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Reviewing a plethora of oxidative-type reactions catalyzed by whole cells of *Streptomyces* species

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Selective oxidation reactions represent a challenging task for conventional organic chemistry. Whole-cell biocatalysis provides a very convenient, easy to apply method to carry out different selective oxidation reactions including chemo-, regio-, and enantio–selective reactions. *Streptomyces* species are important biocatalysts as they can catalyze these selective reactions very efficiently owing to the wide diversity of enzymes and enzymatic cascades in their cell niche. In this review, we present and analyze most of the examples reported to date of oxidative reactions catalyzed by *Streptomyces* species as whole-cell biocatalysts. We discuss 33 different *Streptomyces* species and strains and the role they play in different oxidative reactions over the past five decades. The oxidative reactions have been classified into seven categories that include: hydroxylation of steroids/non-steroids, asymmetric sulfoxidations, oxidation of aldehydes, multi-step oxidations, oxidative cleavage, and *N*-oxidations. The role played by *Streptomyces* species as recombinant hosts catalyzing bio-oxidations has also been highlighted.

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Introduction

Biocatalysis contributes greatly to a growing number of transformations aimed at the conversion of natural and synthetic compounds under benign conditions, and detection and analysis of several compounds.[1] It is turning out to be a key enabling technology in the chemical and pharmaceutical industries, and various industrial bioeconomy sectors. Biocatalysts do not only facilitate the development of new drugs but also play a role in improving the synthesis of existing drugs using selective, 'green' methods.[2] For drug design aspects, biocatalysis is mainly focused in the study of early stage metabolism, which contributes to selection of the final form of a drug to ensure correct design and structure–activity relationship.[3]

Biocatalysis using whole microbial cells is preferably used when substrate and product structures are insensitive to other enzymatic functions present in the cells and when cofactor regeneration systems are required.[4] In general, the use of whole cells is easier and cheaper than the pure enzymes since enzyme

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isolation and purification are avoided and the enzymes retain better stability in their natural environment of cells and are also protected from shear forces in stirred bioreactors.[5,6] The use of whole cells is further motivated if the target enzyme is membrane-bound (intracellular) and in processes involving a cascade of enzymatic reactions *e.g.* for biosynthesis of secondary metabolites like antibiotics.[6] Furthermore, the stoichiometric consumption of nicotinamide cofactors such as NAD(P)H or $NAD(P)^+$ during the enzymatic reaction makes the use of whole cells important as it is capable of continuous cofactor regeneration.[5,6]

Oxidation is an important reaction in drug metabolism, hence a critical step in drug development processes.[7] Carbonyl compounds, dihydroxy compounds and epoxides, resulting from carbon–carbon oxidation, are among the valuable intermediates in the synthesis of pharmaceutical agents.[8] Pharmaceutical industries have started to rely on biocatalysis and to get access to effective enzymes for catalysis.[9] Synthesis of chiral compounds for various pharmaceutical preparations is challenging to achieve through chemical approaches, while the use of biocatalytic approaches provide routes to stereo-, regio-, and enantioselectivity.[10,11] In contrast to chemical oxidation reactions, biocatalytic counterparts can be carried out under ambient conditions giving rise to pure compounds without consuming high energy that affects selectivity.[12,13] Biocatalysts avoid the use of metals and complex chemicals that are problematic for waste treatment, and thus provide economic and environmental benefits over chemical catalysts.[9,12,14]

Oxidation *via* microbial approaches was developed long time ago to constitute key steps in the production of pharmaceutically important vitamins and steroids as well as several other organic compounds.[1] Consequently, such approaches were considered to be of most fundamental scientific interest and a starting point for innovative synthetic pathways. The great diversity of oxidative biocatalysts either in the form of natural producer organisms or as recombinant microbial cells has made the microbial oxidation toolbox quite handy for organic chemistry. Asymmetric oxidations are enabled through various microbial biocatalysts with inherent selective enzymes. In addition, methods of process optimization increase opportunities to enhance the conversion rates and selectivity, and reduce the substrate/product inhibition, while protein engineering also allows tailoring of the biocatalyst to the given reaction. Many microbial oxidations use mild oxidants (*i.e.*, molecular oxygen or hydrogen peroxide) that are benign and harmless, and minimize undesirable side reactions, unlike other oxidants (*e.g.*, peroxyacids). Therefore, replacing strong, non-selective oxidants with simple molecular oxygen represents an excellent practical choice.[4,15] The ability of microbial biocatalysts to conduct difficult oxidation reactions without protection/deprotection of the reactants is highly advantageous as compared to conventional chemical methods.[4,16,17]

Importance of *Streptomyces* species in whole-cell biocatalysis

Streptomyces is the most extensively studied genus of the actinobacteria phylum which represents one of the most diverse phyla in the bacterial domain.[18–21] Their secondary metabolism has been a focus of attention for many years,[19] as they represent three-fourths of the total bioactivity produced by Actinobacteria.[22,23] *Streptomyces* species produce different bioactive molecules including antibiotics such as tetracenomycin and streptomycin, pigments like melanin, and extracellular enzymes like tyrosinase.[24] Their versatile metabolism has further made *Streptomyces* species an important source of enzymes for the biocatalysis toolbox for advanced biotechnological applications.[25] Several *Streptomyces* species contribute to the processes of antibiotics, anticancer drugs, immunosuppressants, steroids, and anthelmintic agents.[26] Furthermore, together with other actinobacterial species, including *Rhodococcus* and *Corynebacterium*, they were use as whole-cell

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biocatalysts in different studies.[22] Genome mining approach and bioinformatic tools play vital roles in the search for novel biocatalysts within the different genomes of *Streptomyces* species. This helps in identifying enzyme functions, efficient enzyme production systems as well as many tools that are useful for rapid prototyping of the biocatalytic reaction.[1,25]

According to other review studies on related topics, there has been a long-term focus on secondary metabolism of *Streptomyces* species for finding novel antibiotics as well as developments for their production. This has overshadowed investigations on their potential for other biotechnological applications.[19,26] Additionally, there is one review that has discussed the applications of various enzymes derived from different *Streptomyces* strains;[25] and another has focused on the contributions of *Streptomyces* P450 enzymes in drug metabolism applications.[27] In the current review, we take a close look on the published works since the 1980s of using whole cells of different *Streptomyces* species as oxidative biocatalysts and highlight the main experimental tricks that allowed successful selective oxidation reactions.

