiated by an ATP-dependent steroid/sterol CoA ligase catalysing the CoA activation of the C27 carboxylic acid intermediate (Si, 1968; Si et al., 1968). Subsequent cleavage of the side-chain to 17-ketosteroid takes place stepwise in three consecutive cycles and two molecules of propionyl-CoA and one molecule of acetyl-CoA are formed from the side-chain (Fig. 3). This process involves two initial steps of typical \( \beta \)-oxidation with the participation of one or two different sets of four enzymes, i.e. acyl-CoA dehydrogenases, enoyl-CoA-hydratases, hydroxyacyl-CoA dehydrogenases and thiolases. These steps will release one propionyl-CoA and one acetyl-CoA. The additional propionyl-CoA cannot be released by a conventional \( \beta \)-oxidation due to the presence of the cyclopentane ring D. Although the two following steps would proceed by the action of an acyl-CoA dehydrogenase and an enoyl-CoA-hydratase, as occurs in a typical \( \beta \)-oxidation, they will render a tertiary alcohol, which is not susceptible to dehydrogenation to form the keto group. Therefore, the last step will require the participation of a hydroxyacyl-CoA lyase similar to other CoA-lyases (e.g. malyl-CoA lyase, citramalyl-CoA lyase or 3-hydroxy-3-methylglutaryl-CoA lyase) that will render androst-4-ene-3,17-dione (AD) and propionyl-CoA. A similar mechanism has been proposed for the metabolism of cyclohexanecetate in Arthrobacter sp. CA1 (Ougham and Trudgill, 1982). A number of genes related to \( \beta \)-oxidation have been recently identified in M. tuberculosis by transposon mutagenesis and suggested to be involved in cholesterol side-chain degradation (Griffin et al., 2011). Some of these genes were located inside the large cholesterol degradation gene cluster (hsd4A, itp2, fadE29, fadE28, fadA5, fadE30, fadE32, fadE33, fadE34 and hsd4B), but others were found outside of the cluster (fadE5, echA9, fadD36 and fadE25).

Our knowledge of genes involved in microbial degradation of cholesterol side-chain is still very limited, but a catabolic gene cluster was identified in R. jostii RHA1 and Mycobacterium smegmatis, encoding several enzymes predicted to be involved in the \( \beta \)-oxidation process (van der Geize et al., 2007; Uhía et al., 2012) (Fig. 4). Recently, the fadD19 gene product of this cluster has been identified as a steroid-CoA ligase with an essential role in the degradation of C-24 branched-chain-sterols (Wilbrink et al., 2011). In addition, the fadA5 (Rv3546) gene from M. tuberculosis H37Rv has been proposed to encode a \( \beta \)-ketoacyl-CoA thiolase that functions in cholesterol side-chain \( \beta \)-oxidation (Nesbitt et al., 2010). This gene is upregulated by cholesterol and repressed by KstR (see below), and is required for utilization of cholesterol as a sole carbon source in vitro and for full virulence of M. tuberculosis in the chronic stage of mouse lung infection (Nesbitt et al., 2010).

It is worth to mention that cholesterol catabolism would follow an obligatory order for side-chain and ring structure modifications depending of the microorganism. In this sense, while the \( \Delta \)cyp125 mutants of BCG (Bacillus Calmette-Guerin, an inactivated form of M. bovis) and Rhodococcus rhodochrous RG32 transformed cholesterol to cholestenone, the corresponding mutant of R. jostii...
RHA1 did not (Capyk et al., 2009a). Furthermore, ring degradation cannot proceed with substrates having a full-length aliphatic side-chain in the BCG mutant as it is not able to grow or further transform cholestenone, while *R. jostii* RHA1 mutant grows in this compound (Capyk et al., 2009a).

The combination of these two first enzymatic steps, i.e. the oxidation of cholesterol together with the degradation of its lateral chain, renders the central intermediate AD [Fig. 2 (5A)] that is further metabolized by the central catabolic pathway.

**Central catabolic pathway**

Once the aliphatic chain has been oxidized, cholesterol catabolism appears to follow the common pathway described for C19 steroids. Briefly, 3-ketosteroid-Δ^{1}\text{-} dehydrogenases, as KsdD in *M. smegmatis* (Brzostek

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**Fig. 3.** Proposed β-oxidation-like reactions for cholesterol side-chain degradation. The Fad proteins have been assigned according to the nomenclature of the *E. coli* genes involved in the β-oxidation of fatty acids. LiuE is the name assigned to 3-hydroxy-3-methylglutaryl-coenzyme A lyases.
et al., 2005), KstD in \textit{R. erythropolis} (van der Geize et al., 2000; 2001; 2002a) and \textit{M. tuberculosis} (Knol et al., 2008) that are equivalent to TesH of \textit{Comamonas testosteroni} (Horinouchi et al., 2003a) transform AD into androsta-1,4-diene-3,17-dione (ADD) \cite[Fig. 2 (5B) and Fig. 4\text{].} Later on, \(9\alpha\)-hydroxylation takes place catalysed by KshAB in \textit{R. erythropolis} \cite{van der Geize et al., 2001; 2002b; 2008a}, KshAB (Rv3526/Rv3571) in \textit{M. tuberculosis} \textit{H37Rv} \cite{Capyk et al., 2009b}, and KstH (KshA-like) in \textit{M. smegmatis} \cite[Fig. 4\text{]} (Andor et al., 2006), followed by a non-enzymatic transformation of the \(9\alpha\)-hydroxyandrosta-1,4-diene-3,17-dione \cite[Fig. 2 (6B)\text{]} to 3-hydroxy-9,10-secoandrosta-1,3,5(10)-trien-9,17-dione \cite[Fig. 2 (7)\text{]} \text{(3-HSA)} has been recently elucidated revealing that it is a trimmer \cite{Capyk et al., 2009b}. Five kshA homologues have been identified in \textit{R. rhodochrous} DSM43269 showing different substrate specificity \cite{Petrusma et al., 2011}.

Subsequently 3-HSA is hydroxylated by enzymes similar to the two-component oxygenase TesA1A2 from \textit{C. testosteroni} \cite{Horinouchi et al., 2004} leading to the production of 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-trien-9,17-dione (3,4-DHSA) \cite[Fig. 2 (8)\text{]} \text{\textit{Mycobacterium tuberculosis} and \textit{R. jostii} RHA1 have a similar enzyme named HsaAB \cite{Dresen et al., 2010}. The crystal structure of HsaA from \textit{M. tuberculosis} revealed that the enzyme is very similar to \(p\)-hydroxyphenylacetate hydroxylase \cite{Dresen et al., 2010}. The last catechol derivative is cleaved by a \textit{meta} extradiol dioxygenase similar to TesB in \textit{C. testosteroni} \cite{Horinouchi et al., 2001}, which was named HsaC in \textit{R. jostii} RHA1 \cite{van der Geize et al., 2007} or \textit{M. tuberculosis} \cite{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) \cite[Fig. 2 (9)\text{]} \text{. This compound is then hydrolysed by enzymes similar to TesD from \textit{C. testosteroni} \cite{Horinouchi et al., 2003b} named HsaD in \textit{M. tuberculosis} \textit{H37Rv} \cite{Lack et al., 2008; 2010} or \textit{R. jostii} RHA1 \cite{van der Geize et al., 2007} yielding 2-hydroxyhexa-2,4-dienoic \text{and DOHNAA acids} \cite[Fig. 2 (10)\text{]} [and 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic (DOHNAA) acids \cite[Fig. 2 (11)\text{]} .}

\textbf{Lower catabolic pathway}

The lower metabolic steps of the cholesterol catabolic pathway should degrade the 2-hydroxyhexa-2,4-dienoic and DOHNAA acids leading to metabolites able to enter
the central metabolic pathways (Kieslich, 1985), but the enzymes involved in this downward pathway have not been yet investigated in detail.

Most likely the catabolism of the 2-hydroxyhexa-2,4-dienoic acid involves genes similar to the tesE, tesF and tesG testosterone catabolic genes from C. testosteroni (Horinouchi et al., 2005). In this sense, the 2-hydroxyhexa-2,4-dienoic acid can be metabolized to 4-hydroxy-2-oxohexanoic acid by the TesE-like hydratase [Fig. 2 (12)] that eventually will be transformed by the action of TesG-like aldolase into pyruvic acid and propionylaldehyde that is further transformed into propionic acid by the action of TesF-like aldehyde dehydrogenase (Horinouchi et al., 2005). Although it has been suggested that TesF could render propionyl-CoA, the similarity of TesF with CoA acylating aldehyde dehydrogenases is very low and therefore, the most probably end-product should be propionic acid.

DOHNAA would be further transformed to succinic acid and it is assumed that the first step in the degradation of this compound could be the elimination of a propionyl moiety, to produce 9,17-dioxo-1,2,3,4,5,6,10,19-octanorandrostan-7-0ic acid [Fig. 2 (13)] through a typical \(\beta\)-oxidation (Kieslich, 1985). This reaction is carried out in two steps, i.e. the activation of DOHNAA by ATP and coenzyme A, followed by a reduction of DOHNAA-CoA by an NADPH-dependent dehydrogenase (Miclo and Germain, 1990). The enzymes specifically involved in these catabolic steps have not been precisely identified nor characterized so far.

Anoxic metabolism of cholesterol

Taylor and colleagues (1981) showed for the first time that bacteria were capable of degrading cholesterol under denitrifying conditions. The most frequent transformation of cholesterol in anoxic habitats is a reduction of its double bond yielding coprostanol (Li et al., 1995; Harder and Probian, 1997). Many intestinal fermenting bacteria are able to catalyse this reaction (Groh et al., 1993; Freier et al., 1994), and the reductase responsible for this reaction has been characterized in Eubacterium coprostanoligenes (Li et al., 1995).

A denitrifying \(\beta\)-proteobacterium, strain 72Chol, was the first bacterium isolated and cultured on cholesterol under strictly anoxic conditions able to mineralize cholesterol to carbon dioxide (Harder and Probian, 1997; Hylemon and Harder, 1998). However, the anoxic metabolism of cholesterol has been mainly studied at the biochemical level in the denitrifying \(\beta\)-proteobacterium Sterolobacter denitrificans, now used as a model organism (Tarlera and Denner, 2003). Interestingly, this bacterium was also able to catabolize anaerobically testosterone (Chiang et al., 2010).

