



# Microbial transformation of steroids, an old and novel drug opportunity strategy: a review

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## Abstract

The microbial transformation (MIT) of compounds that are raw materials for pharmaceuticals is a valuable aspect of white biotechnology. MIT as its main example cycloperhydrophenanthrene which leads to the synthesis of new and well-known pharmaceuticals. MIT reduces the conversion time of base organic compounds to pharmaceuticals compared to the chemical reactions to synthesize them in the long term, allows savings in time, in investment, with an improvement in the effectiveness and biological activity of the drug: hormones, antibiotics for infectious and genetic diseases such as cancer. Specifically, the MIT of steroids improves the therapeutic potential of drugs, currently even for the prevention of obesity. The objective of this short review is to analyze the status of steroids by MIT in the pharmaceutical industry. The analysis indicates that MIT is an ancient and today novel strategy that allows white biotechnology to expand treatment options for all types of infectious and non-infectious diseases in harmony with the environment in favor of the quality of human life and with molecular biology the future is promising for long-term public health solutions.

**Keywords:** Microbial potential, microbial diversity, microbial biochemistry, genetic and molecular biology, pharmaceuticals, health

## Introduction

Steroids comprise a wide range of naturally common compounds distributed in all the animal and plant kingdoms, with huge physiologically active derivatives that play crucial roles in biological systems [1-4]. Steroids are key components of cell membranes, for stability and growth in cellular and development. Steroids are precursors to bile acids and steroid hormones [5-8]. Steroids have base structure consisting of 17 carbon atoms in a tetracyclic ring system well known as cyclopentanoperhydrophenanthrene, now as gonane and estrane [3-6]. Steroid products are found in diversity of living species, ecdysteroids in insects, phytosterols and diosgenin in plants, cholesterol and corticosteroids: glucocorticoids, mineralocorticoids as well as sex hormones, bile acids, and vitamin D; neurosteroids, in vertebrates, and in yeasts and fungi are ergosterol and ergosteroids as part of its membrane cells [7,8,10]. Steroids and its diversity are essential in medical practice, functioning as scaffolds for synthesizing new pharmacologically potent compounds [5,11,13,14]. Steroids control a cascade of physiological activities at target sites and play key roles in cancer research [5,8,11,12]. The physiological activity of steroids is closely associated to their molecular structure, as well as, the number, spatial orientation, and reactivity of functional groups in the steroid nucleus, as well as the oxidation state of the rings [1,4,13,15-17]. For example, the presence of an oxygenated group at C-11 $\beta$  is essential for anti-inflammatory activity, besides a hydroxyl group at C-17 $\beta$  determines androgenic properties [3,5,8,19,20]. Aromatization of steroids at the A-ring affects estrogenic activity, and

corticosteroids feature a 3-keto-5-ene group or a pregnane side chain at C-17 [2,11,14,21,22]. In steroids functional modifications involve simple, chemically defined reactions catalyzed by microbial enzymes [1,4,13,15]. Genetic MIT ability provides these enzymes to facilitate the transformation reactions, enhancing the efficiency and specificity of steroids by MIT [6,7,9,23,24]. The chemical modification of steroids, which requires high temperatures, pH, expensive reagents, and protective groups for reactive centers, has been a chemical method to obtain valuable new or improved drugs [3,8,16,17,25]. However, MIT offers an alternative approach that enables the production of biologically active steroid derivatives with high regio- and stereoselectivity under mild, environmentally friendly conditions [17-19,27-31]. The aim of this short review is to analyze the potential of microbial biotransformation of steroidal compounds of value in the pharmaceutical industry and its connection with other related industries.

## Microbial transformation of steroids

There are currently around 300 known steroidal drugs, used for several aims: immunosuppression, anti-inflammation, and contraception. Steroid applications have expanded to treating cancers, osteoporosis, human immunodeficiency virus (HIV) infections or Acquired Immune Deficiency Syndrome or AIDS [3-5,7,8,32]. The therapeutic effects of certain steroid hormones are related to its interaction to intracellular receptors that regulate gene expression as transcription properties [13,20-22].