Streptomyces culture conditions for induction of relevant enzymes

The application of *Streptomyces* as a whole-cell biocatalyst is in the form of either growing or as resting cells. Growing cultures maintain cofactors needed for recycling or regeneration systems, which ensure completion of reaction such as those dependent on P450 enzymatic systems.[28] On the other hand, the resting cells allow easier isolation of products during subsequent purification steps as the occurrence of side products and bacterial metabolites is much less than in growing cells.[5] It is also possible that the most favorable conditions for growth may not be suitable for the putative reaction. Therefore, optimization of biotransformation conditions is important, to induce and express the target enzymatic system, and to positively influence the biotransformation process.[29] This includes modification of the culture media or fermentation effectors such as pH, temperature, time, use of co-solvents, type of bioreactor, and speed of stirring. For example, the oxidative P450 enzyme system was induced in *S. griseus* upon growing on soybean flour as enriched media.[30–34] Genistein, the major component of soybean flour, was responsible for induction of enzyme expression although it inhibited cell growth due to its bacteriostatic effect.[35] In a study related to hydroxylation of vitamin D_3 derivatives catalyzed by *S. sclerotialus* FERM BP-1370 and *S. roseosporus* FERM BP-1574 grown in two different media, P450 enzyme was produced only in the enriched medium containing bacto-soytone, corn steep liquor and a high concentration of glucose.[36]

The use of low concentrations of substrates as inducers in primary pre-cultures is useful in several cases *e.g.*, with toxic substrates, as this helps in the adaptation of microbial cells. This evokes cells to express enzymes responsible for substrate conversion or metabolism to less toxic compounds as has been reported for transformation of several substrates using the whole cells of *Streptomyces*.[32,37–39] *Streptomyces* species might transform low concentrations of substrates unless the cells are induced by specific substrates. Remarkably, the resting (recombinant) cells of *S. avermitilis*, expressing isoflavone *O*-methyl transferase, oxidized daidzein at a concentration not exceeding 100 μM.[40]

Enzyme production is induced *in vivo* upon addition of specific substrates. Hernandez and coworkers examined a laccase-type phenol-oxidase produced by *S. cyaneus* CECT 3335,[39] where 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) was used as a substrate to induce expression of laccase activity in submerged cultures. The enzyme was applied for bleaching of eucalyptus Kraft pulps using ABTS as a mediator.

Co-solvents can help increase the solubility of hydrophobic or poorly water-soluble substrates, hence the permeability into bacterial cells. However, the choice of the type of co-solvents is crucial to ensure the safety of the process and maintenance of enzyme activity. So low concentrations of polar organic solvents help increase solubility of substrates and reaction rates and maintain selectivity.[4,41,42]

Detergents play critical role in some biotransformations, thanks to their effect on cell permeability, as reported in earlier and recent studies.[37,43–45] Triton X100 (1% v/v) increased the relative hydroxylation activity of *S. avermitilis* MA-4680 to transform daidzein by about 170%, while Brij 35 had no significant effect.[44] On the other hand, 1% (v/v) Brij-35 increased the hydroxylation activity of *S. avermitilis* MA-4680 for the biotransformation of phloretin to the respective regiospecific hydroxylated product.[45] Regarding the biotransformation of adamantane and its derivatives, Tween 60, at a concentration of 3% (v/v) increased the activity of *S. griseoplanus* AC122 for its hydroxylation to 1-adamantanol.[43] In a following study, the authors indicated that such detergents could have effect on the induced enzyme system responsible for the reaction.[37] Among the other detergents tested, Tween 20 doubled the activity of *Streptomyces* sp. SA8 cells for hydroxylation of 1-adamantanol although the substrate is poorly soluble in the detergent. Triton X-100 and Nymeen S-215 inhibited the activity probably due to the denaturation of the responsible enzyme system.[37]

Culture characteristics of *Streptomyces* species

The morphology of *Streptomyces* species was widely studied four decades ago, and they were classified into subgroups based on the nature and flexibility of their hairy spores.[46,47] Most *Streptomyces* exhibit the mycelial growth feature,[48] which represents a major disadvantage for industrial applications due to the formation of highly viscous cultures. This may cause problems in stirred tank reactors and contribute to mass and heat transfer problems. The morphology of *Streptomyces* mycelia grown in liquid media is affected by external factors including: (a) the medium composition and fermentation conditions, (b) chemical signaling molecules (*i.e.*, A-factor),[49] and (c) genetic factors, like the *ssgA* expression level.[50] The latter is critical for septum formation in aerial hyphae according to earlier reports.[51]

The cell morphology of the host also has a significant effect on the level of heterologous protein production since the filamentous nature of the microorganism impedes the

fermentation process due to the formation of large clumps, highly viscous culture, slow growth rates, and stirring problems.[52] Some genetic manipulation strategies positively impacted the morphology of *Streptomyces* species. *S. lividans* 1326 was selected to overexpress a morphogene, *ssgA*, that pleiotropically affects bacterial growth and cell division. This resulted in enhanced septation in vegetative hyphae and the formation of much wider hyphae that is in turn favorable for protein production and secretion.[52] Another species, *S. coelicolor*, has never been utilized in large-scale fermentation due to the formation of large pellets. A variant overproducing *ssgA*, *S. coelicolor* GSA2, produced smaller average-sized mycelia with many protruding hyphae, which enhanced growth rates and reduced lag phase.[50]

The type of culture medium has an essential role, as some proteins that induce hyphal growth were reported to be produced only in rich media. For instance, *SapB*, a surfactant peptide important for differentiation, is produced by *S. coelicolor* to induce efficient formation of aerial hyphae.[53] However, aerial hyphae are formed in minimal medium by other pathways mediated by substitute proteins like chaplins and rodlins. The overexpression of gene encoding *SapB* in chaplin-lacking mutant strains hardly restored aerial formation in minimal medium, indicating that chaplin alone is responsible for aerial morphogenesis on minimal medium. *SapB* and the chaplins are both essential for formation of aerial hyphae on rich medium since strains that did not produce both were bald under all examined growth conditions.[54]

Notably, morphology has a great effect on productivity in *Streptomyces* species. Mycelial mats with reduced branching rates and strong cell walls (which make nutrients be easily available to all hyphae) are more appropriate for efficient enzyme production. In comparison, for production of antibiotics, growth as pellets is more suitable, and greatly depends on the growth phase.[50,55] It was also reported in previous studies (in relation to antibiotic production from *S. antibioticus*) that the mode of growth from adopting different media, was taken into consideration to avoid producing too many mycelial clumps. Cultures containing carbon sources such as glucose and glycerol resulted in high biomass levels, but the oleandomycin production was not high. Contrarily, in cases of growth on nitrogen sources such as aspartic acid, the biomass levels were not as high as in the former case, but the oleandomycin production was higher.[56]

