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专论与综述

甾类化合物微生物转化与分解代谢机制研究进展

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摘 要：甾类化合物具有重要的生理医药作用，市场需求巨大。甾类化合物及其关键甾类药物通过微生物转化制备工艺较化学合成法具有区域立体选择性、减少合成步骤、缩短生产周期、提高收率以及生态友好等优点逐步被应用，然而甾类物质微生物分解代谢机制还有待进一步深入探索研究并确定。本文从甾类化合物结构种类与主要来源、生理功能、微生物转化与分解代谢机制的研究等方面进行了归纳，着重解析甾类化合物分解代谢过程关键酶系及其分子作用机制，为甾药化合物生产菌种改造与工程菌构建，以及微生物转化工业化生产工艺的开发提供参考。

关键词：甾类化合物，微生物转化，侧链降解，核心环降解，代谢机制

Advances in microbial transformation and catabolic mechanism of steroid compounds

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Abstract: Steroid compounds plays an important role in physiological and pharmaceutical effects and the market demand is very huge. The microbial transformation process of steroid compounds and their key steroids is gradually applied since its application offers a number of advantages over chemical synthesis as follows: regio-stereoselectivity, reducing the synthesis procedures, shortening the production cycle, improving yield, more eco-friendly process and so on. However, the mechanism of microbial catabolism of steroids is still to be further explored and determined. The essay comprehensively analyzes the structure types and main sources, physiological functions, microbial transformation and catabolic mechanisms of steroid compounds and focuses on the key enzymes and their molecular mechanisms of metabolic process of steroids, which can provide a reference for construction of engineering strains and the development of industrial production technology for microbial transformation of steroids.

Keywords: Steroid compounds, Microbial transformation, Side-chain degradation, Core-ring cleavage, Metabolic mechanism

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甾类化合物在医药领域的市场需求仅次于抗生素，全球总产值已达 400 亿元，有 400 多种授权甾类药物在流通，而且未来的需求还会递增[1-3]。甾类化合物是重要的生命元素，是构成甾醇(Sterols)、胆汁酸(Bile acid)、维生素 D (Vitamin D)、神经甾体(Neurosteroids)以及类固醇激素(Steroid hormones)的基本结构物质，具有重要的生理医药功能[3-4]。甾体药物大多是利用具有甾体骨架的天然产物进行结构改造及化学合成而成，甾体药物作为重要的激素药物对机体起着重要的调节作用，被开发成为麻醉药、抗心律失常药、抗细菌药、抗胆碱酯酶药、抗凝血剂、抗真菌药、抗肿瘤药、抗原生动物药、胆汁分泌剂、诊断剂、神经调节阻断剂、胆石分散剂、止血剂、钙调节剂、脂调节剂、神经病治疗剂、泻药与安定药等[5]。甾类药物最初是从动物肾上腺提取的，显然实际应用意义并不大。后来也有研究者尝试以有机小分子为原料出发通过化学方法全合成工艺的探索，虽然理论上可行，但是由于合成路线太长、收率低、反应性差且副产物处理困难，加之环境污染问题，严重缺乏工业生产应用价值[2,6-7]。

甾体药物的工业化生产主要有化学合成法和微生物转化法两种。以植物体中提取的薯蓣皂素(Diosgenin)、剑麻皂素(Tigogenin)、蕃麻皂素(Hecogenin)等皂素为主导原料，进一步借助化学方法合成甾药关键中间体的工艺面临生产成本高、步骤较多、过程复杂以及环境污染严重等客观问题[8-11]。1970 年薯蓣皂苷元及其它皂苷元正式被确立为甾醇药物合成的起始物质，但是由于供应量的减少和价格的攀升导致该工艺的利润有所下降，且 1992 年联合国卫生组织宣布禁止使用全化学合成法生产药物，已逐渐不适用甾醇工业化生产[12]。而微生物转化(Microbial transformation)是指利用微生物产生的酶作用于化合物的某一基团，使其转化为结构上类似且更有价值的新型产物，其反应本质为酶促反应，具有较强的专一性与手性选择性，可以弥补化学合成的不足。1908 年 Bondi 研究了胆酸代谢途径以及各种分枝杆菌分解氧化甾醇类化合物，1913 年 Sohngen 发现多种微生物能够以甾醇为唯一碳源生长，1952 年 Murray 与 Petersons 首次利用黑根霉一步催化黄体酮为 11α-羟基黄体酮，开创了微生物转化甾体化合物的先例。1944 年第一次发现分枝杆菌可以降解胆固醇转化为 4-雄烯二酮(4-Androstene-3,17-dione，4-AD)与 1,4-雄烯二酮(Androsta-1,4-diene-3,17-dione，ADD)，研究者相继发现更多不同微生物可以降解不同甾醇类化合物制备甾药关键中间体如 4-AD 或 ADD，而 4-AD 或 ADD 是甾类激素药物不可替代的中间体，几乎可以用于合成所有的甾类激素药物，因此以植物甾醇(Phytosterols)为原料利用微生物降解而制备关键中间体工艺，相对于以皂素为原料的化学合成工艺因具有原料来源稳定、无污染以及成本低的优点而被采用。然而，微生物降解甾类化合物的分解代谢机制仍有待进一步深入探索，而且不同的微生物间还存在一定的差异性。本文介绍了微生物转化甾类化合物及其分解代谢机制方面的研究进展，为进一步研究代谢分子机制及生产应用提供一定参考。

1 甾类化合物的结构种类及主要来源

甾体类化合物属于脂类，通常指一类特殊的多元环萜类化合物，包括核心环(Core ring)、C_{17} 位侧链(Side chain)以及不同官能团取代基(Functional groups)三部分，核心环(Core ring)由 A、B、C 三个完整封闭的六元环和一个五元环 D 环组成，C_{17} 位侧链通常为长烃类，包括 8 个碳链烷基、10 个碳烷基或者烯基等，官能团取代基主要指核心环碳原子连接的相关的官能团，常见位点有 C_3、C_9、C_{11}、C_{17}、C_{18}、C_{19} 等。根据核心环特点通常包括五类基本结构单元(图 1A)，即 Cholest、Chol、Pregn、Androst、Estr 等单元，其余的均为相应结构单元的衍生物结构。通常所说的甾醇类物质结构特点为 C_3 位 β-OH、C_{17} 位为 8–10 个碳链烷基或者烯基，C_{10} 位与 C_{13} 位连有甲基，如胆固醇(Cholesterol) (图 1B)。甾类化合物拥有类似的结构且结构复杂数目繁多，在自然界中存在上千种甾类化合物，普遍存在于动植物和部分微生物中[1,13-16]，胆固醇(Cholesterol)、