The first steps of the anoxic catabolism of cholesterol are similar to those described for the aerobic process and thus, first the hydroxyl group at C-3 is oxidized into the keto group leading to cholest-5-en-3-one followed by a subsequent isomerization to yield cholestene (Chiang et al., 2007). These two reactions are catalysed by the bifunctional enzyme AcmA that belongs to the short-chain dehydrogenase reductase (SDR) superfamily (Chiang et al., 2008a). The oxidation of cholestene to cholest-1,4-diene-3-one, is catalysed by AcmB, a cholest-4-en-3-one-1,4-dihydrogenase (Chiang et al., 2008a,b). The enzyme is very similar to the flavin adenine dinucleotide-dependent 3-ketosteroid-1,4-dihydrogenase from Nocardia corallina (Itagaki et al., 1990).

In contrast with the aerobic pathway where cholestene is hydroxylated by a monoxygenase at C-27 to produce the corresponding primary alcohol, the anaerobic hydroxylation of the cholesterol side-chain is oxygen-independent (Chiang et al., 2007). This step is catalysed by an enzyme belonging to the molybdenum-containing hydroxylase family and involves the hydroxylation of C-25 of cholestene or cholest-1,4-diene-3-one using water as a source of the oxygen atoms (Hille, 2005).

The cholesterol uptake system

Cholesterol as other steroids does not appear to be able to diffuse to the bacterial cytoplasm and needs to be transported by specific uptake systems before being metabolized. A pioneering work in steroid uptake systems was carried by Watanabe and Po (1974) demonstrating the presence of an inducible transport system for testosterone in Pseudomonas testosteroni.

Mce-family proteins are closely involved in the invasion and prolonged existence of M. tuberculosis in host macrophages. The first mce gene to be discovered was found to promote the uptake of bacteria into nonphagocytic cells and was thus designated to function in mycobacterial cell entry (‘mce’) (Arruda et al., 1993). The genome of M. tuberculosis contains four mce operons (mce1-4) each including 9–13 genes with a similar arrangement encoding two transmembrane proteins with homology to the permease subunits of ABC transporters, along with several putative secreted or cell-surface proteins (Pandey and Sassetti, 2008). Recently, it was reported that the mycobacterial gene cluster mce4 encodes a cholesterol import system that enables M. tuberculosis to derive both carbon and energy from this component of host membranes (Pandey and Sassetti, 2008). The energy for the substrate translocation by all four Mce systems in M. tuberculosis is generated by a common ATPase, named MceG, belonging to the Mle family (Joshi et al., 2006). Interestingly, the mceG gene is located far away from the four mce operons in M. tuberculosis (Mohn et al., 2008). Genome sequence analyses revealed that diverse actinobacteria, including members
of the Mycobacterium, Nocardia, Rhodococcus, and Streptomyces genera, harbour mce transporter operons involved in steroid uptake (Casali and Riley, 2007; Garcia et al., 2011). The reason why these Mce systems require many more proteins than classical ABC transporters remains unclear. It has been suggested that these proteins might form a large complex necessary for the movement of the substrate across cell walls (Mohn et al., 2008).

The mce4 locus also encodes a system involved in steroid uptake in R. jostii RHA1 (Mohn et al., 2008) and in Rhodococcus equi (van der Geize et al., 2008b). The mce4 locus of R. jostii RHA1 is upregulated by cholesterol, and mutants with deletions in this operon lost their ability to grow in this compound (van der Geize et al., 2007). A deletion of supAB genes, which form part of the mce4 operon in R. equi, confirmed that these genes are essential for growth of this organism on cholesterol (van der Geize et al., 2008b). Therefore, up to now, the mce4 locus has been associated with steroid or cholesterol metabolism in all annotated bacterial genomes.

On the other hand, the Mce4 uptake system is also essential for growth of R. jostii RHA1 in cholesterol related compounds such as 5α-cholesterol, 5α-cholestanone and β-sitosterol (Mohn et al., 2008). Nevertheless, mutants of this uptake system were unaffected for growth on steroid compounds having shorter polar side-chains (Mohn et al., 2008). These data indicate that only side-chains of at least eight carbons are recognized by Mce4 steroid uptake system.

Regulation of the cholesterol catabolism

From the very beginning it was recognized that expressions of several genes/enzymes involved in steroid degradation were induced by their respective steroid substrates (Marcus and Talalay, 1956; Möbus et al., 1997; Skowasch et al., 2002), but very few data are still available upon the regulation of genes involved in cholesterol degradation. Kendall and colleagues (2007) have recently identified a repressor, named KstR, belonging to the TetR family of transcriptional regulators, which is involved in the regulation of 83 genes in M. smegmatis related with cholesterol and lipid metabolism (Fig. 4). The operator motif of the KstR dimer was predicted in silico as a 14 bp conserved sequence (TnnAACnnGTnnA, where n is any nucleotide) located in the upstream region of the KstR-regulated genes (Kendall et al., 2007; Uhía et al., 2011b). The KstR-dependent promoter of the MSMEG_5228 gene of M. smegmatis, which encodes an HSD responsible for the first step in the cholesterol degradative pathway, has been characterized in more detail (Uhía et al., 2011a). DNaSeI footprint assays have experimentally demonstrated that KstR specifically binds to an operator region of 31 nucleotides containing the predicted palindromic sequence AACTGgAACgtGTTtCAGTT overlapping the −10 and −35 boxes of the P5228 promoter, suggesting that KstR represses the MSMEG_5228 transcription by preventing the binding of RNA polymerase. The repression mediated by KstR is alleviated by the binding of an inducer molecule that still remains unknown.

Using the microarray data from M. smegmatis, a KstR regulon was also deduced for the human pathogen M. tuberculosis due the high degree of sequence similarity. In this bacterium, KstR is involved in the transcriptional repression of 74 genes (Kendall et al., 2007). The KstR regulon in M. tuberculosis contains a large number of genes that have been found to be essential for the survival of this pathogen in both macrophages and mice (Schnappinger et al., 2003). The 3D-structure of KstR from M. tuberculosis has been already determined (PDB ID: 3MNL).

More recently, another transcriptional repressor has been described as KstR2, which controls the expression of other additional 15 genes related to the lower cholesterol catabolic pathway in M. tuberculosis and M. smegmatis, suggesting that this complete pathway is controlled by at least two regulatory proteins (Fig. 4) (Kendall et al., 2010).

The transcriptome of M. smegmatis growing in cholesterol has been compared with that of cells growing in glycerol as the sole carbon and energy sources (Uhía et al., 2012). The results supported the role of KstR and KstR2 as autoregulated repressors of cholesterol catabolism. Microarray analyses revealed that 89 genes were upregulated at least threefold during growth on cholesterol. These upregulated genes are scattered throughout the M. smegmatis genome and likely reflect a general physiological adaptation of the bacterium to grow on this highly hydrophobic polycyclic compound. The catabolic genes are mainly organized in three large clusters that are putatively controlled by KstR and KstR2. Most of the cholesterol upregulated genes within these clusters appear to be responsible for the uptake of cholesterol, the β-oxidation of the branched side-chain at C17, and the catabolism of the rings to central metabolites. However, the transcriptome analysis also revealed the presence of other cholesterol upregulated genes that were not clustered. Within these genes, some are putatively controlled by the KstR repressor whereas others do not contain binding motifs for KstR or KstR2 in their promoter regions. This finding suggests the putative existence of additional regulatory systems different to KstR and KstR2.

The KstR binding motif identified in mycobacteria is also present in the genome of R. RHA1 (van der Geize et al., 2007; Kendall et al., 2007). Moreover, the kstR and kstR2 orthologues in R. jostii RHA1, i.e. ro04482 and ro04598, respectively, are induced by cholesterol (van der Geize
et al., 2007). In R. jostii RHA1 several cholesterol-induced genes do not seem to be regulated by KstR or KstR2, which could also indicate the presence of additional regulators of the catabolic pathway (Uhía et al., 2012).

Pathogenic implications of cholesterol metabolism in mycobacteria

In contrast to previous thoughts (Av-Gay and Sobouti, 2000), it has been recently demonstrated that M. tuberculosis can grow using cholesterol as a sole carbon and energy source (Pandey and Sassetti, 2008). In this sense, cholesterol has recently been identified as an important lipid for mycobacterial infection (de Chastellier and Thilo, 2006; Ouellet et al., 2011). The relatively abundant cholesterol present in host cells is an important growth substrate for these bacteria in different infection stages (e.g. intracellular growth or intracellular persistence).

*Mycobacterium tuberculosis* growing in the human cells appears to obtain the energy from host lipids rather than carbohydrates, supported by the finding that numerous genes involved in lipid metabolism are upregulated during growth in the mammalian macrophage and the mouse lung (Dubnau et al., 2005). Some genes that are critical for survival of *M. tuberculosis* in macrophages are involved in cholesterol degradation (Rengarajan et al., 2005; van der Geize et al., 2007). The Mce4 transport system that, at stated above, is required for cholesterol import into bacterial cells, plays an important role in pathogenesis because a mutant lacking this transporter fails to persist in the lungs of chronically infected mice and cannot grow in (IFN-γ)-activated macrophages (Pandey and Sassetti, 2008). Furthermore, it has been proposed that the ability of Mce4 proteins to bind cholesterol could act as a signal allowing the pathogen to interact with the host (Mohn et al., 2008).

The igr locus, which was identified because it is necessary for intracellular growth of *M. tuberculosis* in macrophages and mice, is absolutely required for cholesterol metabolism, because igr-deficient bacteria cannot grow using cholesterol as a primary carbon source (Chang et al., 2007; Miner et al., 2009). This locus consists of a single operon containing genes for a putative cytochrome P450 (igrA), two acyl coenzyme A dehydrogenases (igrBC), a conserved hypothetical protein (igrD), a putative enoyl coenzyme A hydratase (igrE) and a lipid carrier protein (igrF) (Chang et al., 2007). A homologous operon is present in *R. jostii* RHA1 and its expression is shown to be induced during growth in cholesterol (van der Geize et al., 2007). The igr cluster is encompassing the mce4 genes, which encode the uptake system for cholesterol and related steroids (Mohn et al., 2008). Interestingly, the igrA gene is homologous to the *Rv3545* and *ro04679* genes from *M. tuberculosis* and *R. jostii* RHA1, respectively, and encode the Cyp125 cytochrome involved in cholesterol C27 (or C26) hydroxylation described above (Capyk et al., 2009a; Rosloniec et al., 2009).

*Mycobacterium tuberculosis* lacking the kshA and kshB genes, which encode the two-component system catalysing the NADH dependent 9α-hydroxylation of 3-ketosteroids (Petrusma et al., 2009), failed to use cholesterol and 4-androstene-3,17-dione as carbon sources and its growth and survival is attenuated in macrophages and mice (Hu et al., 2009).