Fermentation parameters, including dissolved oxygen tension, rotation, and rates of oxygen uptake have critical impact on the differentiation of *Streptomyces* cell growth in bioreactors. There is a strong correlation between the hyphal growth and oxygen demand for respiration needed for maintenance of saturation, hence enhancing the catalytic efficiency.[57,58] In-depth studies on factors affecting growth and morphology have helped crucially in the optimization of protein production.[50]

Immobilized whole cells of *Streptomyces*

Application of immobilized cells enable easy removal and reusability, reduction of operational cost, use of continuous flow reactors, and low formation of secondary products due to the non-growing state of the cells. In addition, higher flow rate of reagents can be obtained due to the confinement of cells in a small region resulting in increased specific productivity.[5] However, *Streptomyces* sp. MTCC 7546 directly converted acrylonitrile into acrylic acid using the immobilized or free cells. Unlike other actinobacteria (*e.g.*, *Rhodococcus* and other genera), the acrylonitrile biotransformation was enhanced only by using their immobilized bacterial cells.[22] Other Actinobacteria exhibited higher activity in immobilized form as observed for *Gordonia terrae* catalyzing selective oxidation of prochiral sulfides.[59] As a preliminary test, we performed immobilization of *Streptomyces glaucescens* GLA.0 in calcium alginate beads. To avoid mycelial growth that might hinder confinement of cells on immobilized beads, basal mineral media containing 0.1% (v/v) of cyclohexanol, as a sole carbon source, was used instead of an enriched medium. The cells maintained viability and catalytic activity for up to two weeks compared to free cells exposed to the same conditions of growth (unpublished data).

Recombinant whole cells of *Streptomyces*

The use of recombinant microorganisms is an alternative system that serves for the overexpression of genes of the desired enzymes. This technique ensures the regeneration of cofactors required for reaction of interest. Secondary reactions can be avoided by knocking out genes expressing enzymes responsible for the unwanted pathway(s) or even by selection of another proper host. Such design can be tailored to be suitable at industrial scale levels with regard to the maximum substrate concentration and the physical and chemical environment.[5]

Streptomyces strains were successfully applied as good heterologous hosts for expression and production of target protein.[52] Many advantages of *Streptomyces* as recombinant hosts are that they do not form inclusion bodies, have a high secretion capacity, relatively low activity levels of extracellular proteases,[60] and are well suited for expressing GC-rich genes.[52]

The deletion of genes encoding for specific transcriptional repressors is a good strategy for enhanced protein expression in *Streptomyces* as reported for xylanase enzyme production.[52] As a prominent example, *S. lividans* is one of the most flexible species to be manipulated genetically, among the actinomycetes[61] and is thus considered the appropriate host for proper folding and efficient production of active enzymes.[50,52,62–64] Bifunctional plasmids formed from multi-copy number *E. coli* plasmids and *Streptomyces* shuttle plasmids were constructed and developed with inducible promoters, which enhanced the production of some proteins of interest.[52,65]

Streptomyces species as recombinant hosts for biocatalytic oxidations

Some *Streptomyces* strains have been exploited as heterologous hosts for cytochrome P450 genes from various wild type *Streptomycetes.* They are better than *E. coli* due to high GC contents of the native *Streptomyces* genes (up to more than 70%), which

make functional expression in *E. coli* more complicated.[40] *S. ahygroscopicus* ZB01 has high catalytic activity for the regio-specific hydroxylation of avermectin (1) to 4″-oxo-avermectin (2),[65] which is a key step in the synthesis of emamectin benzoate, a potent semisynthetic insecticide.[89] A cytochrome P450 gene, CYP107Z13, presumed to be responsible for the reaction, was cloned into a shuttle vector pKC1139 to generate pKCZ1, and then transformed into *S. lividans* TK54. The growing cells had higher catalytic activity for avermectin oxidation than resting cells due to effective coenzyme regeneration, electron transformation, and energy production. As cytochrome P450s require cofactor regeneration and exhibit poor stability in isolated forms,[90] use of recombinant whole-cell systems is an efficient approach for biotransformation processes. Since the direct chemical regiospecific oxidation of the 4-OH group in avermectin requires protection–deprotection steps, the biological approach is a greener and cheaper option for conducting such a reaction (Scheme 1).[65]

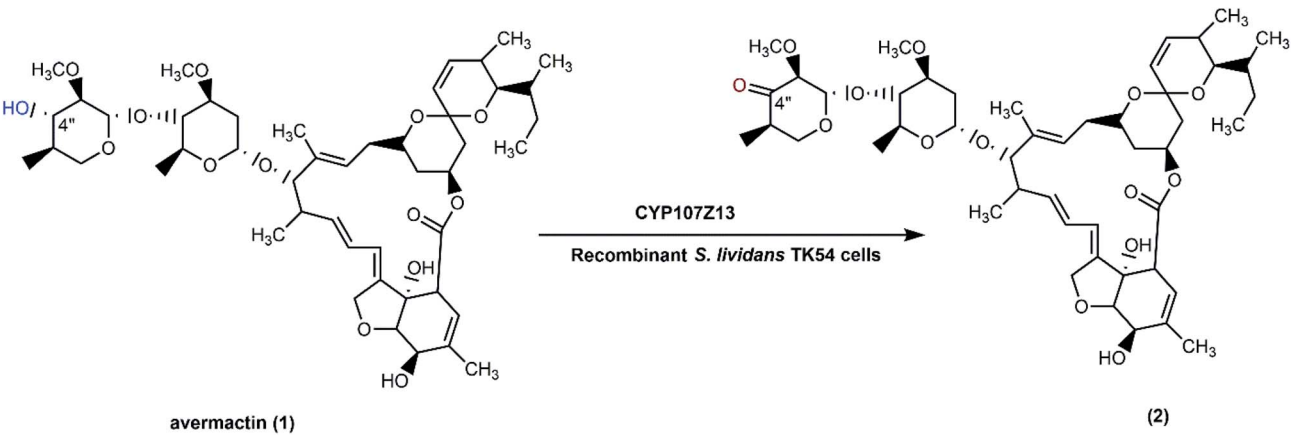
S. lividans TK24, was again exploited as a recombinant host for heterologous expression of two cytochrome P450 genes, CYP105D1 derived from *S. griseus* ATCC 13273, and CYP107B1 from *Saccharopolyspora erythraea* NRRL 2338.[67] To evaluate the activity of both enzymes, the biotransformation of 7-ethoxycoumarin (3) was performed using a whole-cell system upon expression in *S. lividans* TK24 (Scheme 2). Dealkylation of the substrate was directly observed with heterologously expressed CYP107B1, which depended on an endogenous electron transfer partner from the host. On the other hand, CYP105D1 exhibited oxidative activity (*O*-dealkylation activity) upon co-expression with a redox partner (ferredoxin reductase, FdR1) derived from a closely related strain, *S. coelicolor* A3 (II).[67,91] Remarkably, another cytochrome P450 enzyme, CYP107P3, is responsible for mediating the *O*-dealkylation of 7-ethoxycoumarin in the wild-type biotransformation of *S. griseus*.[89]