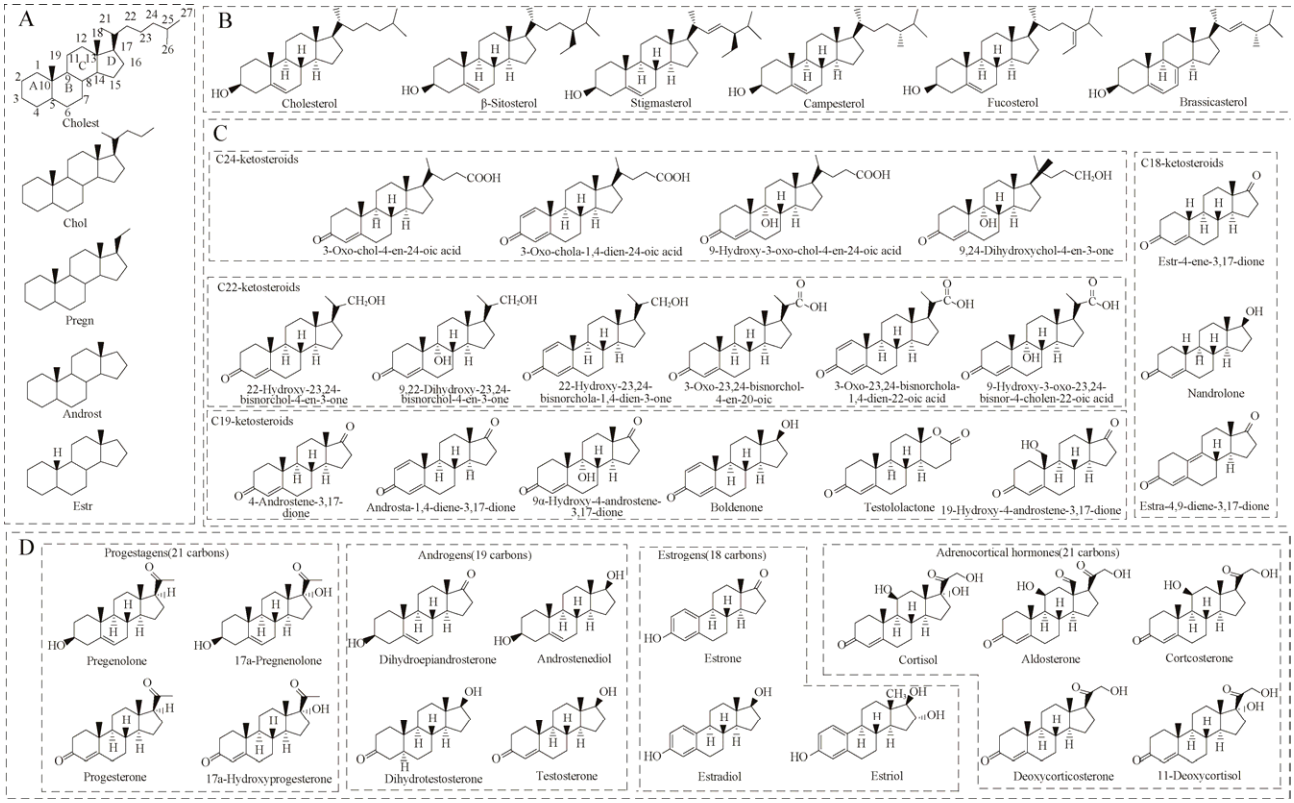

图 1　甾类化合物

Figure 1　Steroid compounds

注：A：甾类化合物常见基本结构单元；B：甾醇化合物；C：甾醇微生物降解关键中间体；D：甾体激素.

Note: A: Common structure unit of steroid compounds; B: Sterols; C: Key intermediate in degradation of sterols by microorganism; D: Steroid hormones.

性激素(Sex hormones)、肾上腺皮质激素(Adrenocortical hormones)、胆汁酸(Bile acids)、维生素 D (Vitamin D)及神经甾体(Neurosteroids)等存在于高等脊椎动物体中，蜕化类固醇(Ecdysteroids)存在于昆虫体内，麦角固醇(Ergosterol)存在于酵母细胞壁中，β-谷甾醇(β-Sitosterol)、豆甾醇(Stigmasterol)、菜油甾醇(Campesterol)、岩藻甾醇(Fucosterol)、菜籽甾醇(Brassicasterol)等(图 1B)存在于植物中。其中植物甾醇(Phytosterols，PSs)多达 250 种以上[1]，由于植物甾醇来源广泛且价格相对便宜，因此用于生产重要的医药甾药化合物或中间体(图 1C)。

2　甾类化合物的生理功能

关于甾体药物的作用机理，大多比较认可的是“基因表达学说”，即由于甾体激素分子量小(300 Da 左右)且脂溶性强，因此很容易透过细胞膜进入细胞内，首先与胞浆受体结合形成复合物，然后穿过细胞核膜与核内受体结合，进而启动或抑制 DNA 的复制转录，最终诱导或抑制新蛋白的生成，从而引起各种生理性状的改变[4,7,17]。甾体化合物的生理功能取决于核心环连接的官能团的种类、数目以及位置的构型[1,18]。甾体化合物主要包括胆固醇、植物甾醇、胆汁酸、维生素 D、神经甾体与类固醇激素等。胆固醇是构成细胞壁膜的成分之一，主要分布在磷脂双分子层之间，可以影响细胞膜的流动性以及调节细胞增殖与分化[19]，主要生理功能体现在参与血浆蛋白的合成，在体内可转化为胆汁酸盐、维生素 D_3 前体以及肾上腺皮质激素与性激素等(图 1D)。植物甾醇在食品中作为添加剂以及在化妆品

中也有重要的应用[3,13,20-21]。尤其是植物甾醇中的豆甾醇可以阻碍胆固醇的吸收，从而降低血清中胆固醇的含量，具有抗氧化活性，预防和治疗高血压、冠心病等心血管疾病，能促进血纤维蛋白溶酶的激素因子，可以预防血栓症，此外还可以抑制癌细胞的形成。胆汁酸是构成胆汁的重要成分，在胆汁中主要以甘氨酸胆甾酸和牛磺酸胆甾酸的钠盐或钾盐的形式存在，减少水与脂肪的表面张力，促进脂溶性维生素的吸收与脂肪的消化，具有调节糖代谢与能量代谢、抗炎症、药物代谢与脱毒的功效[15,22]。维生素 D 是一类具有抗佝偻病价值的维生素，对骨骼的形成有着重要的意义。神经甾体特指神经系统产生的甾体化合物，主要通过进入突触间隙调节 $GABA_A$ (γ-氨基丁酸 A 型)受体的功能而影响神经细胞的兴奋性，对中枢神经系统有着广泛的生理作用，具有保护神经细胞、镇静催眠、麻醉、抗惊厥、抗焦虑、促进学习记忆和改善认知能力等作用，而没有神经毒性和传统甾体激素的副作用[23]。研究表明，去氢表雄酮(Dihydroepiandrosterone，DHEA)可以直接作用于神经元细胞表面，改变神经递质受体结构，从而调节神经刺激在细胞间的传递[1,23-25]。

甾体激素又称类固醇激素，根据生理学功能分为肾上腺皮质激素和性激素，其中肾上腺皮质激素分为糖皮质激素(Glucocorticoids)和盐皮质激素(Mineralocorticoids)，而性激素包括孕激素(Progestogens)、雌性激素(Estrogens)、雄性激素(Androgens)以及蛋白同化激素类(Anabolic steroids)。肾上腺皮质激素基本结构中甾体母核含有 21 个碳，C_3 位与 C_{20} 位均为羰基，$C_{4\text{-}5}$ 为双键。糖皮质激素在 C_{17} 位有 α-羟基以及 C_{11} 位为羟基(如氢化可的松)或者羰基(如可的松)，是机体应激反应最重要的调节激素，参与调节糖类、脂肪与蛋白质代谢，调节机体发育生长以及免疫功能等，具有抗炎、抗毒、抗过敏、抗休克、非特异性抑制免疫及退热等多种作用，也是临床上使用最广泛而有效的抗炎和免疫抑制剂，而盐皮质激素在 C_{17} 位无 α-羟基与 C_{11} 位无氧(如去氧皮质酮)或者在 C_{11} 位有氧但与 C_{18} 位碳结合(如醛固酮)，主要生理作用是促进肾小管重吸收钠而保留水并排泄钾，与下丘脑分泌的抗利尿激素相互协调，共同维持机体电解质平衡和体液容量[5,7,23]。孕激素基本结构中母核含有 21 个碳，C_3 位为羰基，$C_{4\text{-}5}$ 为双键，C_{17} 位为 β-甲基酮，主要生理作用是促进女性附性器官成熟及第二性征出现，并维持正常性欲及生殖功能的激素[26]。雌性激素基本结构中母核含有 18 个碳，A 环为苯环结构，C_3 位为羟基，C_{17} 位为 β-羟基，主要生理作用是促进雌性第二性征的发育和性器官的成熟，促进蛋白质合成，减少碳水化合物利用，调节水、盐分子与钙的平衡，与孕激素一起完成性周期、妊娠、哺乳等，用于治疗女性性功能疾病、更年期综合症、骨质疏松症等[4,7]。雄性激素基本结构中母核含有 19 个碳，C_3 位为羰基，$C_{4\text{-}5}$ 为双键，C_{17} 位为 β-羟基，主要生理功能是促进男性器官及副性征的发育和成熟，对抗雌激素，抑制子宫内膜生长及垂体-性腺功能，增强免疫功能，促进蛋白质合成及骨质形成，刺激骨髓造血功能，使红细胞和血状红细胞增加[7]。蛋白同化激素是由雄性激素衍生出的一系列人工合成类固醇化合物，是一类外源性的以蛋白同化作用为主的甾体激素，可分为睾酮衍生物、雄烷衍生物、诺龙衍生物、杂环衍生物以及杂类合成类固醇，主要生理功能为雄性化作用显著减弱而蛋白同化作用增强，促进蛋白质合成和抑制蛋白质异化，促进组织钙化和生长，刺激骨髓造血功能，增加红血球量，促进组织新生和肉芽形成，降低胆固醇[5,7,18]。因此，开发甾类药物对改善人类健康体质显得尤为重要，具有非常重要的社会意义。