On the other hand, ChOxs have been considered as potential pathogenic determinants. In this sense, the ChOX (ChoE) from *R. equi*, a primary pathogen of horses and an opportunistic pathogen of humans, has been described as the major membrane-damaging factor (Navas et al., 2001), although its role in pathogenesis is controversial (Pei et al., 2006). The ChOX (ChoD) from *M. tuberculosis* also appears to be important for the growth of the pathogen in peritoneal macrophages and lungs of mice (Brzostek et al., 2007). *Mycobacterium leprae*, an obligate intracellular pathogen that shows a dramatic reduction of functional genes when compared with *M. tuberculosis*, still conserves and expresses the choD orthologue (Marques et al., 2008) and although this pathogen cannot catabolize cholesterol, it is a necessary factor for growth in macrophages (Kato, 1978). Nevertheless, the relevance of ChoD in cholesterol metabolism has been recently questioned (Griffin et al., 2011; Uhía et al., 2011a).

Interestingly, a mutation in the HSD (Rv1106c) of *M. tuberculosis* that is required for growing in cholesterol does not reduce its growth in infected macrophages or guinea pigs suggesting that cholesterol is not a sole nutrition source *in vivo* (Yang et al., 2011), consistently with the idea that there are multiple lipids in the host that *M. tuberculosis* would be able to catabolize for surviving (de Carvalho et al., 2010).

As mentioned above, the FadA5 thiolase involved in the side-chain metabolism of cholesterol is required for full virulence of *M. tuberculosis* as a fadA5 mutant showed attenuated phenotype and at 8 weeks after infection, the number of cfu in the mouse lungs decreased about 10-fold compared with the level in the wild-type strain (Nesbitt et al., 2010).

Finally, the development of chemical inhibitors targeting cholesterol catabolic enzymes has been proposed as a novel route for designing new therapeutics to deal with the re-emergent pathogen *M. tuberculosis* (Ouellet et al., 2011).

Biotechnological applications of cholesterol degrading bacteria

Microorganisms capable of degrading and/or transforming cholesterol have been widely used for many years in...
biotechnological applications. These applications include the development of cholesterol biosensors for clinical diagnosis, microbial steroid biotransformations for the production of steroid drugs and hormones, probiotics with cholesterol-lowering effects, and enzymes for insecticide and fungicide purposes.

**Biosensors for cholesterol analysis in biological samples**

The increase of cardiovascular disorders mainly due to hypercholesterolemia make the quantification of cholesterol an important clinical target and therefore several biosensors have been developed for this purpose (Arya et al., 2008; Jubete et al., 2009; Solanki et al., 2011). Biosensors for cholesterol are also useful in the food industry to maintain quality control (Bragagnolo and Rodriguez-Amaya, 2002; Hwang et al., 2003; Jubete et al., 2009). Some of these cholesterol biosensors are based on cholesterol degrading enzymes (Table 2).

ChOxs are widely utilized for the enzymatic analyses of cholesterol. The most common source of ChOxs are from *Streptomyces hygroscopicus, B. sterolicum* and *Pseudomonas fluorescens* (Arya et al., 2008; Nien et al., 2009; Singh et al., 2009). Because 70% of cholesterol exists in ester form and 30% as free form in blood samples, cholesterol esterases are also included in biosensors together with ChOxs (Arya et al., 2008; Shih et al., 2009). In addition, horseradish peroxidase has been used in the fabrication of photometry based biosensors (Arya et al., 2008). This enzyme can be also coupled to 4-aminoantipyrine and phenol to determine the hydrogen peroxide released in the ChOx reaction, a method routinely used in clinical laboratories (Pollegioni et al., 2009). To avoid the interference of blood pigments in colorimetric assays some electrochemical methods based on oxygen consumption by ChOx reaction have been also developed (Cheillan et al., 1989; Pollegioni et al., 2009).

Microbial ChDHs, which catalyse the conversion of 3β-hydroxy-5-ene-steroids to 3-oxo-4-ene-steroids using NAD or NADP as cofactors, are also utilized in cholesterol biosensors (Ishige et al., 2009; Wallace-Davis et al., 2011).

Several authors have also shown that the P450scc cytochrome, an isoform of the P450 (CYP) cytochrome superfamily, which catalyses the side-chain cleavage of cholesterol to pregnenolone in the presence of molecular oxygen and NADPH-ferrihaemoprotein reductase (Carrara et al., 2008), can be utilized for determination of cholesterol with high sensitivity (Antonini et al., 2004; Paternolli et al., 2004; Shumyantseva et al., 2004; 2005). The highly specific conversion of steroid hormones by cytochromes makes feasible a specific substrate

| Sensing element (Transductor) | Range       | Reference                        |
|-------------------------------|-------------|----------------------------------|
| Cholesterol oxidase           | 25–400 mg dl⁻¹ | Dhand and colleagues (2007)      |
| Electrochemical               | 25–400 mg dl⁻¹ | Solanki and colleagues (2007a)   |
| Surface plasmon resonance     | 50–500 mg dl⁻¹ | Solanki and colleagues (2007b)   |
| Amperometric                  | 6–30 mg dl⁻¹  | Özer and colleagues (2007)       |
| Flow injection chronoamperometric | 0.1–50.0 μg dl⁻¹ | Umar and colleagues (2009)    |
| Voltammetry                   | 0.1–10.0 μg dl⁻¹ | Wisitsoraat and colleagues (2010) |
| Cholesterol oxidase + cholesterol esterase | 1200–3600 mg dl⁻¹ | Law and colleagues (1997)      |
| Colorimetric                  | 3–200 mg dl⁻¹  | Situmorang and colleagues (1999)  |
| Amperometric                  | 65–520 mg dl⁻¹ | Singh and colleagues (2004)     |
| Spectrophotometric            | 50–400 mg dl⁻¹ | Arya and colleagues (2007)      |
| Voltammetric                  | 1–6 mg dl⁻¹   | Aravamudhan and colleagues (2007) |
| Polarographic                 | 2–50 mg dl⁻¹  | Basu and colleagues (2007)      |
| Cholesterol oxidase + cholesterol esterase + horseradish peroxidase | 3–20 mg dl⁻¹ | Martin and colleagues (2003) |
| Acoustic                      | 100–400 mg dl⁻¹ | Singh and colleagues (2008)    |
| Amperometric                  | 0.1–65.0 mg dl⁻¹ | Salinas and colleagues (2006) |
| Electrochemical               | 0–300 mg dl⁻¹  | Ahmadalinezhad and Chen (2011)  |
| Cholesterol oxidase + horseradish peroxidase | 130–780 mg dl⁻¹ | Kumar and colleagues (2006)  |
| Amperometric                  | 33–233 mg dl⁻¹ | Ishige and colleagues (2009) |
| Electrochemical               | 65–500 mg dl⁻¹ | Wallace-Davis and colleagues (2011) |
| Cytochrome P450                | 7–50 mg dl⁻¹  | Paternolli and colleagues (2004) |
| Electrochemical               | 0.7–5.0 mg dl⁻¹ | Shumyantseva and colleagues (2005) |
determination in complex media by using CYP based sensors (Bistolasa et al., 2005)

Biotransformations

The synthesis of the complex steroid molecules requires very specific reactions, and the utilization of microorganisms should enable the performance of reactions of high regio- and stereo-selectivity. Moreover, the mild conditions required for bioconversions as well as the more ecological processes compared with chemical synthesis make the high yield biological production an interesting field to develop.

The importance of microbial biotransformations in the production of steroid drugs and hormones became a reality when the 11α-hydroxylation of progesterone by a Rhizopus species was patented in 1952 (Hogg, 1992). Since then, microbial reactions for the transformation of steroids have proliferated and specific transformation steps have been incorporated into numerous partial syntheses of steroids with therapeutic use and commercial value (Mahato and Garai, 1997). Although some of these bioconversions are well-established, increasing the efficiency of the existing processes as well as identifying new potentially useful bioconversions, are two current objectives of the steroid industry (Fernandes et al., 2003).

Microbial bioconversion has been focused mainly in steroid hydroxylation, Δ4-dehydrogenation and sterol side-chain cleavage that associated to chemical synthesis steps, have enhanced the large-scale production of natural and modified steroid analogues. The use of whole cells instead of enzymes is preferred as the production costs are lower and it is possible to perform multi-steps conversions with a single biocatalyst (Bortolini et al., 1997). The manufactured steroid compounds have a wide range of therapeutic applications as anti-inflammatory, immunosuppressive, diuretic, anabolic, contraceptive agents, breast and prostate cancer and anti-obesity agents, among others. The therapeutic action of steroid hormones has been traditionally associated to their binding to the respective intracellular receptors, which act as transcription factors in the regulation of gene expression. Some steroid molecules are also called neurosteroids due to their role as memory enhancers, inducers of endocrine response to stress, anxiolytic agents, anticonvulsants, antidepressives and neuroprotective effect (Fernandes et al., 2003).

Many different steroids have been used for biotransformations, but specifically cholesterol obtained from animal fats and oils, such as lard, beef tallow, milk fat or fish oil has been also employed as starting raw material. It has been biotransformed into different compounds such as 17-ketosterols, AD, 9α-OH-AD and ADD (Fernandes et al., 2003). Microorganisms capable of oxidizing cholesterol have a serious draw-back for steroid synthesis, as they do not only degrade the side-chain but usually also cause the complete undesirable fission of the steroid skeleton (Kieslich, 1980). Some of the microorganisms capable of cleavage of the side-chain of cholesterol and used by their potential interest as biocatalysts are R. coralline, Arthrobacter simplex, R. equi, Mycobacterium fortuitum, R. erythropilus, M. neoaurum or Micrococcus roseus (Liu and Lee, 1992; Shi et al., 1992; Ahmed and Johri, 1993; Ahmed et al., 1993; Lee et al., 1993; Smith et al., 1993; Srivastava and Patil, 1994; Liu et al., 1994a; Mahato and Garai, 1997; van der Geize and Dijkhuizen, 2004; Sripalakit et al., 2006; Molchanova et al., 2007). Mycobacterium sp. NRRL B-3805 (Liu et al., 1994b; Liu and Lo, 1997) and Lactobacillus bulgaricus (Kumar et al., 2001) have been used to produce testosterone from cholesterol using a single strain. Production of ADD from cholesterol using two-step microbial transformation has been reported (Lee et al., 1993). First, cholesterol is converted to cholestenone by the inducible ChOx of A. simplex, and second, cholestenone is transformed into ADD by Mycobacterium sp. (Mahato and Garai, 1997). Some of the microorganisms used to produce cholestenone from cholesterol are Mycobacterium sp. (Smith et al., 1993), R. equi (Ahmed and Johri, 1993), Escherichia (Panchishina, 1992), Nocardia rubra (Osipowicz et al., 1992), A. simplex (Liu et al., 1994a), R. erythropilus (Jadoun and Bar, 1993), R. equi (Myamato and Toyoda, 1994), Agrobacterium sp. M4 (Mahato and Garai, 1997; Yazdi et al., 2000).