In addition, *S. avermitilis* is a useful host strain with various enzymes capable of useful transformations.[40,92] Unnatural *O*-methyl-isoflavone was produced, for the first time, with a genetically-modified construct of *S. avermitilis*.[40] Isoflavone *O*-methyltransferases were isolated from *Streptomyces* species and transformed into *S. avermitilis* ΔSaOMT2 strain (a deletion mutant of the gene encoding for *O*-methyltransferase). This was done in order to cancel the background *O*-methylation activity of the host. Among other constituted recombinant strains of *S. avermitilis*, only SeOMT3 showed the highest conversion yield of 4′-hydroxy-7-methoxyisoflavone (6). Upon coexpression with SAM synthetase gene (metK), the novel 4′,7-dihydroxy-3′-methoxyisoflavone (8) was produced due to 3′*O*-methylation of 3′,4′,7-trihydroxyisoflavone (7), the hydroxylated product of daidzein formed by other oxygenases found in the host (Scheme 3).

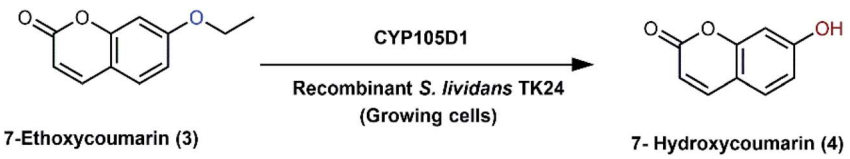
Another species, *S. albus* J1074 was used as a heterologous host for two genes, supposed to encode for crucial enzymes involved in the synthesis of borrelidin,[68] a polyketide macrolide produced by various *Streptomycetes*. To determine the metabolic pathway, the biosynthetic gene cluster was cloned from a producing strain, *S. parvulus* Tü4055.[93] Later, borrelidin non-producing mutants were generated to define and characterize the genes responsible for formation of nitrile moiety in borrelidin, about which little is known concerning the structural characteristics. According to the metabolites detected, one gene was supposed to code for a P450 monooxygenase (borI), and the other for an aminotransferase (borJ). An additional gene was likely encoding for a putative dehydrogenase. The activities of borI and borJ were confirmed by their heterologous expression in *S. albus* J1074, and bioconversion of the starting substrate, 12-desnitrile-12-methyl-borrelidin (9), to borrelidin (15). The reaction also included oxidation steps. As a result, the pathway for the formation of the nitrile moiety was proposed during borrelidin biosynthesis (Scheme 4).[68]

Role of cytochrome P450 enzymes in the oxidative transformations catalyzed by *Streptomycetes*

Streptomyces species are among the microorganisms possessing similar route for drug metabolism as humans, which involves



Scheme 1 Biotransformation of avermectin (1) with whole cells (resting or growing) of *S. lividans* TK54, as a recombinant host expressing CYP107Z13 gene from *S. ahygroscopicus* ZB01.



Scheme 2 Biotransformation of 7-ethoxycoumarin (3) with the growing cells of *S. lividans* TK24 overexpressing CYP105D1 gene derived from *S. griseus* ATCC 13273. This scheme has been adapted, with amendments, from Ueno *et al.*;[67] with permission from Elsevier, copyright 2005.

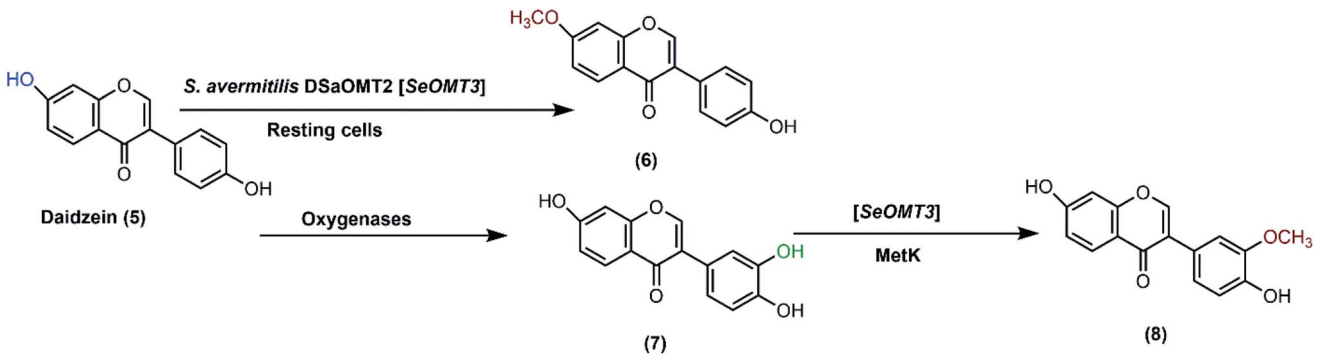
hydroxylation at a saturated carbon atom.[27] Whole-cell reactions using *Streptomyces* species serve to produce good quantities of hydroxylated derivatives that can be scaled for pharmacokinetic analysis since such metabolites are equivalent to those of mammalian ones.[3] As one of the richest producers of natural bioactives, several studies related to the biosynthetic pathways within *Streptomyces* have been set up indicating that P450s are key enzymes in such pathways,[74] which also represent excellent biocatalysts for various selective oxidations.[25,94] Bacterial P450s are more stable and allow for heterologous expression than membrane bound eukaryotic P450s. This enables not only feasible applications in organic and pharmaceutical industries, but also in pharmacological and toxicological studies for the produced drug metabolites.[95] It was noted that P450 enzymes are a major class of biocatalysts responsible for the oxidative metabolism of various drugs depending on electron transport systems.[67,91]