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Some steroids, as well as dehydroepiandrosterone, progesterone, pregnenolone, and its products, like 17 $\beta$ -estradiol and allopregnanolone, are classified as neurosteroids due to steroids activity on the central nervous system [1,2,14]. The MIT of exogenous steroid compounds is commonly by wide groups of bacteria and fungi, to enhance pharmacological activity and efficiency [27,30,33,34]. Several types of MIT reactions, as well as hydroxylation, dehydrogenation, side-chain degradation, ring A aromatization, reduction and esterification are used to achieve specific modifications [16-19, 22,23]. MIT techniques diverse processes in culture media with microorganisms, free enzymes, biphasic systems, liposomes, microemulsions, methods altering cell wall permeability and the use of immobilized cells and enzymes [1,6,15,17,24]. The spectrum of steroids that can be transformed by microbial cells is wide [4,7,9,18,25]. Most advances in steroid happened in 1950 at that time researchers had not clear idea about the pharmacological properties of cortisol and progesterone [8,14,16,30].

Researchers also discovered that genus fungi as well-known species, could biotransform 11 $\alpha$ -hydroxylation, a critical reaction essential for synthesizing biologically active steroids [11,25,34,36,]; including fungal transformation of Azorellane and Aquilinane types diterpenoids have unique tricyclic fused 5-, 6-, and 7-membered systems and a wide spectrum of biological properties: antimicrobial, antiprotazoal, spermicidal gastroprotective [3,8,9,17,26]. These discoveries marked the onset of a basic development of steroids as a pharmaceutical, and the main point potential of microbial systems in the synthesis of valuable steroid compounds [10,11,13,32]. Currently, the main objectives in steroid pharmaceutical research and development in target on identifying, and isolating microbial strains with unique activities or improving transformation capabilities [33-35, 37-39]. Genetic engineering and metabolic engineering of bacteria, fungi, and plants play a key role in these tasks [15,22,24,28,40]. Industrially, microbial hydroxylation activities, like are: C-11 $\alpha$ , C-11 $\beta$ , C-15 $\alpha$ , and C-16 $\alpha$ , are performed with high yields and enantioselectivity [2,9,13,14, 22,27]. Since steroids have hydrophobicity, which caused steroids to be tolerant to biodegradation, the mechanisms of steroid metabolism by both aerobic and anaerobic microorganisms have been investigated [18,23,26,28-30]. For effective MIT, precursor steroids are required, that are then converted into valuable intermediates and final products [7,11,17,25,31]. MIT are: regiospecific and stereospecific, allowing the modification of compounds into suitable isomers through simple, chemically defined reactions catalyzed by microbial enzymes [1,3,15,32-33]. These enzymes act on compounds to design highly selective reactions, with easy techniques of isolation and purification of the new target compounds [3,6,17,19,22,27]. Besides, MIT are easy to use with necessary sterility conditions and allows for repeated working with these enzymes [15,31,34]. Steroid MIT are possible under several conditions of pressure and temperature, which is a viable alternative to chemical and ecological synthesis [2,23,24,40,41]. Although challenges such as productivity and chemical purity of steroids released, have non risk of contamination, microbial cells are systems can optimize and reduce costs by eliminating the need for isolating, purifying and stabilizing pure enzymes [1,7,9,25]. Microbes naturally secrete all necessary cofactors and provide a stable environment for the enzymes, preventing protein structural changes and maintaining enzyme reactivity for many repeated processing to

optimize steroid transformation [26,30,34]. Oxidation of steroid [6,12,35]. Common steroid precursors including cholesterol, steroidal alkaloids, steroidal sapogenins, and phytosterols, are readily available for MIT processes [16,18,19].

## Types of steroids

### Cholesterols and corticosteroid

Figure 1 illustrates the classification of steroids according to their biological functions or activities, including: bile acids, steroid hormones, cardioactive glycosides, aglycones, and steroid saponins [2,6,25].

### Steroid hormones

Estrogens and androgens play a crucial role in maintaining homeostasis and regulating development [34,36,40]. The gut microbiota significantly influences systemic sex hormone levels by metabolizing these hormones into various derivatives [24,27,32,37]. Under normal physiological conditions, estrogens undergo rapid deactivation in the liver through processes such as glycosylation, sulfation or methylation, followed by their elimination via urine and feces [30,31,37,38]. Gut microbes can alter this process by enzymatically reactivating estrogens, thereby modulating their bioavailability [8,32,35].