Microbial transformation of steroids can also be an environmentally beneficial process. Dias and colleagues (2002) studied the composition of different industrial waste materials for their steroid/cholesterol content in order to find out if these industrial by-products were suitable as steroid sources for the microbiological production of AD and ADD. They were used as a substrate for microbial degradation by a Mycobacterium sp. strain and proved to be easily converted to AD and ADD. Thus, a profitable application of these wastes could be the production of sterol-rich fractions, reducing the pollution loads.

Probiotics

Probiotics are living microorganisms, which upon ingestion in certain numbers exert health benefits on the host beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Although many in vitro and in vivo studies have shown that the administration of probiotics reduces serum/plasma total cholesterol, LDL-cholesterol and triglycerides or increases HDL-cholesterol, their hypocholesterolemic effects remain controversial (Ooi and Liong, 2010). The species with cholesterol-lowering effects
Probiotics have been suggested to reduce cholesterol via various mechanisms that do not necessarily imply the transformation of cholesterol by the probiotic strains, e.g. enzymatic deconjugation of bile acids by bile-salt hydrolases of probiotics (Lambert et al., 2008), assimilation of cholesterol (Pereira and Gibson, 2002), co-precipitation of cholesterol with deconjugated bile (Liong and Shah, 2006), cholesterol binding to cell walls of probiotics (Liong and Shah, 2005), incorporation of cholesterol into the cellular membranes of probiotics during growth (Lye et al., 2010a), production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics (De Preter et al., 2007) and conversion into coprostanol (Lye et al., 2010b).

As stated above, the most frequent transformation of cholesterol in the intestinal anoxic habitats is a biogenic reduction of its double bond yielding coprostanol, which is directly excreted in faeces (Li et al., 1995; Harder and Probian, 1997). Many intestinal fermenting bacteria are able to catalyse this reaction (Groh et al., 1993; Freier et al., 1994) decreasing the amount of absorbed cholesterol and leading to a reduced concentration in the physiological cholesterol pool (Ooi and Liong, 2010). Lye and colleagues (2010b) evaluated the conversion of cholesterol to coprostanol by strains of lactobacilli such as L. acidophilus, L. bulgaricus and Lactobacillus casei detecting both intracellular and extracellular cholesterol reductases in these strains, indicating possible intracellular and extracellular conversions of cholesterol to coprostanol. Cholesterol reductase has been also directly administered to humans to convert cholesterol to coprostanol in the small intestines to reach a bloodstream cholesterol-lowering effect (Ooi and Liong, 2010).

One of the best-studied mechanisms proposed for the cholesterol-lowering effects of probiotics is the enzymatic deconjugation of bile acids by bile salt hydrolases (Begley et al., 2006). Bile, a water-soluble end-product of cholesterol in the liver that consists of cholesterol, phospholipids, conjugated bile acids, bile pigments and electrolytes, is released into the duodenum upon ingestion of food. When it is deconjugated by bile salt hydrolases-producing strains, bile acids are less soluble, being no absorbed by intestines and eliminated in the faeces. Cholesterol is then used to synthesise new bile acids as a homeostatic response, resulting in a hypocholesterolemic effect of probiotics (Begley et al., 2006; Ooi and Liong, 2010).

The cholesterol-lowering effects are also attributed to the ability of probiotic strains to bind cholesterol, a process that is growth-dependent and strain-specific. However, although growing cells remove more cholesterol than dead cells, the heat-killed cells can still remove cholesterol from media, indicating that some cholesterol is bound to the cellular surface (Usman and Hosono, 1999). During the probiotic growth, the presence of cholesterol increases the concentration of saturated and unsaturated fatty acids, leading to increased membrane strength and subsequently higher cellular resistance towards lysis (Lye et al., 2010a; Ooi and Liong, 2010).

**Microbial enzymes for insecticides and fungicides**

Besides the nutritional role of ChOx, other roles as biological weapons have been ascribed to these enzymes (Ghoshroy et al., 1997; Martin and Aparicio, 2009). Purcell and colleagues (1993) discovered a highly efficient protein that killed boll weevil (Anthonomus grandis Boheman) larvae in Streptomyces culture filtrates and identified the protein as ChOx. Purified enzyme was active against boll weevil larvae at a concentration comparable with the bioactivity of Bacillus thuringiensis proteins against other insect pests. The enzyme is involved in the lysis of the midgut epithelial cells of the larvae and also exhibits insecticidal activity against lepidopteran cotton insect pests, including tobacco budworm (Heliothis virescens), corn earworm (Helicoverpa zea) and pink bollworm (Pectinophora gossypiella) (Corbin et al., 2001). Recently, it was reported that Chromobacterium subtsugae has insecticidal properties (Martin et al., 2007). ChOx might be involved in this insecticidal activity because it was recently found that Chromobacterium strains produce this enzyme (Doukyu et al., 2008). Some insecticide proteins are vital for pest control strategies employing transgenic crops. The ChOx encoding gene has been expressed in tobacco cells (Corbin et al., 1994; Cho et al., 1995; Doukyu, 2009).

**Future prospects**

Despite the large number of works carried out during the last century, the bacterial catabolism of cholesterol and other steroids is still far for being completely understood. For instance, the enzymes involved in the degradation of the steroid side-chain as well as those responsible for the last steps of the catabolic pathway remain to be precisely established. Moreover, the physiological role played by bacterial ChOxs is still unclear. Very few data are available about the proteins responsible for the bacterial uptake of steroids and this field will require supplementary attention as the possibility to create by metabolic engineering heterologous recombinant bacteria able to transform steroids for biotechnological purposes will depend on the possibility of conferring these bacteria-specific steroid transport capacities. As stated above, only a limited number of regulatory proteins involved in the metabolism of cholesterol have been identified and

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partially characterized and the real inducers of these regulators remain to be elucidated as it has not been determined yet whether the cholesterol or their catabolic intermediates are their effectors. Taking into account that the availability of commercial pathway intermediates is very limited the identification of such putative inducers appears to be a difficult task.

Although cholesterol is equally disseminated both in oxic and anoxic environments only two bacterial strains were reported so far to mineralize cholesterol to carbon dioxide under anoxic conditions. Therefore, the anoxic catabolism of cholesterol remains challenging because compared with the oxic metabolism of cholesterol, the metabolism of this substrate in the absence of oxygen is still very poorly understood (Chiang et al., 2008a).

While powerful technological approaches based on modern omic technologies (genomics, metagenomics, transcriptomics, etc.) will be useful to solve many of the questions raised here, the complexity of the pathways and their intermediates still demand to carry out huge efforts by using conventional biochemical and genetic tools to be able to decipher all the steps and processes involved in steroid catabolism.

In spite of the fact that the increment of cardiovascular diseases makes the availability of cholesterol biosensors of outstanding interest, few have been successfully commercialized. Optimization of critical parameters, such as enzyme stabilization, quality control and instrumentation design is needed. Increased understanding of the immobilized bioreagents, improved techniques for immobilization and technological advances in the microelectronics will lead to the rapid development of more accurate cholesterol biosensors (Arya et al., 2008).

Expected developments in biological production of steroid compounds range from the identification of novel biocatalysts or the improvement of existing ones to the enhancement of the biotransformation processes. The application of genetic engineering and systems biology tools for the upgrading of steroid-transforming microorganisms; solubility improvement of substrates that are sparingly soluble in water; immobilization of enzymes or whole cells in a suitable matrix for repetitive economic utilization of enzymes and development of a continuous process for economic product recovery are some of the biotransformation fields of research.

Lamb and colleagues (1998) have proposed the existence of a sterol biosynthetic pathway in M. smegmatis, after ascertaining a significant number of homologies between the genome sequence of M. tuberculosis and the sterol biosynthetic enzymes of Saccharomyces cerevisiae. Therefore, the development of a sterol cell-factory in M. smegmatis based on the approach followed by Duport and colleagues (1998), who engineered six genes to construct a yeast strain that produces pregnenolone and progesterone from galactose, could be envisaged (Fernandes et al., 2003). The knowledge of microbial cholesterol catabolic pathways could lead to the introduction of several changes in probiotic organisms thus transforming them in strains capable of reducing cholesterol levels in foods and intestines. In this regard, Park and colleagues (2008) constructed a constitutive high-level expression vector for the genus Bifidobacterium and used it to express ChOx from Streptomyces coelicolor. In vivo studies of the cholesterol-lowering effects of this genetically modified organism as well as of the hypocholesterolemic properties of other modified strains that could be constructed in the future would be of outstanding interest.

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References

Ahmadalinezhad, A., and Chen, A. (2011) High-performance electrochemical biosensor for the detection of total cholesterol. Biosens Bioelectron 26: 4508–4513.

Ahmed, S., and Johri, B.N. (1993) Microbial transformation of steroids in organic media. Indian J Chem 32: 67–69.

Ahmed, S., Roy, P.K., and Basu, S.K. (1993) Cholesterol side-chain cleavage by immobilized cells of Rhodococcus equi DSM 89-133. Indian J Exp Biol 31: 319–322.

Andor, A., Jekkel, A., Hopwood, D.A., Jeanplong, F., Ilkoy, E., Konya, A., et al. (2006) Generation of useful insertionally blocked sterol degradation pathway mutants of fast-growing Mycobacteria and cloning, characterization, and expression of the terminal oxygenase of the 3-Ketosteroid 9α-Hydroxylase in Mycobacterium smegmatis mc2155. Appl Environ Microbiol 72: 6554–6559.

Antonini, M., Ghisellini, P., Paternolli, C., and Nicolini, C. (2004) Electrochemical study of the interaction between cytochrome P450scCK201E and cholesterol. Talanta 62: 945–950.

Aravamudhan, S., Ramgir, N.S., and Bhansali, S. (2007) Electrochemical biosensor for targeted detection in blood using aligned Au nanowires. Sens Actuators B 127: 29–35.

Arima, K., Nagasawa, M., Bae, M., and Tamura, G. (1969) Microbial transformation of sterols. Part I. Decomposition of cholesterol by microorganisms. Agric Biol Chem 33: 1636–1643.

Arruda, S., Bomfim, G., Knights, R., Huima-Byron, T., and Riley, L.W. (1993) Cloning of an M. tuberculosis DNA fragment associated with entry and survival inside cells. Science 261: 1454–1457.

Arya, S.K., Pandey, P., Singh, S.P., Datta, M., and Malhotra, B.D. (2007) Dithiobisuccinimidyl propionate self assembled monolayer based cholesterol biosensor. Analyst 132: 1005–1009.
Araya, S.K., Datta, M., and Malhotra, B.D. (2008) Recent advances in cholesterol biosensor. Biosens Bioelectron 23: 1083–1100.