The genome sequences of *Streptomyces* species have revealed multiple cytochrome P450 genes.[96] Species such as *S. coelicolor* A3 (2), *S. avermitilis*, *S. scabies*, *S. hygroscopicus* and *S. peucetius* contain 18–33 P450s within their respective sequences.[97,98] P450s are heme-containing monooxygenases involved in the oxidative transformations in the secondary metabolism for production of bioactive molecules.[91] The oxidative steps catalyzed by P450s enzymes in biosynthetic pathways of antibiotics from different *Streptomyces* species have been recently reviewed.[97] *Streptomyces* P450s were also detected to be responsible for the stereo- and regio-specific oxidation of antibiotics such as oleandomycin.[91] Multiple P450 genes in *Streptomyces* aid in the stereochemistry required for preparing various therapeutic agents and can contribute to engineering strategies in the future.[97]

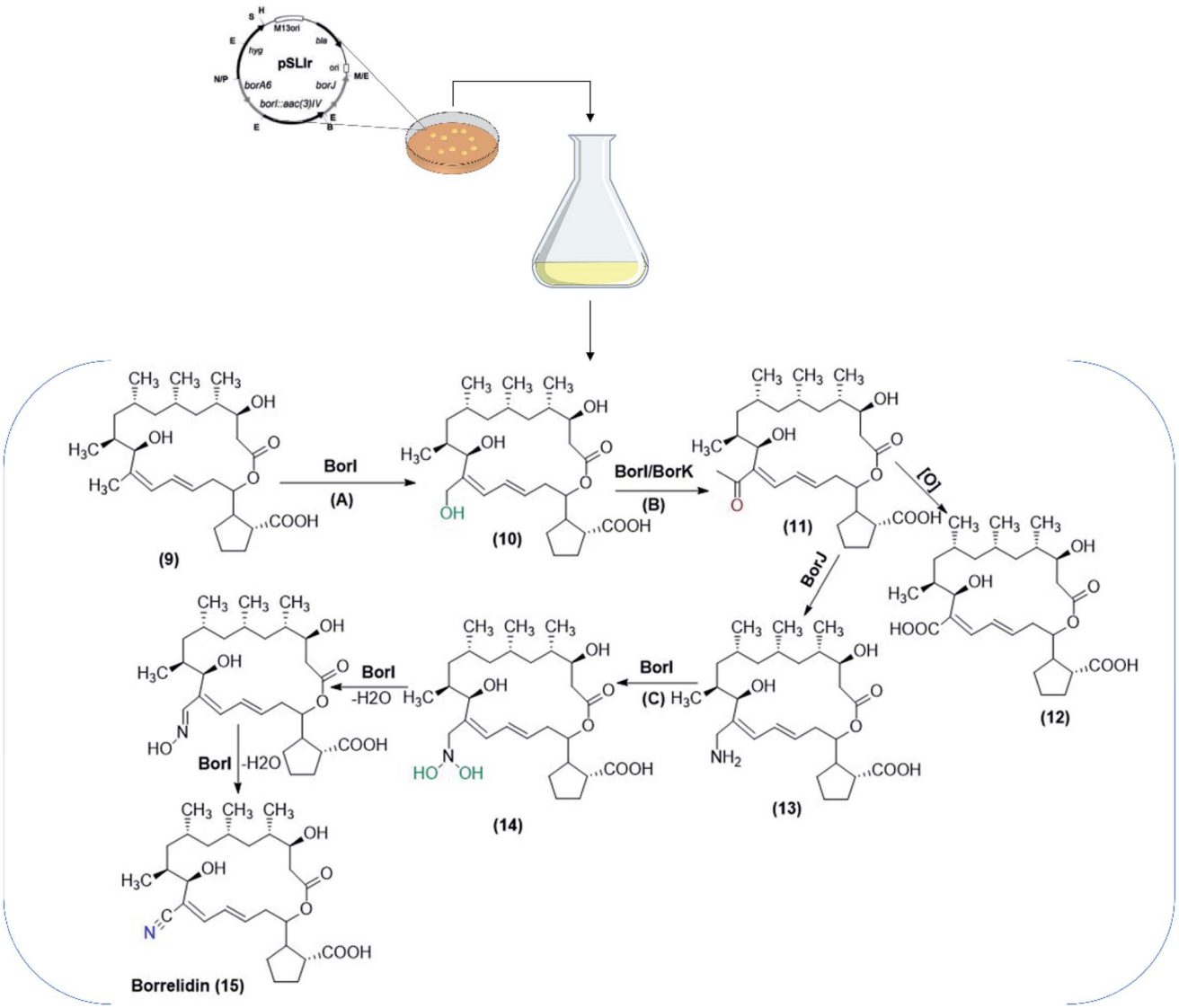
P450s possess highly strict substrate specificities displaying stereo-specific reactions including deamination, dealkylations, dehalogenation, epoxidation, *N*-oxidation, heteroatom oxidation, desaturation and peroxidation[98–100] in addition to hydroxylation as a common *Streptomycetes* catalyzed oxidative reactions.[97] However, P450s are also reported to have promiscuous activities, which enable interaction with diverse xenobiotics,[27,96] especially when involved in the bioactive synthetic pathways.[94]

Many previous studies on steroid biotransformations, catalyzed by *Streptomycetes* verified P450s to be the essential catalytic enzymes. *S. griseus* ATCC 13273 and *S. setonii* ATCC 39116, among others, catalyzed oxidation of five xenobiotics mimicking the mammalian rat liver P450s.[101] Diazepam and testosterone were subjected to aliphatic and aromatic hydroxylation, while warfarin and theophyllin were subjected to *N*-demethylation, but further oxidation of a hydroxyl group and an unsaturated carbon occurred to yield theophyllin metabolites.[76] In another study reported in 2007, *S. virginiae* IBL-14, isolated from soil, transformed diosgenone (16), a spiro steroid, to a rare nuatigenin-type spiro steroid, called isonuatigenone (17).[81] The latter is of pharmacological value in traditional medicine. In a subsequent study, a cytochrome P450 monooxygenase, *FcpC*, from *S. virginiae* IBL-14, was identified as the enzyme responsible for the tertiary hydroxylation activity at C-25 atom in the F-ring (Scheme 5).[82]