3 甾类化合物微生物转化与分解代谢机制

3.1 甾类化合物转化微生物

甾体微生物转化是利用微生物细胞酶系对甾体化合物某一特定部位进行化学反应产生新的产物。微生物对甾类化合物很多位置的原子或基团都有可能进行转化[27-30]，涉及的反应类型有氧化、还原、水解、酯化、缩合、异构化、重排以及侧链降

解等，羟化反应主要位点有 $C_{9\alpha}$、$C_{11\alpha}$、$C_{11\beta}$、$C_{15\alpha}$、$C_{16\alpha}$、$C_{16\beta}$、$C_{17\alpha}$、C_{19}、C_{26}，脱氢反应通常发生在 A 环的 $C_{1,2}$ 和 $C_{4,5}$ 位之间，芳香化反应主要发生在 A 环上，环氧化反应一般发生在 $C_{9,11}$ 和 $C_{14,15}$ 位之间，双键还原反应一般发生在 A 环 $C_{1,2}$、$C_{4,5}$ 和 B 环 $C_{5,6}$ 位之间，酮基还原主要体现在 C_3、C_{17}、C_{20} 位上，羟基氧化为酮基常见位点 $C_{3\alpha}$、$C_{3\beta}$、$C_{17\alpha}$。甾类化合物转化研究较多的主要体现在羟基化反应、脱氢反应以及侧链降解反应等方面。甾体转化微生物包括细菌、放线菌、酵母菌以及霉菌等。化学法除了在甾体 C_{17} 位比较容易引入羟基外，其它位点都很难引入，甾体微生物转化中羟化反应是最早用于工业化生产的，1952 年 Murray 与 Petersons 首次利用黑根霉一步催化黄体酮为 11α-羟基黄体酮，开创了微生物转化甾体化合物的先例。少根根霉(*R. arrhizus*)、黑曲霉(*Aspergillus niger*)、赭曲霉(*A. orchraceus*)、弯孢霉(*Curvularia* sp.)、小克银汉霉(*Cunninghamella* sp.)、金龟子绿僵菌(*Metarhizium anisopliae*)、球孢白僵菌(*Beauveria bassiana*)也可以催化甾类化合物 C_{11} 位羟基化[31-32]。对甾体 $C_{16\alpha}$ 位羟基化转化的微生物有玫瑰色链霉菌(*Streptomyces roseochromogenes*)、绿色链霉菌(*S. argenteolus*)、橄榄链霉菌(*S. olivaceus*)、银色链霉菌(*S. argenteolus*)等[5]。由于抗炎甾体激素药物在母核 $C_{1,2}$ 位引入双键后能成倍地增加抗炎作用，微生物脱氢也成为甾体抗炎激素药物合成不可缺少的一步。研究发现，降解甾类化合物的微生物大多数为 *Arthrobacter* spp.、*Brevibacterium* spp.、*Pseudomonas* spp.、*Norcardia* spp.、*Comamonas* spp.、*Rhodococcus* spp.以及 *Mycobacterium* spp.属等[33-59]。大多数在生产甾类药物中间体时为了防止核心环降解通常需要加入抑制剂[1]。部分真菌经过诱变筛选后也可以降解甾醇转化关键甾类药物前体物质 4-AD[60-61]、9-OHAD[62]和 ADD[63-68]。在上述众多的微生物菌属中，其中红球菌属(*Rhodococcus* spp.)与分枝杆菌属(*Mycobacterium* spp.)能够有效积累甾类药物关键中间体 4-AD、ADD 或 9-OHAD，因而引起研究者的广泛关注。红球菌是介于分枝杆菌与诺卡氏菌之间的一类微生物，与分枝杆菌很相似[69]。红球菌与分枝杆菌用于甾类转化生产主要基于几个方面特性：(1) 多数不具有侵染致病性，且表现为快速生产、易于培养增殖[2]；(2) 拥有完善的甾类降解基因簇，含有丰富的氧化还原酶以及 β-氧化酶系，有利于甾类化合物的快速分解代谢[70-74]；(3) 细胞壁中含有致密的分枝菌酸层，可以提高细胞的耐受力，适用于甾类的油水两相体系发酵工艺[75]，促进细胞对疏水性甾类化合物的吸收与转化[3,76]。

随着诱变育种技术的不断改进以及对微生物降解甾类机制的进一步揭示，研究者们已经选育出高效积累关键甾药前体物质的大量优良菌株[53,77-81]。近些年来，研究的重心又转向分枝杆菌属的新金分枝杆菌(*Mycolicibacterium neoaurum*)[54,56,82-90]与耻垢分枝杆菌(*Mycolicibacterium smegmatis*)[91-95]。

3.2 甾类化合物转化微生物生物信息学

甾类化合物分解代谢通路的推测源自对关键中间体的鉴定基础，因而也导致前期菌种选育方面遭遇到瓶颈。随着基因测序技术的发展，大多数降解甾醇、胆甾酸、睾酮等甾类的微生物也完成了基因测序[41,46,54,56,83,88-90,93,96-101]，其中多数为分枝杆菌(表 1)。

研究显示降解甾类化合物的相关基因通常成簇排列且多数基因会由于甾类化合物诱导显著上调，通过比较红球菌 *Rhodococcus jostii* RHA1 与结核分枝杆菌 *M. tuberculosis* H37Rv 甾醇代谢基因簇显示相似性比较高，且研究显示该基因簇受到甾类等底物诱导的水平也非常相似[91,95,102-104]。基于对红球菌、分枝杆菌等微生物甾类分子降解机制的研究，学者们逐步揭示了甾类降解关键基因簇并阐明了部分关键基因及其功能[52,72,95,104-111] (图 2)。Griffin 通过高密度转座子突变及高深度测序证明，结核分枝杆菌 *M. tuberculosis* H37Rv 与甾醇代谢的基因还有约 60%位于上述基因簇之外的其它位置且呈分散状态分布，一些快速生长型分枝杆菌还因同工酶的存在也给甾类化合物降解机制与工程菌改造等研究增加了

表 1　甾类代谢典型微生物基因组信息

Table 1　Genomic information of typical microorganisms with degradation steroids

Strain	GenBank No.	Size (bp)	CDS	*kstR*	*kstR2*	*igr*
Mycolicibacterium neoaurum ATCC 25795	JMDW00000000.1	5 468 381	5 192	RS0123370	RS0123530	Yes
Mycobacterium tuberculosis H37Rv	NC_000962.3	4 411 532	4 090	Rv3574	Rv3557c	Yes
Rhodococcus jostii RHA1	NC_008268.1	7 804 765	9 189	Ro04482	Ro04598	Yes
Mycolicibacterium neoaurum HGMS2	CP031414.1	5 421 383	5 078	RS00580	RS00705	Yes
Mycolicibacterium neoaurum MN2	LQMX00000000.1	5 383 007	5 073	RS22745	RS22135	Yes
Mycolicibacterium neoaurum MN4	JXYZ00000000.1	5 390 529	5 068	RS14335	RS02720	Yes
Mycolicibacterium neoaurum VKMAc-1815D	NC_023036.2	5 421 267	5 048	RS24245	RS24120	Yes
Mycobacterium sp. VKMAc-1816D	AOHQ00000000.1	5 413 599	5 091	RS6380	RS2135	Yes
Mycolicibacterium neoaurum VKMAc-1817D	CP009914.1	6 324 222	6 024	RS26515	RS26275	Yes
Mycolicibacterium neoaurum DSM 440744	CCDR00000000.1	5 504 703	5 272	RS24985	RS24790	Yes
Mycobacterium sp. NRRL B-3805	CP011022	5 421 338	5 049	RS24255	RS24130	Yes
Mycolicibacterium smegmatis MC2155	NC_008596.1	6 988 209	6 947	MSMEG_6042	MSMEG_6009	Yes
Pimelobacter simplex VKM Ac-2033D	CP009896.1	5 637 360	5 463	KR76_12270	KR76_25115	Lost chsE1

Note: *kstR/2*: TetR-type transcriptional regulator/2; *igr*: Intracellular growth.