Av-Gay, Y., and Sobouli, R. (2000) Cholesterol is accumulated by mycobacteria but its degradation is limited to non-pathogenic fast-growing mycobacteria. Can J Microbiol 46: 826–831.

Baker, M.E., and Blasco, R. (1992) Expansion of the mammalian 3β-hydroxysteroid dehydrogenase/plant dihydroflavon reductase superfamily to include a bacterial cholesterol dehydrogenase, a bacterial UDP-galactose-4-epimerase, and open reading frames in vaccinia virus and fish lymphocystis disease virus. FEBS Lett 301: 89–93.

Basu, A.K., Chattopadhyay, P., Roychoudhuri, U., and Chakraborty, R. (2007) Development of cholesterol biosensor based on immobilized cholesterol esterase and cholesterol oxidase on oxygen electrode for the determination of total cholesterol in food samples. Bioelectrochemistry 70: 375–379.

Begley, M., Hill, C., and Gahan, C.G.M. (2006) Bile salt hydrolase activity in probiotics. Appl Environ Microbiol 72: 1729–1738.

Bistolasa, N., Wollenbergera, U., Jungb, C., and Schellera, F.W. (2005) Cytochrome P450 biosensors: a review. Biosens Bioelectron 20: 2408–2423.

Björkhem, I., and Eggertsen, G. (2001) Genes involved in initial steps of bile acid synthesis. Curr Opin Lipidol 12: 97–103.

Bortolini, O., Medici, A., and Poli, S. (1997) Biotransformation of the steroid nucleus of bile acids. Steroids 62: 564–577.

Bragagnolo, N., and Rodriguez-Amaya, D.B. (2002) Simultaneous determination of total lip, cholesterol and fatty acids in meat and backfat of suckling and adult pigs. Food Chem 79: 255–260.

Brown, R.L., and Peterson, G.E. (1966) Cholesterol oxidation by soil Actinomycetes. J Gen Microbiol 45: 441–450.

Brzostek, A., Sliwiński, T., Rumijowska-Galewicz, A., Korycka-Machala, M., and Dziadek, J. (2005) Identification and targeted disruption of the gene encoding the main 3-ketosteroid dehydrogenase in Mycobacterium smegmatis. Microbiology 151: 2393–2402.

Brzostek, A., Dziadek, B., Rumijowska-Galewicz, A., Pawelczyk, J., and Dziadek, J. (2007) Cholesterol oxidase is required for virulence of Mycobacterium tuberculosis. FEMS Microbiol Lett 275: 106–112.

Capyk, J.K., Kalscheuer, R., Stewart, G.R., Liu, J., Kwon, H., Zhao, R., et al. (2009a) Mycobacterial cytochrome P450 125 (Cyp125) catalyzes the terminal hydroxylation of C27-steroids. J Biol Chem 284: 35534–35542.

Capyk, J.K., D’Angelo, I., Strynadka, N.C., and Ellis, L.D. (2009b) Characterization of 3 ketosteroid 19α-hydroxylase, a Rieske oxygenase in the cholesterol degradation pathway of Mycobacterium tuberculosis. J Biol Chem 284: 9937–9946.

Carrara, S., Shumyantseva, V.V., Archakov, A.I., and Samori, B. (2008) Screen-printed electrodes based on carbon nanotubes and cytochrome P450ssc for highly sensitive cholesterol biosensors. Biosens Bioelectron 24: 148–150.

de Carvalho, L.P.S., Fischer, S.M., Marrero, J., Nathan, C., Ehrt, S., and Rhee, K.Y. (2010) Metabolomics of Mycobacterium tuberculosis reveals compartmentalized co-catabolism of carbon substrates. Chem Biol 17: 1122–1131.

Casali, N., and Riley, L.W. (2007) A phylogenomic analysis of the Actinomycetales mce operons. BMC Genomics 8: 60.

Chang, J.C., Harik, N.S., Liao, R.P., and Sherman, D.R. (2007) Identification of mycobacterial genes that alter growth and pathology in macrophages and in mice. J Infect Dis 196: 786–795.

de Chastellier, C., and Thilo, L. (2006) Cholesterol depletion in Mycobacterium avium-infected macrophages overcomes the block in phagosome maturation and leads to the reversible sequestration of viable mycobacteria in phagolysosome-derived autophagic vacuoles. Cell Microbiol 8: 242–256.

Chellian, V., Lafont, H., Termine, E., Hamann, Y., and Lesgards, G. (1989) Comparative study of methods for measuring cholesterol in biological fluids. Lipids 24: 224–228.

Chiang, Y.R., Ismail, W., Müller, M., and Fuchs, G. (2007) Initial steps in the anoxic metabolism of cholesterol by the denitrifying Sterolibacter denitrificans. J Biol Chem 282: 13240–13249.

Chiang, Y.R., Ismail, W., Heintz, D., Schaeffer, C., van Dorselaer, A., and Fuchs, G. (2008a) Study of anoxic and oxic cholesterol metabolism by Sterolibacter denitrificans. J Bacteriol 190: 905–914.

Chiang, Y.R., Ismail, W., Gallien, S., Heintz, D., Van Dorselaer, A., and Fuchs, G. (2008b) Cholest-4-en-3-one-delta 1-dehydrogenase, a flavoprotein catalyzing the second step in anoxic cholesterol metabolism. Appl Environ Microbiol 74: 107–113.

Chiang, Y.R., Fang, J.Y., Ismail, W., and Wang, P.H. (2010) Initial steps in anoxic testosterone degradation by Steroidobacter denitrificans. Microbiology 156: 2253–2259.

Chipley, J.R., Dreyfuss, M.S., and Smucker, R.A. (1975) Cholesterol metabolism by Mycobacterium. Microbios 12: 199–207.

Cho, H.J., Choi, K.P., Yamashita, M., Morikawa, H., and Murooka, Y. (1995) Introduction and expression of the Streptomyces cholesterol oxidase gene (choA), a potent insecticidal protein active against boil weevil larvae, into tobacco cells. Appl Microbiol Biotechnol 44: 133–138.

Corbin, D., Greenplate, J., Wong, E., and Purcell, J. (1994) Cloning of an insecticidal cholesterol oxidase gene and its expression in bacteria and in plant protoplasts. Appl Environ Microbiol 60: 4239–4244.

Corbin, D.R., Grebenok, R.J., Ohnmeiss, T.E., Greenplate, J.T., and Purcell, J.P. (2001) Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. Plant Physiol 126: 1116–1128.

Coulombe, R., Yue, K.O., Ghisla, S., and Vrieling, A. (2001) Oxygen access to the active site of cholesterol oxidase through a narrow channel is gated by an Arg-Glu pair. J Biol Chem 276: 30435–30441.

Cox, J.S., Chen, B., McNeil, M., and Jacobs, W.R., Jr (1999) Complex lipid determines tissue-specific replication of Mycobacterium tuberculosis in mice. Nature 402: 79–83.

De Preter, V., Vanhoutte, T., Huys, G., Swings, J., De Vuyst, L., Rutgeerts, P., and Verbeke, K. (2007) Effects of Lacto-
bacillus casei Shirata, Bifidobacterium breve, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. Am J Physiol Gastrointest Liver Physiol 292: 358–368.

Dhand, C., Singh, S.P., Arya, S.K., Datta, M., and Malhotra, B.D. (2007) Cholesterol biosensor based on electrophoretically deposited conducting polymer film derived from nanostructured polyaniline colloidal suspension. Anal Chim Acta 602: 244–251.

Dias, A.C.P., Fernandes, P., Cabral, J.M.S., and Pinheiro, H.M. (2002) Isolation of a biodegradable sterol-rich fraction from industrial wastes. Bioresour Technol 82: 253–260.

Doukyu, N. (2009) Characteristics and biotechnological applications of microbial cholesterol oxidases. Appl Microbiol Biotechnol 83: 825–837.

Doukyu, N., and Aono, R. (1999) Two moles of O2 consumption and one mole of H2O2 formation during cholesterol catabolism.

Fernández de Las Heras, L., García Fernández, E., and Fernández, E., Navarro-Llorens, J.M., and Pinheiro, H.M., and D’Angelo, I., Tocheva, E.I., Strynadka, J.N. (2010) Structural and biochemical characterization of KshA and KshB, encoding two homologues of 3-ketosteroid 9α-hydroxylase, in Gordonia cholesterolivorans sp. nov., a cholesterol-reducing anaerobe. Int JYST Syst Bacteriol 60: 137–142.

Gagné, F., Blaise, C., and André, C. (2006) Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (Oncorhynchus mykiss) hepatocytes. Ecotox Environ Saf 64: 329–336.

García, J.L., Uhia, I., García, E., and Galán, B. (2011) Bacterial degradation of cholesterol and other contaminant steroids. In Microbial Bioremidiation of Nonmetals: Current Research. Koukkou, A.-I. (ed.). Ioannina, Greece: Caister Academic Press, pp. 23–43. ISBN: 978-1-904455-83-7.

Ge, F., Li, W., Chen, G., Liu, Y., Zhang, G., Yong, B., et al. (2011) Draft genome sequence of Gordonia neofellicaeis NRRL B-59395, a cholesterol-degrading actinomycete. J Bacteriol 193: 5045–5046.

van der Geize, R., and Dijkhuizen, L. (2004) Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. Curr Opin Microbiol 7: 255–261.

van der Geize, R., Hessels, G.I., van Gerwen, R., Vrijbloed, J.W., van der Meijden, P., and Dijkhuizen, L. (2000) Targeted disruption of the kstD gene encoding a 3-ketosteroid 9α-dehydrogenase isoenzyme of Rhodococcus erythropolis strain SQ1. Appl Environ Microbiol 66: 2029–2036.

van der Geize, R., Hessels, G.I., van Gerwen, R., van der Meijden, P., and Dijkhuizen, L. (2001) Unmarked gene deletion mutagenesis of kstD, encoding 3-ketosteroid 9α-dehydrogenase, in Rhodococcus erythropolis SQ1 using sacB as counter-selectable marker. FEMS Microbiol Lett 205: 197–202.

van der Geize, R., Hessels, G.I., and Dijkhuizen, L. (2002a) Molecular and functional characterization of the kstD2 gene of Rhodococcus erythropolis SQ1 encoding a second 3-ketosteroid 9α-dehydrogenase isoenzyme. Microbiology 148: 3285–3292.

van der Geize, R., Hessels, G.I., van Gerwen, R., van der Meijden, P., and Dijkhuizen, L. (2002b) Molecular and functional characterization of kshA and kshB, encoding two components of 3-ketosteroid 9α-hydroxylase, a class IA monoxygenase, in Rhodococcus erythropolis strain SQ1. Mol Microbiol 45: 1007–1018.

van der Geize, R., Yam, K., Heuser, T., Wilbrink, M.H., Hara, H., Anderton, M.C., et al. (2007) A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages. Proc Natl Acad Sci USA 104: 1947–1952.

van der Geize, R., Hessels, G.I., Nienhuis-Kuiper, M., and Dijkhuizen, L. (2008a) Characterization of a second Rhodococcus erythropolis SQ1 3-ketosteroid 9α-hydroxylase activity comprising a terminal oxygenase homologue, KshA2, active with oxygenase-catalysed component KshB. Appl Environ Microbiol 74: 7197–7203.
van der Geize, R., de Jong, W., Hessels, G.I., Grommen, A.W.F., Jacobs, A.A.C., and Dijkhuizen, L. (2008b) A novel method to generate unmarked gene deletions in the intracellular pathogen *Rhodococcus equi* using 5-fluorocytosine conditional lethality. *Nucleic Acids Res* 36: e151.