Microbial  $\beta$ -glucuronidase enzymes, primarily found in genera such as *Clostridium* and *Bacteroides*, facilitate the removal of glucuronides from deactivated estrogens, thereby restoring their activity [1,3,42]. Similarly, sulfatase-producing bacteria like *Bacteroides fragilis* and *Peptococcus niger* have been shown to convert estrone sulfate back into estrone, further influencing systemic estrogen levels [9,11,39]. Additionally, *Bacteroides thetaiotaomicron* and other gut microbes can act on sulfonated estrogen precursors, such as dehydroepiandrosterone (DHEA), altering hormone metabolism and potentially affecting inflammatory pathways [12-14,24]. Estrogen metabolism, gut microbes also impact androgen regulation [16,40-43]. For example, *Mycobacterium neoaurum*-derived 3 $\beta$ -HSDH (3 $\beta$ -hydroxysteroid dehydrogenase) has been implicated in the conversion of testosterone to androstenedione, a process linked to behavioral changes, including depression-like phenotypes in animal studies [16,40-43]. Collectively, these microbial interactions with sex steroid hormones highlight the critical role of the gut microbiome in endocrine regulation, with potential implications for metabolic, reproductive, and neuropsychiatric health.

### Steroid saponins

For the steroid industry, natural steroid sapogenin, diosgenin is one more important raw material and it can be used for the microbial production of some new steroids of useful therapeutic action [21,44-45]. The developing alternative methods for synthesizing therapeutically effective steroids, such as prednisone and prednisolone, has gained significant attention [42,46,47]. The glucocorticoids cortisone and hydrocortisone exhibit potent anti-inflammatory activity; however, their clinical use is often limited due to numerous side effects. Consequently, the development of improved derivatives, such as prednisone and prednisolone, allows for lower therapeutic doses, thereby reducing adverse effects [18,19,21].

Recent studies have highlighted the potential of microbial biotransformation in steroid synthesis. Among 13 screened *Rhodococcus* strains, *Rhodococcus coprophilus* DSM 43347 demonstrated the highest catalytic activity, efficiently

performing  $\Delta 1$ -dehydrogenation of cortisone and hydrocortisone. This process resulted in the formation of prednisone with a 94% yield and prednisolone with a 97% yield, offering a promising biotechnological approach to steroid drug development [47-49].

### Phytosterols

Phytosterols are plant-derived sterols, primarily sourced from *Glycine max* (soybean) or produced from tall oil or pitch [9,20,34,55]. These plant sterols are extracted from specific parts of domestic plants for this purpose, as well as from press mud, generated during the extraction of edible oils [5,37,45,50]. MIT of phytosterols has high economic value for the pharmaceutical industry, especially those derived from the activity of *Mycobacterium* spp [12,27,51-53]. Besides sterol-containing waste from food, agricultural, and cellulose manufacturing processes can be utilized to produce valuable steroid compounds without requiring extensive purification of phytosterols [28,33,35,47,54].

### Ecdysterols

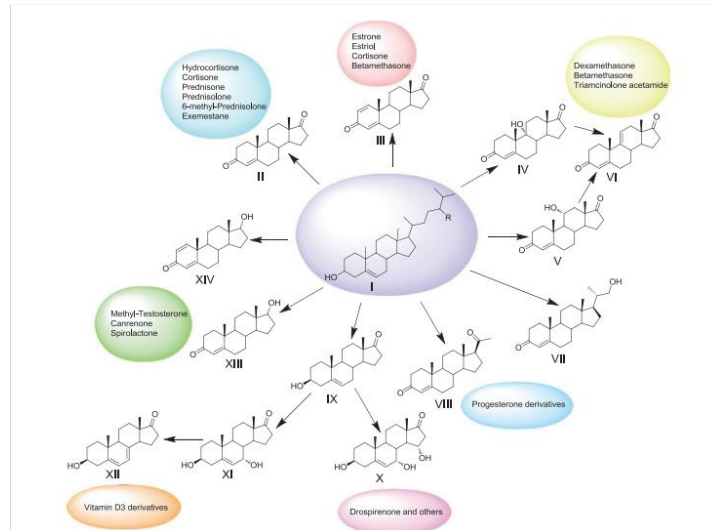
Ecdysteroids are defined according to their chemical structure and/or biological action (metamorphosis hormones), in insects and crustaceans, that is a cause of controversy [22,30,33,34,57]. Ecdysteroids are organic compounds structurally similar to ecdysone, they are polyhydroxysteroids and are widely distributed in nature [4,5,55,56,58]. More than 250 ecdysteroids of different origins have been extracted and purified, especially from plants [7,8,10]. Ecdysteroids possess one or more vicinal diols, notably at the C-2 and C-3 positions of the A ring in the steroid nucleus, as well as at C-20 and C-22 in the side chain. Recent findings indicate that the molecular chaperone 4-phenylbutyrate (PBA) enables the selective extraction of ecdysteroids containing a C-20,22 diol group, while those with only a C-2,3 diol structure remain unextracted. This selective affinity offers a promising approach for the targeted isolation of specific ecdysteroid derivatives [14,17,30, 32,42]. In plants, it is attributed to chemical defense activity against predatory insects [34,37,43,55-58].