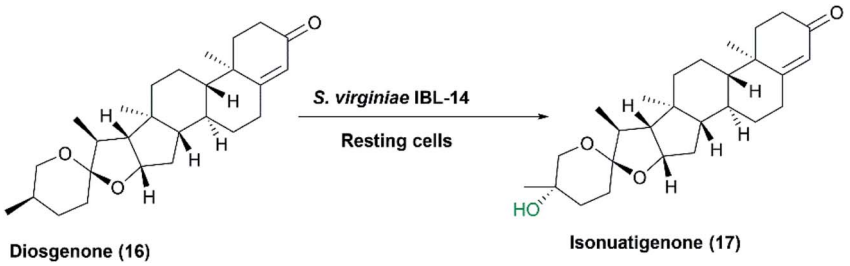
Recently, a novel cytochrome P450 enzyme, 154C2, from *S. avermitilis* has been identified and characterized for



Scheme 3 Biotransformation pathways for daidzein (5) catalyzed by the resting cells of *S. avermitilis* ΔSaOMT2 to 4′-hydroxy-7-methoxyisoflavone (6). Daidzein (5) was converted by the endogenous oxygenases of the host to the corresponding 3′*O*-methylated derivatives of 3′,4′,7-trihydroxyisoflavone (7), then further methylated to 4′,7-dihydroxy-3′-methoxyisoflavone (8) upon coexpression with SAM synthetase gene (metK). This scheme has been adapted, with amendments, from Choi *et al.*;[40] with permission from John Wiley and Sons, copyright 2013.



Scheme 4 The biotransformation pathway for the formation of nitrile moiety included in the borrelidin biosynthesis. The biocatalytic conversions were performed utilizing *S. albus* J1074 co-expressing biosynthetic genes (borI, borJ and borK), isolated from *S. parvulus* Tü4055. The process was performed through incorporation of substrates/intermediates in a solid medium for bacterial growth. The pathway involved *S. albus* J1074 co-expressing borI (A), *S. albus* J1074 co-expressing borI/borK (B) and *S. albus* J1074 co-expressing borI (C) as oxidation steps included in the biosynthetic pathways. The scheme has been adapted, with minor modifications, from Olano *et al.*;[68] with permission from John Wiley and Sons, copyright 2004.



Scheme 5 Biotransformation of diosgenone to the corresponding hydroxylated derivative, isonuatigenone, by the resting cells of *S. virginiae* IBL-14. This scheme has been adapted, with amendments, by permission from Springer, Applied Microbiology and Biotechnology; Wang, FQ., Li, B., Wang, W. *et al.* Biotransformation of diosgenin to nuatigenin-type steroid by a newly isolated strain, *Streptomyces virginiae* IBL-14.,[81] Copyright 2007.

regioselective 2α-hydroxylation of testosterone.[102] Hydroxylation of flavonoids by *S. avermitilis* MA4680 is attributed to such enzymes,[45] as indicated in the examples below. A cytochrome P450, CYP154C3, was identified from *S. griseus* and characterized as a 16α-specific hydroxylase. It was overproduced in *E. coli* and catalyzed the 16α hydroxylation of the D-ring of a range of steroids such as testosterone, estrone and deoxycorticosterone. Ferredoxin and ferredoxin reductase either acted as electron transporters or the isolated enzyme was fused with a P450 reductase from a *Rhodococcus* species.[103]

Präg and co-workers studied the ability of *S. afghaniensis* NC 5228 and *S. aurantiacus* JA 4570 to catalyze the regio- and stereoselective intermolecular oxidative phenol coupling.[104] This is the first detected activity performed by bacteria thus far. *In vitro* analysis and heterologous expression proved that a cytochrome P450 monooxygenase catalyzed such a selective reaction to produce axially chiral biaryl compounds. This enzyme could be comparable to the analogous ones from fungi or plants.[104] Moreover, P450s are believed to have essential role in bio-oxidation of chemicals such as coumarins, retinoids and alkaloids catalyzed by *Streptomyces*.[91]

Studies on electron transport systems of *Streptomyces* P450s involved in secondary metabolism have been done in the last fifteen years to evaluate their roles in biocatalysis of exogenous substrates.[91] The primary electron transfer pathway for a cytochrome P450 from *S. coelicolor*, P450105D5, which catalyzes the hydroxylation of endogenous fatty acids utilizing specific ferredoxin and ferredoxin reductase as redox partners, was established.[105,106] CYP107Z13 was found earlier to be responsible for regio-specific oxidation of avermectin to 4″-oxo-avermectin in *S. ahygroscopicus* ZB01.[65] In a following study, whole-cell recombinant systems were constructed co-expressing CYP107Z13 genes and the putative electron transporter proteins (*i.e.*, ferredoxin (Fd68) and ferredoxin reductases (FdR18/FdR28)). Both FdR18 and FdR28 were found to be critical for electron transfer in the oxidation of avermectin since the conversion was lowered by up to 60% in the gene-deleted mutants compared to the wild-type strain.[89]

CYP105 and CYP107 are the most prevalent and well-studied P450 families from *Streptomyces* species.[98] These enzymes tolerate a broad range of substrates correlating with the biosynthesis of natural products, such as antibiotics (*e.g.* erythromycin).[27,95] The CYP105 subfamilies have been detected in the genomes of all *Streptomycetes* associated with either biosynthesis of natural products or biotransformation of xenobiotics, which proves the structural variation within the members of this family.[100] CYP105D7, involved in the biosynthesis of pentalenic acid in *S. avermitilis*, has also exhibited regiospecific hydroxylation of isoflavones in both whole cells and as an isolated enzyme.[100,107,108]

To verify the role of P450s in hydroxylation activity catalyzed by whole *Streptomyces* cells, many studies relied on the action of different P450 inhibitors on the biotransformation process. Mitsukura, and co-workers found that menadione and 1-aminobenzotriazole inactivated the hydroxylation of adamantane catalyzed by *S. griseoplanus* AC122, which proved that the reaction proceeded *via* P450 cooperating systems.[43] Among other inhibitors, ketoconazole significantly inhibited the activity of *S. avermitilis* MA-4680 towards the hydroxylation of daidzein,[44] while quinidine completely blocked the hydroxylation of phloretin catalyzed by the organism.[45] Transformation of 1-adamantanol catalyzed by *Streptomyces* sp. SA8 was attributed to P450-dependent oxidizing enzymes, since the reaction was completely inactivated by specific or non-specific P450 inhibitors such as clotrimazole, menadione, and 1-aminobenzotriazole.[37] In an earlier study, carbon monoxide, metyrapone, and SKF-525-A, as specific P450 inhibitors inactivated the hydroxylating activity of *S. roseosporus* FERM BP-1574 and *S. sclerotialus* FERM BP-1370 for biotransformation of vitamin D3 derivatives, which indicated that such enzyme systems are involved in the oxidation process.[36]