Ghoshroy, K.B., Zhu, W., and Sampson, N.S. (1997) Investigation of membrane disruption in the reaction catalyzed by cholesterol oxidase. *Biochemistry* 36: 6133–6140.

Griffin, J.E., Gawronski, J.D., Dejesus, M.A., Ioerger, T.R., Akerley, B.J., and Sassetti, C.M. (2011) High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. *PLoS Pathog* 7: e1002251.

Groh, H., Schade, K., and Horhold-Schubert, C. (1993) Steroid metabolism with intestinal microorganisms. *J Basic Microbiol* 33: 59–72.

Guamer, F., and Schaafsma, G. (1998) Probiotics. *Int J Food Microbiol* 39: 237–238.

Harder, J., and Probian, C. (1997) Anaerobic mineralization of cholesterol by a novel type of denitrifying bacterium. *Arch Microbiol* 167: 269–274.

Hayami, M., Okabe, A., Sacia, K., Hayashi, H., and Kane-masa, Y. (1979) Presence and synthesis of cholesterol in stable staphylococcal L-forms. *J Bacteriol* 140: 859–863.

Hille, R. (2005) Molybdenum-containing hydroxylases. *Arch Biochem Biophys* 433: 107–116.

Hogg, J.A. (1992) Steroids, the steroid community, and Upjohn in perspective: a profile of innovation. *Steroids* 57: 593–616.

Horinouchi, M., Yamamoto, T., Taguchi, H., Arai, H., and Kudo, T. (2001) Meta-cleavage enzyme gene tesB is necessary for testosterone degradation in *Comamonas testosteroni* TA441. *Microbiology* 147: 3367–3375.

Horinouchi, M., Hayashi, T., Yamamoto, T., and Kudo, T. (2003a) A new bacterial steroid degradation gene cluster in *Comamonas testosteroni* TA441 which consists of aromatic-compound degradation genes for seco-steroids and 3-ketosteroid dehydrogenase genes. *Appl Environ Microbiol* 69: 4421–4430.

Horinouchi, M., Hayashi, T., Koshino, H., Yamamoto, T., and Kudo, T. (2003b) Gene encoding the hydrolase for the product of the meta-cleavage reaction in testosterone degradation by *Comamonas testosteroni*. *Appl Environ Microbiol* 69: 2139–2152.

Horinouchi, M., Hayashi, T., and Kudo, T. (2004) The genes encoding the hydroxylase of 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione in steroid degradation in *Comamonas testosteroni* TA441. *J Steroid Biochem Mol Biol* 92: 143–154.

Horinouchi, M., Hayashi, T., Koshino, H., Kurita, T., and Kudo, T. (2005) Identification of 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-10-acid, 4-hydroxy-2-oxoheptane-2,4-dienoic acid and related enzymes involved in testosterone degradation in *Comamonas testosteroni* TA441. *Appl Environ Microbiol* 71: 5275–5281.

Horinouchi, S., Ishizuka, H., and Beppu, T. (1991) 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* 57: 1386–1393.

Horvath, J., and Krallmi, A. (1947) Microbiological oxidation of cholesterol with *Azotobacter*. *Nature* 160: 639.

Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., et al. (2008) Fifteen years after ‘wingspread’ environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. *Toxicol Sci* 105: 235–259.

Hu, Y., van der Geize, R., Besra, G.S., Gurcha, S.S., Liu, A., Rohde, M., et al. (2009) 3-ketosteroid 9α-hydroxylase is an essential factor in the pathogenesis of *Mycobacterium tuberculosis*. *Mol Microbiol* 75: 107–121.

Hwang, B.S., Wang, J.T., and Choong, Y.M. (2003) A simplified method for the quantification of total cholesterol in lipids using gas chromatography. *J Food Compost Anal* 16: 169–178.

Hyneman, P.B., and Harder, J. (1998) Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems. *FEMS Microbiol Rev* 22: 475–488.

Ishige, Y., Shimoda, M., and Kamahori, M. (2009) Extended-gate FET-based enzyme sensor with ferrocenyl-alkanethiol modified gold sensing electrode. *Bioens Bioscience* 24: 1096–1102.

Itagaki, E., Wakabayashi, T., and Hatta, H. (1990) Purification and characterization of 3-ketosteroid-Δ5-dehydrogenase from *Nocardia corallinae*. *Biochim Biophys Acta* 1038: 60–67.

Jadoun, J., and Bar, R. (1993) Microbial transformations in a cycloextrin medium. Part 3. Cholesterol oxidation by *Rhodococcus erythropolis*. *Appl Microbiol Biotechnol* 40: 230–240.

Johnston, J.B., Ouellet, H., and de Montellano, P.R. (2010) Functional redundancy of steroid C26-monoxigenase activity in *Mycobacterium tuberculosis* revealed by biochemical and genetic analyses. *J Biol Chem* 285: 36352–36360.

Joshi, S.M., Pandey, A.K., Capite, N., Fortune, S.M., Rubin, E.J., and Sassetti, C.M. (2006) Characterization of mycobacterial virulence genes through genetic interaction mapping. *Proc Natl Acad Sci USA* 103: 11760–11765.

Jubete, E., Loaiza, O.A., Ochoteco, E., Pomposo, J.A., Grande, H., and Rodriguez, J. (2009) Nanotechnology: a tool for improved performance on electrochemical screen-printed (bio) sensors. *J Sens Article ID 842575*, 13 pages doi:10.1155/2009/842575.

Kato, L. (1978) Cholesterol, a factor which is required for growth of mycobacteria from leporous tissues. *Int J Lepr Other Mycobact Dis* 46: 133–143.

Kendall, S.L., Withers, M., Soffair, C.N., Moreland, N.J., Gurcha, S., Sidders, B., et al. (2007) A highly conserved transcriptional repressor controls a large regulon involved in lipid degradation in *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. *Mol Microbiol* 65: 684–699.

Kendall, S.L., Burgess, P., Balhana, R., Withers, M., Ten Bokum, A., Lott, J.S., et al. (2010) Cholesterol utilisation in mycobacteria is controlled by two TetR-type transcriptional regulators: kstR and kstR2. *Microbiology* 156: 1362–1371.

Kieslich, K. (1980) Industrial aspects of biotechnological production of steroids. *Biotechnol Lett* 2: 211–217.
Liong, M.-T., and Shah, N.-P. (2006) Effects of a Lactobacillus casei symbiotic on serum lipoprotein intestinal microflora, and organic acids in rats. J Dairy Sci 89: 1390–1399.

Liu, W.-H., and Lee, C.-Y. (1992) Production of androst-4-ene-3,17-dione from cholesterol by Mycobacterium sp. in a synthetic medium. J Chin Agr Chem Soc 30: 52–58.

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

Liu, W.-H., Tsai, M.-S., and Lee, C.-Y. (1994a) Bioconversion of cholesterol to cholest-4-ene-3-one using free and immobilized growing cells of Arthrobacter simplex. J Chin Agr Chem Soc 32: 190–198.

Liu, W.-H., Kuo, C.-W., Wu, K.-L., Lee, C.-Y., and Hsu, W.-Y. (1994b) Transformation of cholesterol to testosterone by Mycobacterium sp. J Ind Microbiol 13: 167–171.

Lye, H.-S., Rusul, G., and Liong, M.-T. (2010a) Mechanisms of cholesterol removal by Lactobacilli under conditions that mimic the human gastrointestinal tract. Int Dairy J 20: 169–175.

Lye, H.-S., Rusul, G., and Liong, M.-T. (2010b) Removal of cholesterol by Lactobacilli via incorporation of and conversion to coprostanol. J Dairy Sci 93: 1383–1392.

McLean, K.J., Lafite, P., Levy, C., Cheesman, M.R., Mast, N., Pikuleva, I.A., et al. (2009) The Structure of Mycobacterium tuberculosis CYP125: molecular basis for cholesterol binding in a P450 needed for host infection. J Biol Chem 284: 35524–35533.

Mahato, S.B., and Garai, S. (1997) Advances in microbial steroid biotransformation. Steroids 62: 332–345.

Marcus, P.I., and Talalay, P. (1956) Induction and purification of α- and β-hydroxysteroid dehydrogenases. J Biol Chem 218: 661–674.

Marques, M.A., Neves-Ferreira, A.G., da Silveira, E.K., Valente, R.H., Chapeaurouge, A., Perales, J., et al. (2008) Deciphering the proteomic profile of Mycobacterium leprae cell envelope. Proteomics 8: 2477–2491.

Martin, C.K.A. (1976) Microbial transformation of β-sitosterol by Nocardia sp. M29. Eur J Appl Microbiol 2: 243–255.

Martin, C.K.A. (1977) Microbial cleavage of sterol side chains. Adv Appl Microbiol 22: 29–58.

Martin, J.F., and Aparicio, J.F. (2009) Enzymology of the polyenes pimaricin and candidicin biosynthesis. Methods Enzymol 459: 215–242.

Martin, P.A., Gundersen-Rindal, D., Blackburn, M., and Buyer, J. (2007) Chromobacterium subsugae sp. nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. Int J Syst Evol Microbiol 57: 993–999.

Martin, S.P., Lamb, D.J., Lynch, J.M., and Reddy, S.M. (2003) Enzyme-based determination of cholesterol using the quartz crystal acoustic wave sensor. Anal Chim Acta 487: 384–389.

Lee, C.Y., Chen, C.D., and Liu, W.H. (1993) Production of androsta-1,4-diene-3,17-dione from cholesterol using two-step microbial transformation. Appl Microbiol Biotechnol 38: 447–452.