### Ergosterols

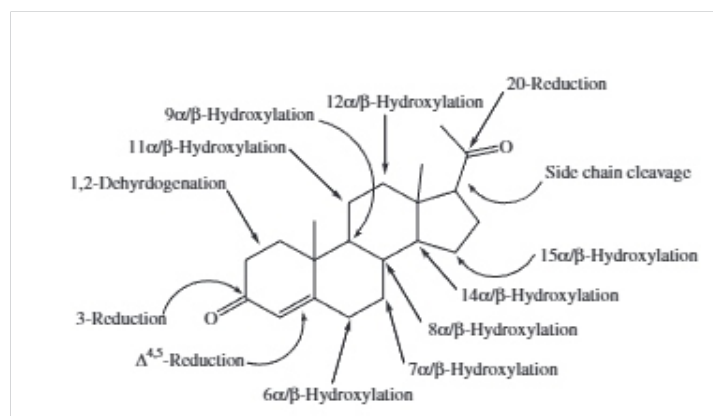
Ergosterol (ergosta-5,7,22-trien-3 $\beta$ -ol) is a sterol that serves as a biological precursor to vitamin D<sub>2</sub>, by the action of ultraviolet light into ergocalciferol, or vitamin D<sub>2</sub>, through a photochemical reaction involving the cleavage of the B ring [7,9,10,12]. Ergosterol is the sterol that composes the cell membranes of fungi and certain protists such as trypanosomatids [5,6,14,17,32]. For these organisms, the synthesis of ergosterol is essential, it is a source of sterols [22,35,36,40,50]. Ergosterol is synthesized and exists in the cell membranes of fungi and plants as beta-sitosterol [53,55,56,59-61].

### Types of steroids and its microbial transformation

Currently, there is a growing trend to isolate, analyze, and heterologously express enzymes that catalyze the microbial transformation of steroids [44,45,47,51,62,63]. This approach not only expands the wide specter of target steroids but also holds the potential for scaling up production in the future [33,52,53]. Several reactions are involved in the biotransformation of steroids, with key sites of these reactions on this specific molecule illustrated in Figure 2 [44,52,54,61,64].



**Figure 1.** Valuable steroids produced from phytosterol (I) using microbiotechnologies, producer strains and active pharmaceutical ingredients (APIs) on their base: II, androst-4-ene-3,17-dione (AD) produced by *Mycobacterium neoaurum* VKM Ac-1815D; III, androsta-1,4-diene-3,17-dione (ADD), by *M. neoaurum* VKM Ac-1816D; IV, 9 $\alpha$ -hydroxy-androst-4-ene-3,17-dione (9-OH-AD), by *Mycobacterium* sp VKM Ac-1817D; V, 11 $\alpha$ -hydroxy-androst-4-ene-3,17-dione, by one-pot two-stage bioconversion with *M. neoaurum* VKM Ac-1815D and *Aspergillus ochraceus* VKM F-830; VI, androst-4,9(11)-diene-3,17-dione, by chemoenzymatic synthesis (via IV or V); VII, 20-hydroxymethylpregn-4-ene-3-one (20-HMP, BA), by *M. neoaurum* VKM Ac-1815D and relative strains; VIII, progesterone, by engineered *Mycobacterium smegmatis* mc2 155 strain; IX, 3 $\beta$ -hydroxyandrost-5-en-17-one (dehydroepiandrosterone; prasterone, DHEA), by *M. neoaurum* VKM Ac-1815D using chemical protection/ deprotection of 3 $\beta$ -hydroxyl function; X, 3 $\beta$ ,7 $\alpha$ ,15 $\alpha$ -trihydroxyandrost-5-ene-17-one, by *Fusarium graminearum* and relative fungal strains; XII, 3 $\beta$ -hydroxy-5,7-diene-17-one, by bioconversion with 7 $\beta$ -hydroxylating fungal strain followed by chemical modification; XIII, androst-4-ene-3-one-17 $\beta$ -ol (testosterone), by engineered *M. neoaurum* strain; XIV, androst-1,4-diene-3-one-17 $\beta$ -ol, boldenone, by recombinant *M. neoaurum* strain [126-130].