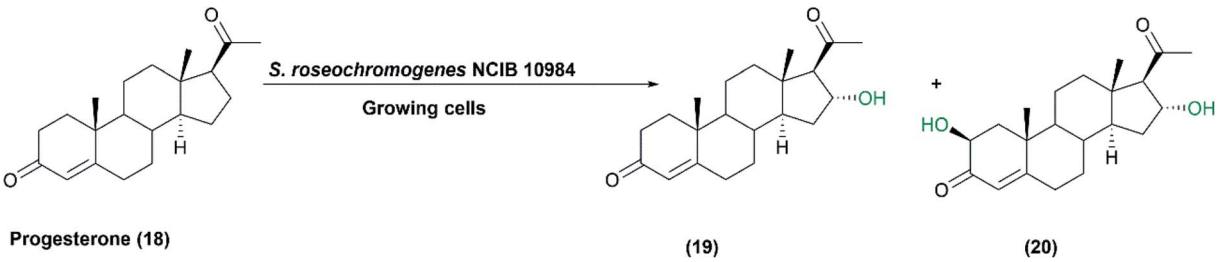
Examples of bio-oxidation using *Streptomyces* species

(1) Whole-cell hydroxylation of steroids catalyzed by *Streptomycetes*

Microbial biotransformation of natural steroids into pharmaceutically active intermediates has been practiced for many years.[22] Regarding hydroxylation of steroids, various *Streptomyces* species have been reported as excellent biocatalysts. The growing cells of *S. roseochromogenes* NCIB 10984 were used for the transformation of progesterone (18) to the respective monohydroxy and dihydroxy products.[74] 16α-Monohydroxy progesterone (19) and 2β, 16α-dihydroxyprogesterone (20) were produced as metabolites as shown in Scheme 6.[5] The progesterone16α-hydroxylase activity was determined *via* the sodium periodate method and the enzyme was purified for characterization.[5]

In a recently published study, *Streptomyces* sp. W2233-SM was checked for its capability to transform steroids, and for the occurrence of a P450 enzyme prior to proceeding to cloning and expression in *E. coli* as a host.[76] Progesterone (18), testosterone (21), and androstenedione (23) were converted to the respective mono-hydroxylated products (19), (22) and (24), using the resting bacterial culture (Scheme 7). CYP154C8 gene was cloned and over expressed in *E. coli* and confirmed as a steroid hydroxylating cytochrome P450.[76]

Hydroxylation of steroid compounds *via* microbial approaches has been widely used to obtain anti-inflammatory agents with high glucocorticoid activities. *S. roseochromogenes* ATCC 13400 was exploited by Restaino and coworkers in the hydroxylation of hydrocortisone (25) at the 16α position[75] (Scheme 8). The ability of the strain to convert steroid substrates was studied earlier,[109] and was found to depend on a P450 cytochrome multi-enzymatic complex including a specific 16α-hydroxylase and two other proteins, roseoredoxin and roseoredoxin reductase, involved in electron transportation. The process parameters were designed wisely to achieve the highest conversion to 16α-hydroxy hydrocortisone (26), a key intermediate in the production of the anti-inflammatory agent desfluorotriamcinolone. Screening of various cultivation parameters showed higher concentration of glucose, malt

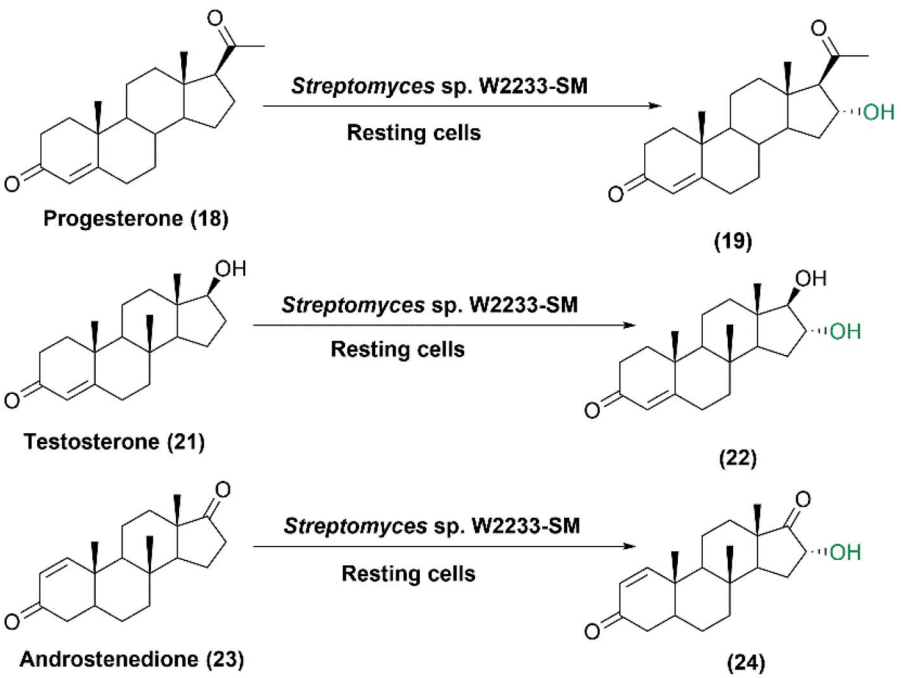


Scheme 6 Biotransformation of progesterone (18) with the growing cells of *S. roseochromogenes* NCIB 10984. This scheme has been adapted, with minor modifications, from Carballeira *et al.*;[5] with permission from Elsevier, copyright 2005.