Li, J., Vrielink, A., Brick, P., and Blow, D.M. (1993) Crystal structure of cholesterol oxidase complexed with a steroid substrate: implications for flavin adenine dinucleotide dependent alcohol oxidases. Biochemistry 32: 11507–11515.

Li, L., Freier, A.T., Hartman, P.A., Young, J.W., and Beltz, D.C. (1995) A resting-cell assay for cholesterol reductase activity in Eubacterium coprostanogenes ATCC 51222. Appl Microbiol Biotechnol 43: 887–892.

Liong, M.-T., and Shah, N.-P. (2005) Acid and bile tolerance and cholesterol removal ability of Lactobacilli strains. J Dairy Sci 88: 55–66.

Liong, M.-T., and Shah, N.-P. (2006) Microbial side-chain degradation of sterols. J Basic Microbiol 7: 461–474.

Knol, J., Bodewits, K., Hessel, G., Dijkhuizen, L., and van der Geize, R. (2008) 3-Keto-5α-steroid Δα-dehydrogenase from Rhodococcus erythropolis SQ1 and its orthologue in Mycobacterium tuberculosis H37Rv are highly specific enzymes that function in cholesterol catabolism. Biochem J 410: 339–346.

Kreit, J., and Sampson, N.S. (2009) Cholesterol oxidase: physiological functions. FEBS J 276: 6844–6856.

Kumar, A., Pandey, R.R., and Brantley, B. (2006) Tetraethylthio-silicate film modified with protein to fabricate cholesterol biosensor. Talanta 69: 700–705.

Kumar, R., Dahiya, J.S., Singh, D., and Nigam, P. (2001) Biotransformation of cholesterol using Lactobacillus bulgaricus in a glucose-controlled bioreactor. Biore Sci Technol 78: 209–211.

Lack, N., Lowe, E.D., Liu, J., Eltis, L.D., Noble, M.E.M., Sima, E., and Westwooda, I.M. (2008) Structure of HsaD, a steroid-degrading hydrolase, from Mycobacterium tuberculosis. Acta Crystallogr Sect F Struct Biol Cryst Commun 64: 2–7.

Lack, N.A., Yam, K.C., Lowe, E.D., Horsman, G.P., Owen, R.L., Sim, E., and Eltis, L.D. (2010) Characterization of a carbon-carbon hydrolase from Mycobacterium tuberculosis involved in cholesterol metabolism. J Biol Chem 285: 434–443.

Lamb, D.C., Kelly, D.E., Manning, N.J., and Kelly, S.L. (1998) A sterol biosynthetic pathway in Mycobacterium. FEBS Lett 437: 142–144.

Lambert, J.M., Bongers, R.S., de Vos, W.M., and Kleerebezem, M. (2008) Functional analysis of four bile salt hydrolase and penicillin acylase family members in Lactobacillus plantarum WCFS1. Appl Environ Microbiol 74: 4719–4726.

Lario, P.I., Sampson, N., and Vrielink, A. (2003) Sub-atomic resolution crystal structure of cholesterol oxidase: what atomic resolution crystallography reveals about enzyme mechanism and the role of the FAD cofactor in redox activity. J Mol Biol 326: 1635–1650.

Law, W.T., Doshi, S., McGeehan, J., McGeehan, S., Gibboni, D., Nikoloukine, Y., et al. (1997) Whole-blood test for total cholesterol by a self-metering, self-timing disposable device with built-in quality control. Clin Chem 43: 384–389.

Lee, C.Y., Chen, C.D., and Liu, W.H. (1993) Production of androsta-1,4-diene-3,17-dione from cholesterol using two-step microbial transformation. Appl Microbiol Biotechnol 38: 447–452.

Li, J., Vrielink, A., Brick, P., and Blow, D.M. (1993) Crystal structure of cholesterol oxidase complexed with a steroid substrate: implications for flavin adenine dinucleotide dependent alcohol oxidases. Biochemistry 32: 11507–11515.

Li, L., Freier, A.T., Hartman, P.A., Young, J.W., and Beltz, D.C. (1995) A resting-cell assay for cholesterol reductase activity in Eubacterium coprostanogenes ATCC 51222. Appl Microbiol Biotechnol 43: 887–892.

Liong, M.-T., and Shah, N.-P. (2005) Acid and bile tolerance and cholesterol removal ability of Lactobacilli strains. J Dairy Sci 88: 55–66.

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Miner, M.D., Chang, J.C., Pandey, A.K., Sassetti, C.M., and Sherman, D.R. (2009) Role of cholesterol in *Mycobacterium tuberculosis* infection. *Indian J Exp Biol* **47**: 407–411.

Möbus, E., Jahn, M., Schmid, R., Jahn, D., and Maser, E. (1997) Testosterone regulated expression of enzymes involved in steroid and hydrocarbon catabolism in *Corymnonas testosteroni*. *J Bacteriol* **179**: 5951–5955.

Mohn, W.W., van der Geize, R., Stewart, G.R., Okamoto, S., Liu, J., Dijkhuizen, L., and Ellis, L.D. (2008) The actinobacterial mce4 locus encodes a steroid transporter. *J Biol Chem* **283**: 35368–35371.

Molchanova, M.A., Andryushina, V.A., Savinova, T.S., Styttenko, T.S., Rodina, N.V., and Voishvilho, N.E. (2007) Preparation of androsta-1,4-diene-3,17-dione from sterols using *Mycobacterium neoaurum* VKPM Ac-1656 strain. *Russ J Bioorg Chem* **33**: 379–384.

Motteran, L., Pilone, M.S., Molla, G., Ghisla, S., and Pollegioni, L. (2001) Cholesterol oxidadse from *Brevibacterium steroidicum*. *J Biol Chem* **276**: 18024–18030.

Myamato, I., and Toyoda, K. (1994) Cholestenone manufacture with cholesterol oxidadse of *Rhodococcus*. Japanese Patent 06, 157, 585.

Nagasawa, M., Bae, M., Tamura, G., and Arima, K. (1969) Microbial transformation of steroids. Part II. Cleavage of sterols side chains by microorganisms. *Agric Biol Chem* **33**: 1644–1650.

Navas, J., Gonzalez-Zorn, B., Ladron, N., Garrido, P., and Vazquez-Boland, J.A. (2001) Identification and mutagenesis by allelic exchange of choE, encoding a cholesterol oxidadse from the intracellular pathogen *Rhodococcus equi*. *J Bacteriol* **183**: 4796–4805.

Nebit, N.M., Yang, X., Fontán, P., Kolesnikova, I., Smith, I., Sampson, N.S., and Dubnau, E. (2010) A thiolase of *Mycobacterium tuberculosis* is required for virulence and production of androstenedione and androstadienedione from cholesterol. *Infect Immun* **78**: 275–282.

Nien, P.C., Chen, Y.P., and Ho, K.C. (2009) Fabricating an amperometric cholesterol biosensor by a covalent linkage between poly(3-thiopheneacetic acid) and cholesterol oxidadse. *Sensors* **9**: 1794–1806.

Norouzi, P., Faridbod, F., Nasli-Esfahani, E., Larijani, B., and Norouzi, P.C., Chen, P.Y., and Ho, K.C. (2009) Fabricating an amperometric cholesterol biosensor by a covalent linkage under the control of 16S rRNA gene promoter of bifidobacteria. *Biotechnol Lett* **30**: 165–172.

Paternolloli, C., Antonini, M., Ghisellini, P., and Nicolini, C. (2004) Recombinant Cytochrome P450 immobilization for biosensor applications. *Langmuir* **20**: 11706–11712.

Pei, Y., Dupont, C., Sydor, T., Haas, A., and Prescott, J.F. (2006) Cholesterol oxidadse (ChoE) is not important in the virulence of *Rhodococcus equi*. *Vet Microbiol* **118**: 240–246.

Pereira, D.I.A., and Gibson, G.R. (2002) Effects of consump- tion of probiotics and prebiotics on serum lipid levels in human. *Crit Rev Biochem Mol Biol* **37**: 259–281.

Petrusma, M., Dijkhuizen, L., and van der Geize, R. (2009) *Rhodococcus rhodochrous* DSM 43269 3-ketosteroid 9x-hydroxylase, a two component iron-sulfur-containing monoxgenase with a subtle steroid substrate specificity. *Appl Environ Microbiol* **75**: 5300–5307.

Petrusma, M., Hessels, G., Dijkhuizen, L., and van der Geize, R. (2011) Multiplicity of 3-ketosteroid-9x-hydroxylase enzymes in *Rhodococcus rhodochrous* DSM43269 for specific degradation of different classes of steroids. *J Bacteriol* **193**: 3931–3940.

Pollegioni, L., Wels, G., Pilone, M.S., and Ghisla, S. (1999) Kinetic mechanisms of cholesterol oxidadse from *Streptomyces hygroscopicus* and *Brevibacterium sterolicum*. *Eur J Biochem* **264**: 140–151.

Pollegioni, L., Piubelli, L., and Molla, G. (2009) Cholesterol oxidadse: biotechnological applications. *FEBS J* **276**: 6857–6870.

Poole, A.J., and Cord-Ruwisch, R.C. (2004) Treatment of strong flow wool scouring effluent by biological emulsion destabilization. *Water Res* **38**: 1419–1426.

Purcell, J.P., Greenplate, J.T., Jennings, M.G., Ryerse, J.S., Persching, J.C., Sims, S.R., et al. (1993) Cholesterol oxidadse: a potent insecticidal protein active against boll weevil larvae. *Biochem Biophys Res Commun* **196**: 1406–1413.

Renganarajan, J., Bloom, B.R., and Rubin, E.J. (2005) Genome-wide requirements for *Mycobacterium tuberculo- sis* adaptation and survival in macrophages. *Proc Natl Acad Sci USA* **102**: 8327–8332.
Cholesterol degradation 698

Rosloniec, K.Z., Wilbrink, M., Capyk, J.K., Mohn, W.W., Ostendorf, M., van der Geize, R., et al. (2009) Cytochrome P450 125 (CYP125) catalyzes C26-hydroxylation to initiate sterol side chain degradation in *Rhodococcus jostii* RHA1. *Mol Microbiol* **74**: 1031–1043.

Salinas, E., Rivero, V., Torriero, A.A.J., Benuzzo, D., Sanz, M.I., and Raba, J. (2006) Multienzymatic-rotating biosensor for total cholesterol determination in a FIA system. *Talanta* **70**: 244–250.

Schnappinger, D., Ehrt, S., Voskuil, M.I., Liu, Y., Mangan, J.A., Monahan, I.M., et al. (2003) Transcriptional adaptaion of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. *J Exp Med* **198**: 693–704.