**Figure 2.** Show main sites of MIT reactions, it has been described and discovered as a result of extensive

and detailed research with a wide diversity of microorganisms that indicate that these changes could have occurred in the evolutionary past of these biological processes [9,40,49,50,65,66-70]. With the use of genetically improved microorganisms or those obtained by genetic engineering to produce molecules that are naturally difficult to obtain, as

results indicate that it is even feasible to synthesize these molecules into bioreactors for safe pharmaceutical use, 7 $\alpha$ -Dihydroxylation [11,19,31,71,72]. This pathway facilitates the removal of the 7 $\alpha$ -hydroxy or 7 $\beta$ -hydroxy group from primary bile acids, leading to the formation of secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA) [8,16,18,20,22,73]. While the production of secondary bile acids via 7 $\alpha$ -dehydroxylation is specific, deconjugation, oxidation, and epimerization are commonly carried out by several genera and species of intestinal bacteria, such as *Bacteroides*, *Clostridium*, *Bifidobacterium* and others bacterial genus [64,66,70,73-75].

The secondary bile acids, DCA and LCA, undergo oxidation and epimerization mediated by 3 $\alpha$ / $\beta$ -HSDH, resulting in the formation of various bile acid derivatives, including isoDCA, isoLCA, 3-oxoLCA, and isoalloLCA, which have recently been identified for their immunomodulatory functions in the gut [54,73,75-77,81]. Besides this other source of these organic compounds as derivatives of cholesterol and hormones such as sulfonated cholesterol and estrogen, can be readily isolated from waste blood collected at slaughterhouses [24,69,71,78-81]. In vertebrates, microbial lipids not only modulate host metabolism but also act as immune signaling molecules in the gut mucosa. Microorganisms regulate the development and functionality of wide intestinal immune cells and contribute to mucosal homeostasis and disease susceptibility [17, 20,37, 77,79,80]. Recent research has shown that working with *Rhodococcus* pre-grown on n-alkanes can increase the yield of 9 $\alpha$ -hydroxy and rostenedione, achieving double the efficiency compared to cells grown on glucose [30,33,35,8,87].

### Oxidation

A fundamental aspect for the MIT of steroids is to select microorganisms, that not only have the capacity in the molecular structure of these compounds but, also have or develop natural or induced tolerance of their enzymes that, facilitate MIT [1,5,8,9,29,33], under different chemical conditions, biochemical actions that are necessary for microorganisms involved in MIT of steroids [12,44, 46,82]. As well as oxidation of alcohols to ketone: 3 $\beta$ -OH to 3-keto by *Aspergillus* spp or *Rhizopus* spp genus fungi [7,35,44,47,52,61].

### Hydroxylation

The substitution of a hydroxyl group directly for a hydrogen atom at a specific position in a steroid molecule, whether in the  $\alpha$  or  $\beta$  configuration, can be achieved while retaining the molecule's overall structure. Hydroxylation processes at positions 11 $\alpha$ , 11 $\beta$ , 15 $\alpha$ , and 16 $\alpha$  are well-developed and are primarily used in the production of adrenal cortex hormones and their analogs [10,14,53,54,58]. In that sense, fungi are among the most active microorganisms for catalyzing hydroxylation reactions [25,32,43,46,48,83]. Certain bacteria genera, as well as *Pseudomonas aeruginosa* [3,7,11,84] the spore-forming *Bacillus* or genus of actinomycetes and its species like: *Streptomyces* and *Nocardia*, exhibits notable hydroxylation activity [32,34,41]. Specifically, 11 $\alpha$ - and 11 $\beta$ -hydroxylations are generally performed by some fungi genus as well known: as *Rhizopus* spp or *Aspergillus* spp [6,9,13,32,85]. While 16 $\alpha$ -hydroxylation is carried out by other fungi genus as: *Curvularia* spp., and *Cunninghamella* spp [36,44,51] also is included the actinomycete genus *Streptomyces* sp [2,3,16, 40,47].