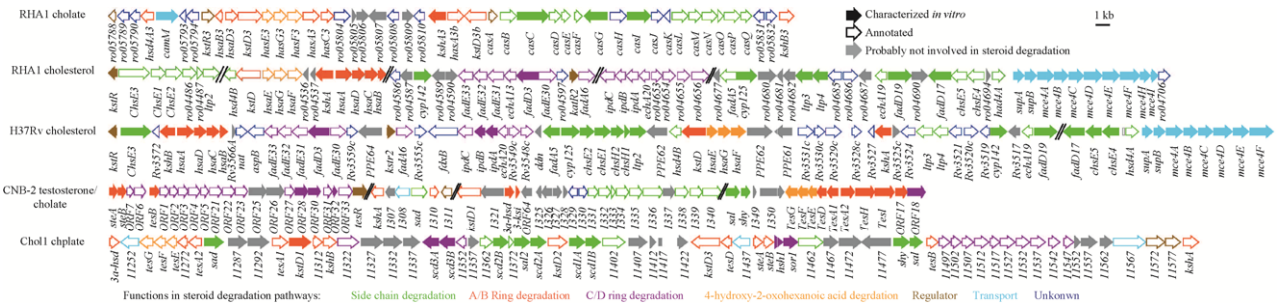

图 2　甾类降解关键基因[109]

Figure 2　The key genes in degradation of steroids[109]

困难[2,82,84,111-129]。随着转录组学与蛋白组学的发展，部分微生物也进行了甾类化合物诱导下的转录组、蛋白组以及基因微阵列的测定[74,91-92,102-103,130-132]。

3.3　甾类化合物微生物分解代谢机制解析

通常甾类化合物如甾醇等在微生物体内分解代谢过程大致包括 4 个阶段：(1) 甾类化合物的摄取与转运(Uptake and transportation)；(2) 底物活化(Substrate activation)；(3) 侧链降解(Side-chain degradation)；(4) 核心环降解(Core ring cleavage)。甾类化合物在微生物体内分解代谢过程属于典型的有氧代谢，前期的研究主要是基于某些代谢中间体推测可能的代谢途径[58,133-154]。但随着基因测序以及甾类化合物降解基因簇的揭示，结合核磁共振、高效液相色谱、高效液相-质谱联用、气相色谱以及气相色谱-质谱联用技术，借助基因工程手段，甾类化合物分解代谢的关键酶及其功能结构被逐步鉴定，实现大部分基因与代谢途径的准确匹配关联，多步反应机制得以准确阐明，从而进一步明确甾类化合物降解机制[2,27,45-46,71-73,82,85,94-95,102,105-106,109-111,115,122,155-175]。根据代谢过程中一些中间体，将代谢路径及方向大致拟定为 4-AD 途径、ADD 途径、9-OHAD 途径、HMP 途径、HMPD 途径、9-DHMP 途径、4-BNC 途径、1,4-BNC 途径、Ts 途径、BD 途径[2,63,82,86,119,123,176-179]。以 β-Sitosterol 和 Cholesterol 为例，结合基因功能从分子水平探讨甾醇分解代谢机制(图 3)。