Schoemer, U., and Martin, C.K.A. (1989) Microbial transformation of sterols. *Biotechnol Bioeng* **22**: 11–25.

Sedlacek, L., and Smith, L.L. (1988) Biotransformations of steroids. *Crit Rev Biotechnol* **7**: 187–236.

Shi, J., Chu, Z., Ju, Y., Mo, G., and Cheng, W. (1992) Degradation of cholesterol to androsta-1,4-diene-3,17-dione by *Rhodococcus corallina*. *Zhongguo Yiyaogongye Zazhi* **23**: 204–207.

Shih, W.C., Yang, M.C., and Lin, M.S. (2009) Development of disposable lipid biosensor for the determination of total cholesterol. *Biosens Bioelectron* **24**: 1679–1684.

Shumantseva, V., Deluca, G., Bulko, T., Carrara, S., Nicolini, C., Usanov, S.A., and Archakov, A. (2004) Cholesterol amperomeric biosensor based on cytochrome P450scc. *Biosens Bioelectron* **19**: 971–976.

Shumantseva, V.V., Carrara, S., Bavastrello, V., Jason Riley, D., Bulko, T.V., Skryabin, K.G., et al. (2005) Direct electron transfer between cytochrome P450scc and gold nanoparticles on screen-printed rhodium-graphite electrodes. *Biosens Bioelectron* **21**: 217–222.

Sih, C.J. (1968) Mechanisms of steroid oxidation by microorganisms. XIII. C22 acid intermediates in the degradation of the cholesterol side chain. *Biochemistry* **7**: 796–807.

Sih, C.J., Tai, Y.Y., Tsong, Y.Y., Lee, S.S., and Coombe, R.G. (1968) Mechanisms of steroid oxidation by microorganisms. XIV. Pathway of cholesterol side degradation. *Biochemistry* **7**: 808–818.

Singh, K., Basu, T., Solanki, P.R., and Malhotra, B.D. (2009) Poly (pyrrole-co-N-methyl pyrrole) for application to cholesterol sensor. *J Mater Sci* **44**: 954–961.

Singh, S., Chaubey, A., and Malhotra, B.D. (2004) Amperometric cholesterol biosensor based on immobilized cholesterol esterase and cholesterol oxidase on conducting polypyrrole films. *Anal Chim Acta* **502**: 229–234.

Singh, S., Solanki, P.R., Pandey, M.K., and Malhotra, B.D. (2006) Cholesterol biosensor based on cholesterol esterase, cholesterol oxidase and peroxidase immobilized onto conducting polyaniline films. *Sens Actuators B* **115**: 534–541.

Singh, S., Singhal, R., and Malhotra, B.D. (2007) Immobilization of cholesterol esterase and cholesterol oxidase onto sol-gel films for application to cholesterol biosensor. *Anal Chim Acta* **582**: 335–343.

Situmorang, M., Alexander, P.W., and Hibbert, D.B. (1999) Flow injection potentiometry for enzymatic assay of cholesterol with a tungsten electrode sensor. *Talanta* **49**: 639–649.

Skowasch, K., Möbus, E., and Maser, E. (2002) Identification of a novel *Comamonas testosteroni* gene encoding a steroid inducible extradiol dioxygenase. *Biochem Biophys Res Commun* **294**: 560–566.

Smith, M., Zahnley, J., Pfeifer, D., and Goff, D. (1993) Growth and cholesterol oxidation by *Mycobacterium* species in Tween 80 medium. *Appl Environ Microbiol* **59**: 1425–1429.

Söhngen, N.L. (1913) Benzin, petroleum, paraffinöl und paraffin als kohlenstoff- und energierquelle für mikroben. *Zbl Bakteriol Parasitenk Abt II* **27**: 595–609.

Solanki, P.R., Arya, S.K., Singh, S.P., Pandey, M.K., and Malhotra, B.D. (2007a) Application of electrochemically prepared poly-N-methylpyrrole-p-toluene sulfonate films to cholesterol biosensor. *Sens Actuators B* **123**: 829–839.

Solanki, P.R., Arya, S.K., Nishimura, Y., Iwamoto, M., and Malhotra, B.D. (2007b) Cholesterol biosensor based on amino-undecanethiol self-assembled monolayer using surface plasmon resonance technique. *Langmuir* **23**: 7399–7403.

Solanki, P.R., Kaushik, A., Agrawal, V.V., and Malhotra, B.D. (2011) Nanostructured metal oxide-based biosensors. *NPG Asia Materials* **3**: 17–24.

Srivalakit, P., Wichai, U., and Saraphanchotithawhya, A. (2006) Biotransformation of various natural sterols to androstenedones by *Mycobacterium* sp. and some steroid-converting microbial strains. *J Mol Catal B Enzym* **41**: 49–54.

Srivastava, A., and Patil, S. (1994) Investigation of some physicochemical parameters involved in the biotransformation of cholesterol to 17-ketosteroids. *J Microbiol Biotechnol* **9**: 101–112.

Staytor, M., and Bloch, K. (1965) Metabolic transformation of cholestadienols. *J Biol Chem* **240**: 4598–4602.

Szentirmai, A. (1990) Microbial physiology of sidechain degradation of steroids. *J Ind Microbiol* **6**: 101–116.

Tak, J.D. (1942) On bacteria decomposing cholesterol. *Anatok van Leeuwenhoek* **8**: 32–40.

Turler, S., Denner, E.B.M. (2003) *Sterolibacterium denitricans* gen. nov., sp. nov., a novel cholesteroloxidizing, denitrifying member of the β-Proteobacteria. *Int J Syst Evol Microbiol* **53**: 1085–1091.

Taylor, C.D., Smith, S.O., and Gagosian, R.B. (1981) Use of microbial enrichments for the study of the anaerobic degradation of cholesterol. *Geochim Cosmochim Acta* **45**: 2161–2168.

Thomas, S.T., Yang, X., and Sampson, N.S. (2011) Inhibition of the *M. tuberculosis* 3β-hydroxysteroid dehydrogenase by azasteroids. *Biorg Med Chem Lett* **21**: 2216–2219.

Turfitt, G.E. (1944) Microbiological agencies in the degradation of steroids: I. The cholesterol-decomposing organisms of soils. *J Bacteriol* **47**: 487–493.

Turfitt, G.E. (1948) The microbiological degradation of steroids 4. Fission of the steroid molecule. *Biochem J* **42**: 376–383.

Uhía, I., Galán, B., Morales, V., and García, J.L. (2011a) Initial step in the catabolism of cholesterol by *Mycobacterium smegmatis* mc²155. *Environ Microbiol* **13**: 943–959.

Uhía, I., Galán, B., Medrano, F.J., and García, J.L. (2011b) Characterization of the KsrR-dependent promoter of the first step of the cholesterol degradative pathway in *Mycobacterium smegmatis*. *Microbiology* **157**: 2670–2680.
Uhía, I., Galán, B., Kendall, S.L., Stoker, N.G., and García, J.L. (2012) Cholesterol metabolism in *Mycobacterium smegmatis*. *Environ Microbiol Rep* doi:10.1111/j.1758-2229.2011.00314.x.

Umar, M., Rahman, M., Vaseem, M., and Hahn, Y.B. (2009) Ultra-sensitive cholesterol. Biosensor based on low temperature grown ZnO nanoparticles. *Electrochem Commun* 11: 118–121.

Usman, H.A., and Hosono, A. (1999) Bile tolerance, taurocholate deconjugation, and binding of cholesterol by *Lactobacillus gasseri* strains. *J Dairy Sci* 82: 243–248.

Veiga, P., Juste, C., Lepercq, P., Saunier, K., Beguet, F., and Gerard, P. (2005) Correlation between faecal microbial community structure and cholesterol-tocoprostanol conversion in the human gut. *FEMS Microbiol Lett* 242: 81–86.

Vrielink, A., Lloyd, L.F., and Blow, D.M. (1991) Crystal structure of cholesterol oxidase from *Brevibacterium sterolicum* refined at 1.8 Å resolution. *J Mol Biol* 219: 533–554.

Wallace-Davis, E.N.K., Murphy, L.J., and Allan, A.W. (2011) Cholesterol sensor. United States Patent 0086373 A1.

Watanabe, M., and Po, L. (1974) Testosterone uptake by membrane vesicles of *Pseudomonas testosteroni*. *Biochim Biophys Acta* 345: 419–429.

Whitmarsh, J.M. (1964) Intermediates of microbiological metabolism of cholesterol. *Biochem J* 90: 23.

Wilbrink, M.H., Petrusma, M., Dijkhuizen, L., and van der Geize, R. (2011) Fad19 of *Rhodococcus rhodochrous* DSM43269, a steroid-Coenzyme A ligase essential for degradation of C24 branched sterol side chains. *Appl Environ Microbiol* 77: 4455–4464.

Wisitsoraat, A., Sritongkham, P., Karuwan, C., Phokharatkul, D., Matusro, T., and Tuantranont, A. (2010) Fast cholesterol detection using flow injection microfluidic device with functionalized carbon nanotubes based electrochemical sensor. *Biosens Bioelectron* 15: 1514–1520.

Yam, K.C., D’Angelo, I., Kalscheuer, R., Zhu, H., Wang, J.X., Snieckus, V., *et al*. (2009) Studies of a ring-cleaving dioxygenase illuminate the role of cholesterol metabolism in the pathogenesis of *Mycobacterium tuberculosis*. *PLoS Pathog* 5: e1000344.

Yam, K.C., van der Geize, R., and Eltis, L.D. (2010) Catabolism of aromatic compounds and steroids by *Rhodococcus*. In *Biology of Rhodococcus*. Alvarez, H.M. (ed.). Heidelberg, Germany: Springer Berlin, pp. 134–160. ISBN 978-3-642-12936-0.

Yang, X., Dubnau, E., Smith, I., and Sampson, N.S. (2007) Rv1106c from *Mycobacterium tuberculosis* is a 3β-hydroxysteroid dehydrogenase. *Biochemistry* 46: 9058–9067.

Yang, X., Gao, J., Smith, I., Dubnau, E., and Sampson, N.S. (2011) Cholesterol is not an essential source of nutrition for *Mycobacterium tuberculosis* during infection. *J Bacteriol* 193: 1473–1476.

Yazdi, M.T., Malekzadeh, F., Khatami, H., and Kamranpour, N. (2000) Cholesterol degrading bacteria: isolation, characterization and bioconversion. *World J Microbiol Biotechnol* 16: 101–106.

Yue, Q.K., Kass, I.J., Sampson, N.S., and Vrielink, A. (1999) Crystal structure determination of cholesterol oxidase from *Streptomyces* and structural characterization of key active site mutants. *Biochemistry* 38: 4277–4286.