### Dehydrogenation

An example of bacterial dehydrogenation is the  $\Delta$ 1-dehydrogenation of 6 $\alpha$ -methyl-cortisol, to 6 $\alpha$ -methyl-prednisolone by resting cells of *Arthrobacter globiformis* and another bacterial genus [7,59,61,86]. Based on a gene encoding 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD), that was identified in the genome of *Rhodococcus* sp P14. Recombinant of *E. coli* BL21 cells, expressing this enzyme successfully transformed estradiol, into estrone with up to 94% efficiency. The *Rhodococcus* sp P14, could utilize other steroids, such as estriol and testosterone, as sole carbon sources [50,52,53,87]. The genome screening also led to the identification of a gene for a short-chain dehydrogenase, that catalyzes the conversion of estradiol to estrone, estriol to 16-hydroxyestrone and testosterone to androst-4-en-3,17-dione [62,63,88-89]. Microbial dehydrogenation using *Escherichia coli* genetically modified has yielded promising results.[90]also reported in other bacteria[54,57,59,64,65,91], can also be applied to produce derivatives of cortisone and hydrocortisone having improved anti-inflammatory properties and reduced side effects [5,8,10]. This is achieved by introducing double bonds at specific positions in the steroid's ring A structure [74,85,86,88,92]. In that sense genera of both fungi and bacteria are able to dehydrogenate the secondary alcohol groups of steroids, generating corresponding carbonyl derivatives. It is well-known that microbial whole cells are particularly effective at performing  $\Delta$ 1-dehydrogenation [22,43,92,100,102].

### Side chain biodegradation

Side-chain cleavage of steroids is performed generally by fungi genus: *Curvularia* spp. or *Cunninghamella* spp, however [3,7,9,16]: such reactions are described also different another genus of bacteria for example: actinobacteria, that is able to perform the basic step of the side-chain oxidation of sterols and other C-27 steroids is hydroxylation at C 26 or C-27 [27-30,34]. The aliphatic side-chain is degraded by an array of  $\beta$ -oxidation reactions [36,41,48,102]. The terminal hydroxylation of C-27sterol is catalyzed by an enzyme known as cytochrome P450125 [97] That enzyme, also known as steroid 26-monooxygenase [51-53,59,64], is produced by various actinomycetes, including *Rhodococcus jostii*. It has been successfully purified and characterized [71,72,84,93].

### Oxidation to ketone through hydroxylation

The chemical functionalization of different carbon atoms in the sterane skeleton is closely related to the MIT activity of the molecule [88,89,93,99,102]. Microbial transformations MIT's play a crucial role in obtaining these compounds through chemical processes, including the oxidation of hydroxyl groups at C-3 and C-17, isomerization of the double bond from  $\Delta$ 5-6 to  $\Delta$ 4-5, hydrogenation of double bonds at  $\Delta$ 1-2 and  $\Delta$ 4-5, and reduction of the carbonyl group at C-17 and C-20 with a  $\beta$  orientation [82-6,94,]; with relative success, reason why new strategies with natural microorganisms and genetically modified microorganisms of plant origin, are still being investigated at MIT [95,96,100-103]. Specifically, to achieve MIT in specific steroid sites that ensure chemical stability and sufficient product performance, through processes based on enzymes and microorganisms. That involve those naturally selected by genetic engineering to achieve efficiency in an effective hydroxylation of the steroid [14,16,97,98, 104-107].

### Ring A aromatization

Ring aromatization of suitable substrates by microorganisms forms aromatic compounds. For example, steroids like estrogens and estrones can be produced by ring Aromatization of steroid precursors or intermediates [21-25]. MIT of steroids has been successful even in aromatic molecules on functional groups, that determine specific pharmaceutical properties, which increases the spectrum of use in medicine [48,50,99-100]. This is the case of MIT from diosgenin successfully in other steroid compounds [13,51-53,101].