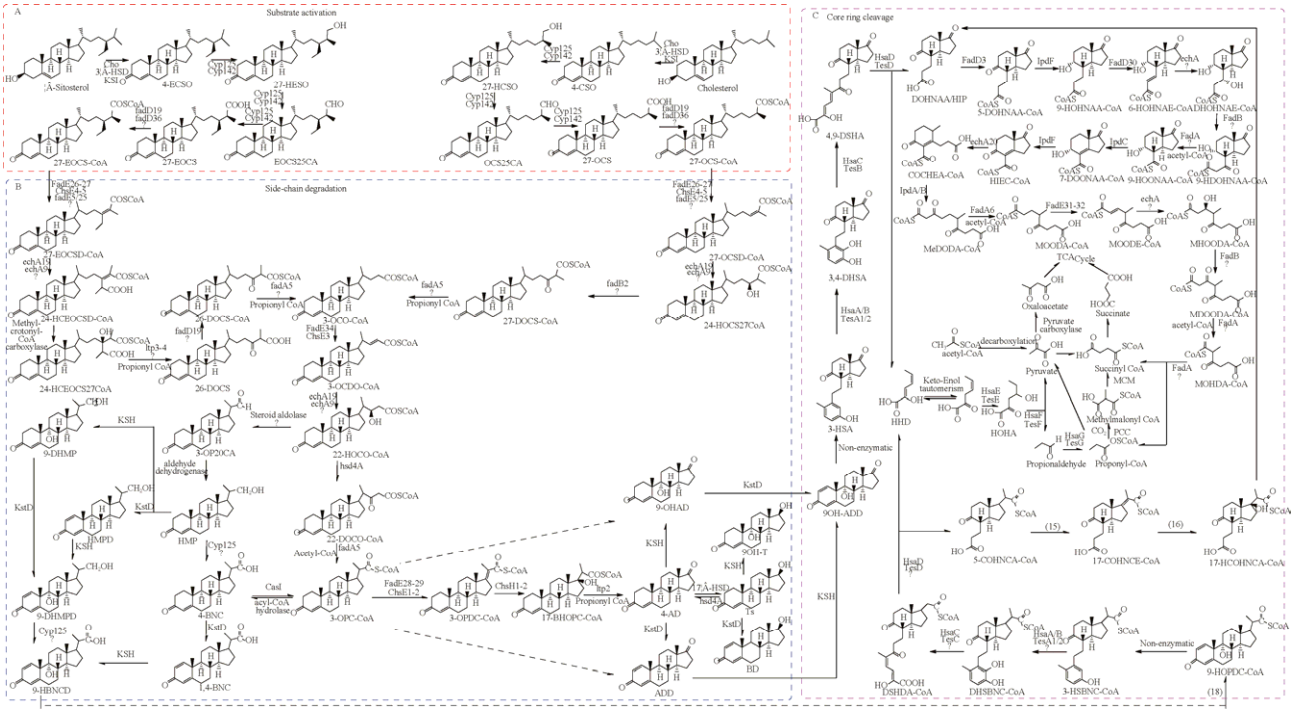

图 3　甾醇微生物分解代谢机制

Figure 3　Speculated catabolic mechanism of sterols in microorganism

Note: A: Sterol substrate activation; B: Side-chain degradation; C: Core ring cleavage. 1,4-BNC: 3-Oxo-23,24-bisnorchola-1,4-dien-22-oic acid; 1,4-BNC-CoA: 3-Oxo-23,24-bisnorchola-1,4-diene-22-oyl-CoA; 3,4-DHSA: 3,4-Dihydroxy-9,10-secoandrost-1,3,5(10)-triene-9,17-dione; 3-HSA: 3-Hydroxy-9,10-secoandrost-1,3,5(10)-triene-9,17-dione; 3-HSBNC-CoA: 3-Hydroxy-9-oxo-9,10-seco-23,24-bisnorchola-1,3,5(10)-trien-22-oyl-CoA; 3-OBNCT-CoA: 3-Oxo-23,24-bisnorchola-1,4,17-trien-22-oyl-CoA; 3-OP20CA: 3-Oxo-pregn-4-en-20-carbaldehyde; 3-OCO-CoA: 3-Oxo-chol-4-en-24-oyl-CoA; 3-OCDO-CoA: 3-Oxo-chola-4,22-dien-24-oyl-CoA; 3-OPC-CoA: 3-Oxo-4-pregnene-20-carboxyl-CoA; 3-OPDC-CoA: 3-Oxo-4,17-pregnadiene-20-carboxyl-CoA; 4,9-DSHA: 4,5-9,10-Diseco-3-hydroxy-5,9,17-trioxo-androsta-1(10),2-diene-4-oic acid; 4-AD: 4-Androstene-3,17-dione; 4-BNC: 3-Oxo-23,24-bisnorchol-4-en-20-oic acid; 4-CSO: Cholest-5-en-3-one; 4-ECSO: 24-Ethyl-cholest-4-en-3-one; 4-CSO: Cholest-4-en-3-one; 5-COHNCA-CoA: 5-Carboxy-9-oxo-1,2,3,4,10,19-hexanor-cholan-22-oyl-CoA; 5-DOHNAA-CoA: 9,17-Dioxo-1,2,3,4,10,19-hexanorandrostan-5-oyl-CoA; 5-ECSO: 24-Ethyl-cholest-5-en-3-one; 6-HOHNAE-CoA: 9-Hydroxy-17-oxo-1,2,3,4,10,19-hexanor-androst-6-en-oyl-CoA; 7-HDONAA-CoA: 7-Hydroxy-9,17-dioxo-1,2,3,4,5,6,10,19-octanorandrostan-7-oyl-CoA; 9,17-DHOPC-CoA: 9α,17β-Dihydroxy-3-oxo-pregn-4-ene-20-carboxyl-CoA; 9-DHMPD: 9,22-Dihydroxy-20-methylpregna-1,4-diene-3-one; 9-DHMP: 9,21-Dihydroxy-20-methylpregn-4-en-3-one; 9-DHMPD: 9,22-Dihydroxy-20-methylpregna-1,4-diene-3-one; 9-HDOHNAA-CoA: 9-Hydroxy-7,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oyl-CoA; 9-HBNCD: 9-Hydroxy-3-oxo-23,24-bisnorchola-1,4-diene-20-oic acid; 9-HOHNAA-CoA: 9-Hydroxy-17-oxo-1,2,3,4,10,19-hexanor-androstan-5-oyl-CoA; 9-HOONAA-CoA: 9-Hydroxy-17-oxo-1,2,3,4,5,6,10,19-otanorandrostan-7-oyl-CoA; 9-HOPDC-CoA: 9-Hydroxy-3-oxo-pregna-1,4-diene-20-carboxyl-CoA; 9-HOPC-CoA: 9α-Hydroxy-3-oxo-4-pregnene-20-carboxyl-CoA; 9-HOPCD-CoA: 9α-Hydroxy-3-Oxo-4,17-pregnadiene-20-carboxyl-CoA; 9-OHAD: 9α-Hydroxy-4-androstene-3,17-dione; 9OH-ADD: 9α-Hydroxy-androsta-1,4-diene-3,17-dione; 9-OHTs: 9α-Hydroxy-testosterone; 17-BHBNCD-CoA: 17β-Hydroxy-3-oxo-23,24-bisnorchola-1,4-dien-22-oyl-CoA; 17-BHOPC-CoA: 17β-Hydroxy-3-oxo-4-pregnene-20-carboxyl-CoA; 17-COHNCE-CoA: 5-Carboxy-9-oxo-1,2,3,4,10,19-hexanor-chol-17-en-22-oyl-CoA; 22-DOCO-CoA: 3,22-Dioxo-chol-4-en-24-oyl-CoA; 22-HOCO-CoA: 22-Hydroxy-3-oxo-chol-4-en-24-oyl-CoA; 17-HCOHNCA-CoA: 17-Hydroxy-5-carboxy-9-oxo-1,2,3,4,10,19-hexanor-cholan-22-oyl-CoA; 24-HOCS27CoA: 24-Hydroxy-3-oxo-4-cholesten-27-oyl-CoA; 24-HCEOCS27CoA: 24-Hydroxy-24-(1-carboxyethyl)-3-oxo-4-cholesten-27-oyl-CoA; 26-DOCS: 3,24-Dioxo-4-cholesten-26-oic acid; 26-DOCS-CoA: 3,24-Dioxo-4-cholesten-26-oyl-CoA; 27-CEOCSD-CoA: 24-(1-Carboxyethyl)-3-oxo-4,24-cholestadien-27-oyl-CoA; 27-DOCS-CoA: 3,24-Dioxo-4-cholesten-27-oyl-CoA; 27-EOCS-CoA: 24-Ethyl-3-oxo-4-cholestene-27-oyl-CoA; 27-HCSO: 27-Hydroxycholest-4-en-3-one; 27-HECSO: 24-Ethyl-27-hydroxycholest-4-en-3-one; 27-EOCS: 24-Ethyl-3-oxo-4-cholestene-27-oic acid; 27-OCS: 3-Oxo-4-cholestene-27-oic acid; 27-OCS-CoA: 3-Oxo-4-cholestene-27-oyl-CoA; 27-OCSD-CoA: 3-Oxo-4,24-cholestadien-27-oyl-CoA; ADD: Androsta-1,4-diene-3,17-dione; BD: Boldenone; COCHEA-CoA: (R)-2-(2-Carboxyethyl)-3-methyl-6-oxocyclohex-1-ene-1-carboxyl-CoA; DHOHNAA-CoA: 7,9-Dihydroxy-17-oxo-1,2,3,4,10,19-hexanorandrostan-5-oyl-CoA; DHSBNC-CoA: 3,4-Dihydroxy-9-oxo-9,10-seco-23,24-bisnorchola-1,3,5(10)-trien-22-oyl-CoA; DSHDA-CoA: 4,5-9,10-Diseco-3-hydroxy-5,9-dioxo-androsta-1(10),2-dien-4-oyl-CoA; DOHNAA: 9,17-Dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid; EOCS25CA: 24-Ethyl-3-oxo-4-cholestene-25-carbaldehyde; HHD: 2-Hydroxy-cis-hex-2,4-dienoate; HIEC-CoA: (7aS)-7a-Methyl-1,5-dioxo-2,3,5,6,7,7a-hexahydro-1H-indene-4-carboxyl-CoA; HMP: 21-Hydroxy-20-methylpregn-4-en-3-one; HMPD: 20-Hydroxymethylpregna-1,4-dien-3-one; HOHA: 4-Hydroxy-2-oxohexanoic acid; MDOODA-CoA: 4-Methyl-5,7-dioxo-octanedioyl-CoA; MHOODA-CoA: 4-Methyl-7-hydroxy-5-Oxo-octanedioyl-CoA; MeDODA-CoA: 6-Methyl-3,7-dioxodecanedioyl-CoA; MOHDA-CoA: 4-Methyl-5-oxo-hexanedioyl-CoA; MOODA-CoA: 4-Methyl-5-oxo-octanedioyl-CoA; MOODE-CoA: 4-Methyl-5-oxo-7-octenedioyl-CoA; OCS25CA: (20S,25S)-3-Oxo-4-cholestene-25-carbaldehyde; Ts: Testosterone.

3.3.1 摄取与转运(Uptake and transportation)

研究表明在 *M. tuberculosis* 中 Mce4 操纵子编码的功能蛋白与甾醇转运密切相关，是 *M. tuberculosis* 摄取宿主细胞胆固醇的关键系统[180-181]。Mce (Mammalian cell entry) 操纵子存在于 *Mycobacteriaceae*、*Nocardiaceae*、*Intrasporangiaceae*、*Norcardioidaceae*、*Pseudonocardiaceae*、*Streptomycetaceae* 中，通常包含 2 种 *yrbE* 基因(*yrbEA*、*yrbEB*)与 6 种 *mce* 基因(*mceA*、*mceB*、*mceC*、*mceD*、*mceE*、*mceF*)[182-183]。在 *M. tuberculosis* 中存在 4 套 Mce 操纵子[184]，即 Mce1、Mce2、Mce3、Mce4，均可编码与 ABC 转运体(ATP-binding cassette transporters)类似的跨膜蛋白以及其它一些必需的膜表蛋白[2,185]。微生物对甾体底物的主动摄取主要跟 Mce4 密切相关，这也是部分致病菌侵染哺乳动物细胞后依靠降解细胞膜内胆固醇而长期寄生的关键[186]。研究显示在 *M. smegmatis* 中 Mce4 操纵子与胆固醇的转运也密切相关[187-188]。研究发现在 *Rhodococcus jostii* RHA1 也存在类似 *M. tuberculosis* 的 Mce4 功能蛋白家族，受 Cholesterol 显著诱导，当部分基因或全部基因缺失时菌株无法利用 Cholesterol、β-Sitosterol、5α-Cholesterol、5α-Cholestanone 等物质而生长，但对 4-AD、Progesterone、Cholic acid 等甾类物质的利用几乎无影响，Mce4 编码的转运子仅能识别 C_{17} 取代有至少 8 个碳原子的非极性类基团如胆固醇等物质[2,104,185]。

3.3.2 底物活化(Substrate activation)