### Reduction

This type of MIT as well as the reduction of ketones and aldehydes to alcohols [62-65,69-71]. This process could be done by some genera and species of: algae, bacteria and fungi, which commonly undergo the reduction of exogenous compounds, like some important androgens can be produced by making use of these processes [7,84-86,93, 100,102], especially under the consideration of the natural microbial potential, to make direct modifications on steroids directly or indirectly with the immobilized enzymes, thus, the genetic design of microorganisms that make precise changes in the molecules to expand, or improve their pharmaceutical spectrum in medicine [12,13,16,17,30,103].

### Hydrolysis of esters

The systemic side effects of steroids applied as drugs is to design molecules, of relatively easy metabolic deactivation after performing the action, in the pharmaceutical target site [104-105]. For this purpose, during the MIT of steroids, it is feasible to apply: esterase enzymes for control and regulation of the activity of the steroid, with pharmacological value, since the esters are located in specific sites [106]. It is possible to use microorganisms that act, on ester bonds sensitive to enzymatic activity, it has been a subject of research, thus microbial actions have been evaluated, with enzymes of such selectivity that its facilitate the hydrolysis activity of esters, with esterases due bacterial genera and species as well as other microbial species in the generation of polyester, today with better results with the use of genetic engineering [21,64,107].

### Esterification

It is possible to obtain, through MIT, molecules similar to those reported in steroids, to make esterification with esterase enzymes by protein engineering in enteric bacteria, that synthesize them by optimizing genetic potential for manipulation and complementing it with the use of organic solvents [108-110]. In this sense, microbial synthesis of esters is relevant, from organic acids and alcohols, that accumulate in high concentration by bacteria such as *Escherichia coli*, capable of direct esterification by means of esterase enzymes efficient by expressing genes of the ethanol and lactic acid metabolic pathway for MIT steroids [1,64,121].

### Microbial transformation of Steroid Compounds

For sterol MIT, some bacterial genera and species have been identified, as effective biocatalysts as well as: *Arthrobacter oxydans* 317, *A. rubellus*, *Brevibacterium* sp., *Mycobacterium smegmatis* [27,48,67,100,111-113]; *Pseudomonas* spp and *Rhodococcus* spp. However, to prevent the degradation of the steroid nucleus during these transformations, inhibitors are often required [16-18,22-25]. For example, estrogen, a major component in orally administered contraceptives, also plays a crucial role in hormone replacement therapy for menopause [3,5,8,40]. Estrone can be produced through the biotransformation of 19-nor-testosterone by cell-free extracts of *Pseudomonas testosterone*, with a small amount of estradiol-17 $\beta$  also produced in this reaction [40-43,92,94]. Recently, the 4,5-seco pathway of 17 $\beta$ -estradiol biodegradation by *Rhodococcus equi* DSSKP-R-001 was discovered, identifying the enzymes and genes involved in the initial stages of catabolism, this process begins with the dehydrogenation of 17 $\beta$ -estradiol by a short-chain dehydrogenase encoded by the *hsd17b14* gene, to form estrone [5,10]. Also, estrone could be transformed by flavin-binding monooxygenase (*At1g12200*), into 4-hydroxyestrone [28,29,31,33].

Subsequent cleavage of the steroid ring A, is catalyzed by 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase, encoded by the *hsaC* and catechol-1,2-dioxygenase encoded by the *catA* [35,43,49,112]. Özçınaret *et al.*, 2018 identified 14 biotransformed products based on neoruscogenin, a major spirostanol steroid from *Ruscus aculeatus* (butcher's broom), as a substrate [36]. As well as with *Rhodococcus equi* [87]. *R. aculeatus* this plant, used in pharmaceutical preparations to treat conditions such as: chronic venous insufficiency, varicose veins, hemorrhoids, and orthostatic hypotension, was processed by the endophytic fungus *Alternaria eureka*, that primarily facilitated oxygenation, oxidation, and epoxidation reactions. Recent metabolomics and bioinformatics studies, have revealed that certain uncultured members of Cluster IV of bacterial anaerobic genus, belong to *Clostridium* within the human gut microbiota carry genes for cholesterol dehydrogenases, specifically intestinal sterol metabolism A (*ismA*), that can convert cholesterol to coprostanol [11,16,17,114-115]. The regulation and transcription mechanisms of various isoforms of the *KshAB* gene are of particular interest to researchers [62-64]. According to data obtained by Baldanta *et al.* (2021), the *KshA2* and *KshA3* isoforms serve as the primary enzymes responsible for the degradation of androst-4-ene-3,17-dione and cholesterol, respectively, while *KshA1* plays a supportive role in these processes [85,86,9,116]. Beyond C-19 steroids, valuable 23,24-dinorcholane derivatives—important precursors for corticosteroid synthesis—have been microbially derived from sterols [4,66,86,93,97,103,117-118,123]. These transformations have also been reported in other bacterial genera [91,100-103,107-108,122], as well as through the catabolism of diosgenin by *Mycobacterium* sp. mHust- $\Delta$ *kstD1,2,3* [68-69,101,124-130].