甾醇底物活化主要包括 3 个阶段(图 3A)：

第一阶段：甾醇首先氧化为胆甾-5-烯-3-酮(5-Cholesten-3-one)，然后异构化为胆甾-4-烯-3-酮(4-Cholesten-3-one)，这一步主要由胆固醇氧化酶(Cholesterol oxidase，ChO)催化完成。ChO 为寡聚黄素氧化酶，以 FAD 为辅基，可同时催化甾醇 C_3 位的羟基氧化脱氢与 C_5 位向 C_4 位的异构化，包括 GMC (Glucose-methanol-choline，与 FAD 非共价结合)和 BOC2 (与 FAD 共价结合)两大类。不同来源的胆固醇氧化酶均可以催化多种 3β-羟基底物，上述两大类 ChO 对底物动力学和氧化还原能力差异性比较显著。研究显示 ChO 除上述功能外还可以催化胆固醇生成 6β-羟基-胆甾-4-烯-3-酮[2,189-191]。在 *M. neoaurum* ATCC 25795 基因组中存在两种类型的 ChoM1 与 ChoM2，ChoM2 虽然位于 kstR (TetR-type transcriptional regulator)调控区外但属于胞外分泌型，是参与氧化脱氢与异构化这一阶段的关键酶，而 ChoM1 位于 kstR 调控区内，与膜结合起辅助作用[2]。然而，在 *M. tuberculosis* 与 *M. smegmatis* 等微生物中催化这一阶段的关键酶并不是 ChO，而是由另一类甾类脱氢酶 3β-Hydroxysteroid dehydrogenase (3β-HSD)，以类似 NAD 或者 NADP 为辅基，该类蛋白家族普遍存在一段甘氨酸密集区(GX_2GX_2G)与活性中心(YX_2K)，虽然位于降解甾醇基因簇之外，却受到 kstR 转录因子的调控[111,192-193]。姚抗发现在 *M. neoaurum* ATCC 25795 基因组中也存在一种 3β-HSD，但证实不是甾醇代谢所必需的酶[2]。除了上述的 ChO 与 3β-HSD 具有 C_5 位向 C_4 位异构化功能外，在 *Mycobacterium*、*Pseudomonas putida*、*Comamonas testosteroni* 等菌中还存在另一类甾酮异构酶 Δ^5-3-Ketosteroid isomerase (KSI)，也可以直接催化胆甾-5-烯-3-酮向胆甾-4-烯-3-酮转化[194-204]，部分分枝杆菌还存在多种同工酶，分析推测可能在异构化这一步起协助催化作用。

第二阶段：胆甾-4-烯-3-酮 C_{27} 羟基化再氧化成甾醛(3-Oxo-4-cholestene-25-carbaldehyde)，最后氧化为甾酸(3-Oxo-4-cholestene-27-oic acid)，这是启动侧链降解的关键第一步。这一阶段主要由 Cytochrome P450 催化完成，主要包括 Cyp125 与 Cyp142，存在于 *M. tuberculosis*、*M. smegmatis*，两者催化位点在 C_{27} 位，但 Cyp125 催化产生 25S 羟化构型产物，而 Cyp142 产生 25R 羟化构型产物[74,107,126,205-208]。然而 *Rhodococcus jostii* RHA1[54,106] 与 *Mycobacterium bovis* BCG[209] 的 Cyp125 却主要催化 C_{26} 羟基化。Su 等研究 *M. neoaurum* TCCC 11978 发现存在 3 种 Cyp125，其中 Cyp125-1 位于 kstR 调节区 igr 因子，过表达引起 ADD(D)产率提升[84]，

通过同源性以及注释比对分析 *M. neoaurum* ATCC 25795 基因组也发现存在 3 种 Cyp125。

第三阶段:甾酸在乙酰辅酶 A 合成酶(Acyl-CoA synthetase)下发生转乙酰作用而被活化,侧链进一步进行类似脂肪酸的 β-氧化(β-Oxidation)过程,与这一步催化反应相关的酶为 FadD19 (Steroid-26-oyl-CoA synthetase),位于 *kstR* 调控区[102]。Yang 等[166]证实 FadD19 可以催化 3-Oxo-cholest-4-ene-26-oic acid 转化为 3-Oxo-cholest-4-ene-26-oyl CoA,但 Wilbrink 等[210]在 RG32 研究中发现敲除 *fadD19* 基因后不影响胆固醇的侧链降解,但可以阻断含有 C_{24} 支链的菜油甾醇与 β-谷甾醇的侧链降解,与 Wrońska 等研究 *M. smegmatis* MC^2 155 结论基本一致[171]。姚抗研究 *M. neoaurum* ATCC 25795 敲除 *kshA-fadD19-echA19* 后发现对植物甾醇转化效果不佳,但不影响胆固醇代谢[2]。虽然 Cyp125 产生 25S 构型甾酸产物,Cyp142 产生 25R 构型甾酸产物,研究显示 *M. tuberculosis* H37Rv 的 FadD19 对构型没有选择性,可以同时催化这两种构型产物[128,166]。可推断 FadD19 是植物甾醇降解的关键酶,而不是胆固醇代谢的限速因子,因此可能还存在其它催化该步骤的同工酶 FadD36[111],植物甾醇和胆固醇差异性体现在 C_{24} 存在支链,可知 FadD19 应该是一个多功能酶。

3.3.3 侧链降解(Side-chain degradation)

胆固醇的侧链降解类似于脂肪酸的 β-氧化(β-Oxidation)过程,共进行三轮氧化过程,前两轮基本上与 β-氧化一致,最后一轮脱丙酰辅酶 A (Propionyl CoA)属于反醇醛反应,此轮反应不同于脂肪酸的 β-氧化而有显著差异性,经过三轮氧化胆固醇依次释放出各 1 分子丙酰辅酶 A (Propionyl CoA)、乙酰辅酶 A (Acetyl CoA)和丙酰辅酶 A (Propionyl CoA),而 β-谷甾醇发生氧化作用则依次释放出 2 分子丙酰辅酶 A (Propionyl CoA)、1 分子乙酰辅酶 A (Acetyl CoA)、1 分子丙酰辅酶 A (Propionyl CoA) (图 3B)。脂肪酸的 β-氧化酶系包括 FadD family (Fatty-acid-CoA ligase)、FadE family (Acyl-CoA dehydrogenase)、EchA family (Enoyl-CoA hydratase)、FadB family (β-hydroxyacyl-CoA dehydrogenase)、FadA family (Acetoscetyl-CoA thiolase)。由 3.3.1 所述可知 FadD19 参与了第一步活化反应,因而推动了后续催化反应。

第一轮脱氢酶为 FadE26-FadE27 (ChsE4-ChsE5),第二轮脱氢酶为 FadE28 (ChsE3),第三轮脱氢酶为 FadE28-FadE29 (ChsE1-ChsE2),全部位于 kstR 调控区[2,46,71-72,102,110,166,175,211]。Yang 等研究 *M. tuberculosis* H37Rv 表明 ChsE4-ChsE5 对第一步 26-OCS-CoA 的脱氢反应活力最强,ChsE3 专一性催化第二步 3-OCO-CoA 的脱氢反应,而 ChsE1-ChsE2 专一性催化 3-OPC-CoA 的脱氢反应,此外 ChsE4-ChsE5 也可以催化 3-OCO-CoA 与 3-OPDC-CoA 的脱氢反应,对 ChsE3 与 ChsE1-ChsE2 起到一定的协助作用[166]。而 Wilbrink 等研究发现 RG32 敲除 *fadE26-fadE27* 后对菜油甾醇失去降解能力,而对 β-谷甾醇与胆固醇降解几乎没有影响[210]。同样情况也发生在 *M. neoaurum* ATCC 25795 中,敲除 *fadE26-fadE27* 后对主产物 4-AD 与 ADD 影响比较少,只是不再积累宝丹酮(Boldenone,BD)与睾酮(Testerone,Ts)[2]。虽然 *M. tuberculosis* H37Rv 的 FadD19 对 C_{27} 甾酸构型没有选择性,但是 ChsE4-ChsE5 却只专一性催化 25S 构型产物,而 25R 构型产物需要在 α-Methyl acyl CoA (MCR)异构酶转化为 25S 构型才能被进一步降解[128]。FadE26-FadE27 并非显著作用于 β-谷甾醇与胆固醇侧链降解,也可能还存在其它同工酶 FadE5 或 FadE25 发挥相关作用[111]。ChsE1-ChsE2 催化 3-OPC-CoA 为 3-OPDC-CoA 也得到了证实[166,170,175]。

Yang 等证实 ChsH1-ChsH2 可以催化 3-OPDC-CoA 为 17-BHOPC-CoA[175]。Wrońska 等研究发现 *M. smegmatis* MC^2 155 敲除 *echA19* 基因后对 β-谷甾醇与胆固醇降解几乎无影响[171],这与姚抗研究 *M. neoaurum* ATCC 25795 的结论一致[2]。综上可知,对于甾醇侧链降解前两步催化 Enoyl-CoA hydratase 相关酶如 EchA9[111]还有待进一步深入研究。

在 *M. tuberculosis* H37Rv 基因组中 β-Hydroxyacyl-CoA dehydrogenase 共有 5 种(FadB1、FadB2、FadB3、FadB4、FadB5)[101,110]。Taylor 等研究发现当敲除 *fadB2* 基因后几乎无影响[212]。截至目前，分枝杆菌第一个氧化过程催化 β-羟基转化为酮基的 β-Hydroxyacyl-CoA dehydrogenase 尚未报道。姚抗研究 *M. neoaurum* ATCC 25795 时证实 Hsd4A 是第二轮氧化中催化 22-HOCO-CoA 为 22-DOCO-CoA 的关键酶，敲除该基因后 C22 中间体 β-氧化代谢完全被抑制，转向新的旁路代谢途径 HMP 途径而生成 HMPD，此外该酶还具有 C17-β-羟基氧化作用，为双功能酶[2,165]。

Nesbitt 等研究发现 *M. tuberculosis* H37Rv 的 FadA5 具有 Thiolase 功能，它是转化胆固醇为 4-AD 与 ADD 产物的关键酶[172]。姚抗研究发现当敲除 *M. neoaurum* ATCC 25795 的 *fadA5* 基因后植物甾醇代谢过程不再积累 4-AD 与 ADD 产物，反而大量积累 HMPD，应该是转向旁路代谢途径 HMP 途径所致，说明发生 β-氧化的 4-AD 途径与 ADD 途径完全被抑制[2]。Schaefer 等证实 *M. tuberculosis* H37Rv 的 FadA5 具有催化 22-DOCO-CoA 为 3-OPC-CoA 的功能，推测可能也具有催化 27-DOCS-CoA 为 3-OCO-CoA 的功能，也就是第一轮 β-氧化的最后一步[173]。第三轮 β-氧化最后一步则由醛缩酶 Ltp2 完成，*ltp2* 属于 *igr* 操纵子，姚抗研究发现当敲除 *M. neoaurum* ATCC 25795 的 *ltp2* 基因后代谢产物发生显著变化，不再积累 4-AD 与 ADD 产物，而出现未见报道的 6 种复杂物质，但由于没有分离出单体物质而无法表征[2]。Gilbert 等证实 Ltp2 可以转化 17-BHOPC-CoA 为 4-AD 产物，证实 *M. tuberculosis* H37Rv 的 Ltp2 协同 ChsH2 形成复合体发挥最大催化作用，在生物体内可能形成 Ltp2-ChsH2-ChsH1 复合体发生作用[170]。

β-谷甾醇发生氧化作用与胆固醇氧化作用差异性源于 C_{24} 存在乙基支链，在第一轮脱氢后并非直接发生水合作用，而是 C_{28} 位首先发生羧化作用，这一步由 Methyl-crotonyl-CoA carboxylase 催化完成，该酶受到 β-Sitosterol 严格诱导，而 Cholesterol 则不能诱导[148]。接着由 Ltp3-Ltp4 发生反醛醇反应脱去 1 分子丙酰辅酶 A，Wilbrink 等在研究 RG32 时证实 Ltp3-Ltp4 是催化含有 C_{24} 支链 β-Sitosterol 与 Campesterol 等降解的关键酶系，具有醛缩酶功能[129]，而姚抗研究发现当敲除 *M. neoaurum* ATCC 25795 的 *ltp3-ltp4* 基因后植物甾醇代谢仍旧积累 4-AD 与 ADD 产物，与野生型基本一致，说明在该菌中 Ltp3-Ltp4 并非是植物甾醇降解的关键酶系，可能还存在其它同工酶作用，可能是侧链降解的关键酶系[2]。

综上所述，经过侧链降解后，1 分子胆固醇可以产生 2 分子丙酰辅酶 A 和 1 分子乙酰辅酶 A，而 1 分子 β-谷甾醇则可以产生 3 分子丙酰辅酶 A 和 1 分子乙酰辅酶 A，丙酰辅酶 A 与乙酰辅酶 A 最后会进入 TCA (Tricarboxylic acid cycle)循环进一步氧化分解。有研究者估算 1 分子的胆固醇经过氧化最后可得 7 分子的 $FADH_2$ 和 18 分子的 NADH，至少产生 80 单位的 ATP[148]。

3.3.4 核心环降解(Core ring cleavage)

核心环降解代谢(图 3C)是 3-甾酮-Δ^1-脱氢酶(3-Ketosteroid-Δ^1-dehydrogenase，KstD)与 3-甾酮-9α-羟化酶(3-Ketosteroid-9α-hydroxylase，KSH)共同作用引起的，KstD 使 A 环的 $C_{1(2)}$ 发生脱氢作用，而 KSH 则是使 B 环 C_9 发生羟基化作用，从而产生 9α-羟基-1,4-二烯甾体的不稳定结构，由于过高的分子势能自发引发 B 环 $C_{9(10)}$ 开环裂解，通常称为 9,10-开环降解途径(9,10-sec-pathway)。因此 KstD 与 KSH 成为重要甾药化合物筛选需要考虑的关键酶，KstD 的失活或缺失可以通过发酵富集制备 9-OHAD、9-DHMP、4-BNC 以及 Ts 等，而 KSH 的失活或缺失可以通过发酵富集制备 ADD、HMPD、1,4-BNC 以及 BD 等，当 KstD 与 KSH 两者同时缺失或失活时可以制备 4-AD、HMP 等。然而不同微生物中 KstD 与 KSH 的种类与功能存在较大的差异性，对底物的选择性有显著性差异，在生物体内是否受甾类物质诱导也存在明显不同。不同微生物的 KstD 与

KSH 可能存在多种同工酶基因。研究表明，KstD 多数为寡聚膜蛋白，结构中存在穿膜螺旋区，以黄素蛋白 FAD 为辅酶，在 N 端存在高度保守的 GSG(A/G)(A/G)(A/G)X_{17}E 结合区，该酶经纯化后未检测到活性，可能原因在于该酶的功能与细胞膜的生理状态密切相关[2,169]。Rohman 等从结构推测出 KstD 的催化机制为 Tyr^{487} 的羟基和 Gly^{491} 的酰胺键与底物 C_3 的羰基作用导致底物 C_3 酮式-烯醇式互变异构，Tyr^{318} 与 Tyr^{119} 通过氢键作用夺取 C_2 的 β-氢质子而 C_2 变成碳负离子，当负电荷转移到 C_1 时，FAD 的异咯嗪环 N_5 原子夺取 C_1 的 α-氢，此时在 C_1 与 C_2 之间形成双键[169]。KSH 属于 Class IV 型单加氧酶，由末端加氧酶(KshA)与铁流还原酶(KshB)双组分组成，KshB 存在 3 处参与电子传递的高度保守功能区，即 RCYSL 为 FAD 结合区，GSGITP 为 NAD 结合区，$CX_4CX_2CX_{29}C$ 为电子传递性铁流蛋白[2,213]。KSH 羟基化反应源于 NADH 的氧化，自由电子通过 KshB 辅酶 FAD 传递到铁流还原中心，过渡到 KshA 的 Rieske 结构域，最后在 KshA 活性中心 Fe^{2+} 处结合氧分子，从而完成 C_9-α 位的羟基化反应[2,122,162]。