Table 1. Microorganisms diversity, substrate, and fermentation condition for steroid Biotransformation

| Genus and species of microorganisms            | Steroid substrate            | Steroid product                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Fermentation conditions                                                     | Ref.          |
|------------------------------------------------|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|---------------|
| <i>Alternaria eureka (fungi)</i>               | Neoruscogenin (sapogenine)   | cholesta-5,25(27)-diene-1β,3β,7β,14α,26-pentaol 16,22-dione, cholesta-5,25(27)-diene-1β,3β,7α,14α,26-pentaol 16,22-dione, cholesta-5,25(27)-diene-1β,3β,14α,26-tetraol-7,16,22-trione, 26-O acetylcholesta-5,25(27)-diene-1β,3β,14α,26-tetraol-7,16,22-trione, cholesta-4,6,25(27)-triene-1β,14α,26 triol-3,16,22-trione, 18(22)-epoxyfurosta-5,25(27)-diene 1β,3β,7α,26-tetraol, spirost-5,25(27)-diene-1β,3β,7β-triol, spirosta-5,25(27)-diene-1β,3β,7α,14α-tetraol, spirost 5,25(27)-diene-1β,3β,7α,12β,14α-pentaol, spirosta-5,25(27)diene 1β,3β,7α,12β-tetraol, spirosta-5,25(27)-diene-1β,3β,7α,11α-tetraol, spirosta-5,25(27) diene-1β,3β,8β,14α-tetraol-7-one, spirosta-5,25(27)-diene-1β,3β,14α-triol-7-one and 5β(6)-epoxyspirost-25(27)-ene-1β,3β,7α,14α-tetraol. | 10 days, 25 °C and 180 rpm                                                  | [36,123]      |
| Rumen mixed anaerobicmicroorganisms (bacteria) | Progesterone                 | Testosteroni and androsta-1,4-diene-3,17-dione                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 18 days; five lactating, nonpregnant Holstein cows of 2 breeds (BW and DIM) | [119]         |
| <i>Bacillus megaterium</i> ATCC13368           | Prednisone and dexamethasone | 15β-hydroxydexamethasone                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 10 days, 25 °C and 180 rpm                                                  | [120,121,122] |

Conclusion

Steroids are naturally occurring compounds found throughout all living kingdoms. The chemical modifications of various carbon atoms, in the steroid framework are closely linked to the biological activity of these molecules. This is why MIT is a very important for producing these compounds, since the improvements facilitate specific chemical alterations that enhance its biological functions. Steroids can be synthesized through both chemical and microbial processes. Industrially, while well-known chemical methods are commonly used, microbial processes have interesting and valuable alternatives by enabling biotransformation or bioconversion. MIT has better and certain advantages over the chemical transformation, because its ability to produce steroids with precise modifications and high selectivity, making it essential for developing steroid-based pharmaceuticals, with better and widely targeted therapeutic effects. Microbial diversity such as microalgae, bacteria, and fungi are commonly employed for steroid biotransformation. These processes are fundamental for releasing steroids used in a wide range of therapeutic applications, more than drug manufacturing, partial synthesis of new steroids, and evaluation for diverse applications such as hormones, diuretics, anabolic agents, anti-inflammatory agents, anti-androgens, contraceptives, and anti-tumor treatments. Interest in steroid MIT has increased in recent years due to the development of new, pharmacologically active compounds. This growth is supported by advancements in MIT strategies, as well as techniques of genetically modified microbial, newly isolated strains, immobilized enzymes, and optimized microbiological culture conditions. These improvements enhance the efficiency and specificity of the MIT processes, making them quite essential for producing high-value new steroid products.

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