在 *Rhodococcus erythropolis* SQ1 中发现分别存在两种 KstD[214-215]与 KSH[117,122]。大多数微生物一般只含有一种 KshB 组分，部分微生物可能存在多种 KshB 组分[54,105]，目前大多数研究主要集中在 KshA 组分及其功能与结构的研究，而对 KshB 组分、功能与结构研究较少，此外也有研究表明 KshB 组分甾体 C_{26}-羟基化密切相关[122,216]。魏巍在 *M. neoaurum* NwIB-01 中发现各存在 1 种 KstD、KshA 及 KshB，KstD 对 4-AD 催化效率较高，敲除或者使 KSH 失活会导致 ADD 产量增加[7,217-219]。而在 *M. tuberculosis* H37Rv 基因组中也仅存在 1 种 KstD、KshA 及 KshB，虽然 KstD 不能催化 4-AD 转化为 ADD[123]，但 KstD 与 KSH 催化关键中间 4-BNC 与 4-BNC-CoA 表现出较高酶活性[176,220]，同样在 HGMS2 中也发现仅存在 1 种 KstD[221]。在 *M. neoaurum* ATCC 25795 中存在 3 种 KstD 与 2 种 KshA 及 1 种 KshB[2,179]，MN-KstD1 显著受甾醇与 9OHAD 底物诱导，而 MN-KstD3 受 4-AD 显著诱导，MN-KstD2 则诱导作用不明显，推测 MN-KstD1 是 9-OHAD 途径关键脱氢酶，MN-KstD3 则是参与 4-AD 途径起到协助降解甾醇作用，MN-KstD1 对 9-OHAD 体现出高催化活性，而 MN-KstD3 对 4-AD 具有高催化能力；MN-KshA1 受到甾醇底物的显著诱导，MN-KshA2B 催化能力总体较弱，而 MN-KshA1B 对 4-BNC 与 1,4-BNC 表现出高催化能力，其次为 ADD，而对 4-AD 催化作用较弱。在 *M. neoaurum* DSM1381 中存在 3 种 KstD，其中 KstD1 受植物甾醇的显著诱导对 HMP 催化作用强，而 KstD2 对 4-AD 催化能力较 HMP 强[82]。在 *Rhodococcus ruber* Chol-4 中存在 3 种 KstD[119]与 3 种 KshA 及 1 种 KshB[114]，KstD1 对 9OHAD 与 Ts 具有高催化能力，KstD2 则对 Ts 与 Deoxycorticosterone (DOC)显示高活性，而 KstD3 仅仅体现在 5α-Ts；KshA2 对含有短支链的甾类底物催化能力强，KshA3 则对长支链甾醇底物体现出高活力，KshA1 可能与胆甾酸代谢有关。在 *Rhodococcus rhodochrous* DSM 43269 中存在 3 种 KstD[120]与 5 种 KshA 及 2 种 KshB[116]，KstD1 是导致 9-OHAD 降解的主要原因，其次是 KstD2，而 KstD3 无催化作用；5 种 KshA 同工酶显示出截然不同的底物特性，各自具有特定的底物诱导机制。9-OHAD 高产菌种 *M. neoaurum* VKM Ac-1817D 中也存在 5 种 KstD 与 5 种 KshA 组分及 2 种 KshB 组分，可以看出 5 种同工酶 KstD 对 4-AD 与 9OHAD 催化能力很弱，KSH 催化 4-AD 的能力可能相对较强，可以催化 Ts 为 9OH-Ts，也可以催化 HMP 为 9-DHMP[54-55,87,222-223]。在 4-AD 高产菌种 *M. neoaurum* VKM Ac-1815D 中仅存在 1 种 KstD 与 2 种 KshA 及 2 种 KshB 组分，表明 KstD 对 4-AD、9-OHAD、HMP 催化能力很弱，KSH 催化 4-AD 的能力也可能比较弱[53-54,90,222,224]。在 ADD 高产菌种 *M. neoaurum* VKM Ac-1816D 中仅存在 1 种 KstD 与 2 种 KshA 及 2 种 KshB 组分，表明 KstD 对 4-AD 催化能力很强，且同时也可以催化 HMP 为 HMPD，

而 KSH 催化 4-AD 的能力也可能比较弱[54,222]。

KstD 与 KSH 是核心环降解的两个关键酶，现在一致认同的是 9-OHADD 自发发生 B 环 $C_{9(10)}$位断链为 3-HSA，然后在 *M. tuberculosis* 的 Hsa 家族蛋白 HsaA/B、HsaC、HsaD 或者是 *C. testosteroni* 的 Tes 家族蛋白 TesA1/2、TesB、TesD 催化降解为 HHD (原 A 环结构)与 DOHNAA，HHD 在 *M. tuberculosis* 的 HsaE、HsaF、HsaG 或者是 *C. testosteroni* 的 TesE、TesG、TesF 进一步降解为丙酰辅酶 A 与丙酮酸，而 DOHNAA 在 FadD3、IpdF、FadE30、FadE33、FadE31-32、IpdC、IpdA/B、FadA6、EchA20 等酶系进一步转化为丙酰辅酶 A 和琥珀酰辅酶 A (Succinyl-CoA)[45,73,95,104,157-161,225-228]。Capyk 等研究发现胆固醇环降解酶系对含有支链的侧链代谢产物(如 9-HBNCD，9-Hydroxy-3-oxo-23,24-bisnorchola-1,4-diene-20-oic acid；9-HOPDC-CoA，9-Hroxy-3-oxo-pregna-1,4-diene-20-carboxyl-CoA)的活力要高于侧链完全降解产物(如 4-AD)[176]。另外，*Rhodococcus* spp.在胆甾酸降解中存在类似中间体降解开环途径[229-230]。此外，也有研究表明甾醇侧链降解与核心环部分降解几乎同时进行[176]，这也是导致部分关键甾药中间体摩尔收率下降的主要原因。

在 *Steroidobacter denitrificans* 研究中发现还存在 2,3-开环降解途径(2,3-sec-pathway)，Yang 等[155]研究该菌降解雄性激素(睾酮)发生 2,3-开环降解途径(图 4A)，Wang 等[156]在研究该菌降解胆固醇代谢时也发现发生了 2,3-开环降解途径(图 4B)。

综上所述，1 分子的胆固醇经过完全降解后将产生 4 分子丙酰辅酶 A、4 分子乙酰辅酶 A、1 分子丙酮酸和 1 分子琥珀酰辅酶 A，而 1 分子的 β-谷甾醇经过完全降解后将产生 5 分子丙酰辅酶 A、4 分子乙酰辅酶 A、1 分子丙酮酸和 1 分子琥珀酰辅酶 A，丙酰辅酶 A、乙酰辅酶 A 与琥珀酰辅酶 A 最后进入 TCA 循环发生彻底氧化，产生大量的 $FADH_2$、NADH 以及 ATP。

4 展望

微生物在甾类化合物转化过程中发挥了重要的作用，将先进的生物制造理念引进甾体药物生产工业，从而提高资源利用率，降低能耗，实现绿色制造可持续发展。因此，基于基因组学、蛋白组学以及转录组学进一步揭示关键酶系及其催化机理不仅具有重要的理论意义，而且具有重要的产业化价值。一方面，在已有的研究基础上进一步研究并探明甾类降解微生物分解代谢 HMP 途径、4-AD 途径、ADD 途径、9-OHAD 途径、HMP 途径等关键酶系及分流机制，借助基因工程手段，通过基因敲除构建新型甾药中间体生产工程菌，实现 4-AD、ADD、9-OHAD、DHEA、HMP 等甾药关键中间体单一产物的生产，提高转化率的同时实现破解产物分离纯化瓶颈难题，几乎所有的甾类激素药物都可以基于 4-AD 或 ADD 为原料合成，开发酶法或化学法新型制备工艺。另一方面，基于对已有转化微生物或者酶系结构功能的研究，通过转录组学与蛋白组学测定分析获得微生物转化酶基因序列

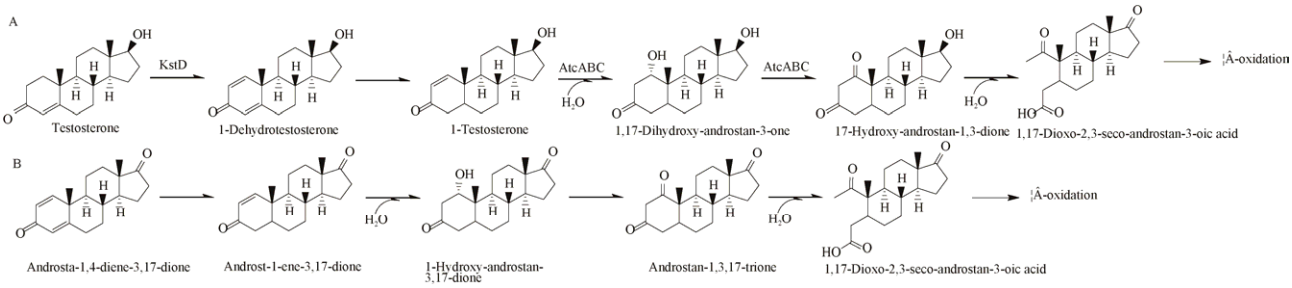

图 4　2,3-开环降解途径

Figure 4　2,3-sec-pathway of degradation

并克隆构建新型工程菌表达体系，进一步采用基因突变技术筛选高催化活性酶，借助酶工程技术实现酶法转化甾体药物，完成化学合成法不能完成的反应，以及克服化学合成法的区域与立体选择性不足，拓展 KstD、KSH、$C_{11/16/17}$ 位羟化酶等甾药转化酶系的应用研究，用于生产甾药活性成分(Active pharmaceutical ingredients，APIs)如 Testosterone、Boldenone、Dexamethasone 等，克服微生物细胞水平转化副产物的生成，提高甾药活性的同时避免化学法有害试剂残留对人体的危害。

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