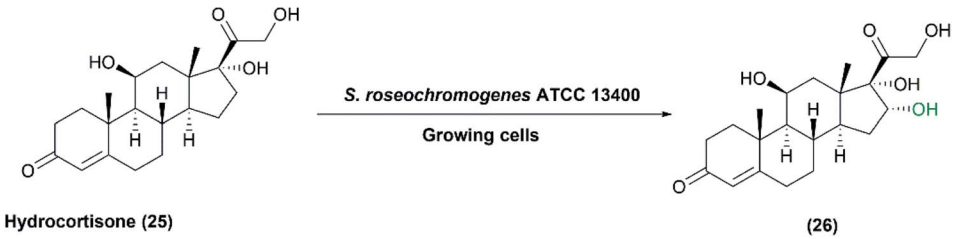
extract and yeast extract in a newly formulated media led to formation of 0.23 ± 0.01 g L^{-1} of 16α-hydroxy hydrocortisone in shake flasks.[75] Also, supplementation of hydrocortisone at the beginning of the experiment increased the conversion rates due to the induction of the P450 cytochrome expression as proved earlier.[109] Furthermore, maximum product concentration was obtained in a pulsed-batch strategy, reaching levels up to 0.508 ± 0.01 g L^{-1} starting from 1 g L^{-1} of the substrate.[75]

Later in 2016, another study was performed to improve the transformation of hydrocortisone (25) by the whole cells of *S. roseochromogenes*.[29] Physiological parameters were first studied in shake flasks to maximize conversion rates and to minimize the formation of by-products. The biomass density of 10.8 ± 0.2 g cell dry weight/L was obtained at pH 6 and 26 °C on rich medium reaching conversion rates above 70% on small scale. Besides, a conversion of approximately 77.5%, equivalent to 0.387 ± 0.01 g L^{-1} of 16α-hydroxy hydrocortisone (26), was obtained in batch fermentation (2.5 L) at the same pH and temperature. Fed-batch methods were employed with agitation at 400–600 rpm and air flow at 4–8 min^{-1} to maintain the dissolved oxygen (DO) concentration greater than 20%. This resulted in the highest production yield (0.804 ± 0.02 g L^{-1}) with lower by-product formation (5%), which was unprecedented according to steroid biotransformation by *Streptomyces* species. This could be due to the enhanced performance of the P450 enzymatic complex by a constant amount of oxygen compared to the pulsed batch strategy performed previously. The product was purified with a yield of 85% by reverse phase chromatography directly from the broth supernatant and separated from the substrate to reach a purity grade of 93%.

Coupled biocatalytic reactions are attractive for improving individual processes since they can overcome incomplete bioconversions.[1] In a recent study, the whole-cell system of *S. roseochromogenes* ATCC13400 was coupled to that of *Arthrobacter simplex* ATCC31652 sequentially or concurrently for transformation of hydrocortisone (25) at different temperatures.[28] *S. roseochromogenes* was responsible for the 16α-hydroxylation, while *A. simplex* performed 1,2-dehydrogenation



Scheme 7 Biotransformation of progesterone, testosterone, and androstenedione with resting cells of *Streptomyces* sp. W2233-SM.



Scheme 8 Biotransformation of hydrocortisone with the growing cells of *S. roseochromogenes* ATCC 13400.

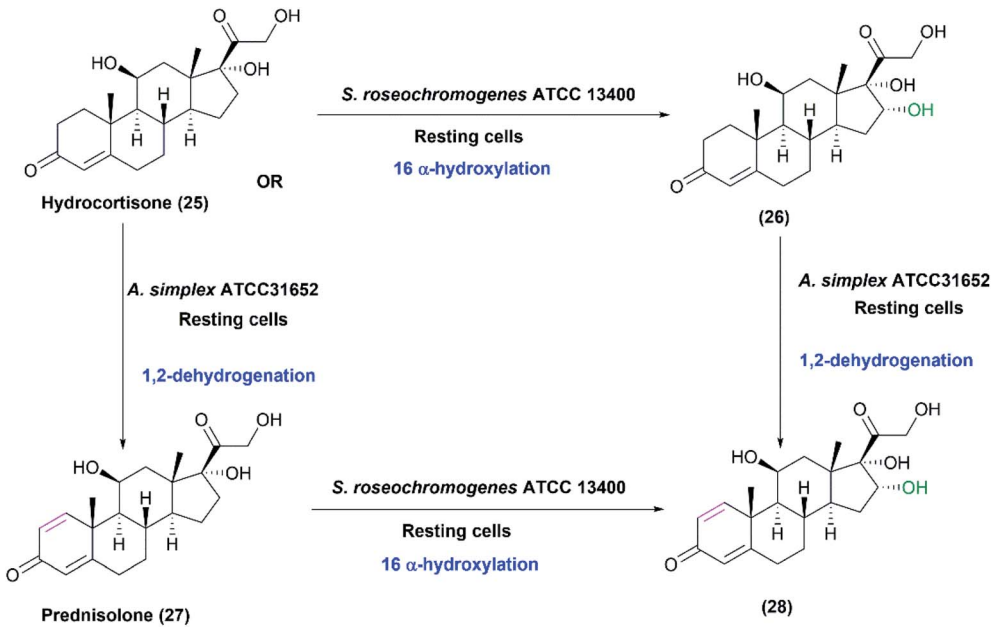
reaction. Starting from hydrocortisone, 16α-hydroxy prednisolone (28) was obtained at a yield of 68.8% after 120 h of the coupled reactions catalyzed by the two strains at pH 6.0 and 26 °C (Scheme 9).[28]

(2) Whole-cell hydroxylation of non-steroids catalyzed by *Streptomycetes*

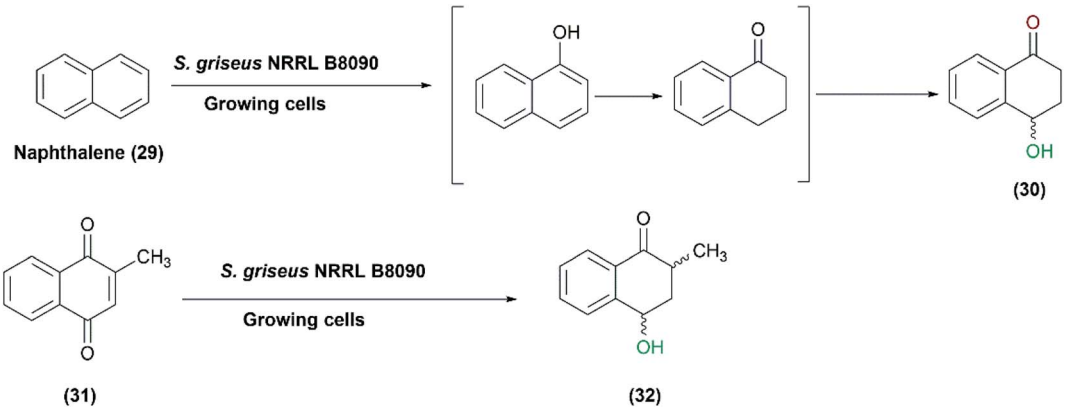
Streptomyces species can hydroxylate various compounds other than steroids such as iso-flavones, admantane derivatives, and daidzein and its analogues. In a study reported in 2007, growing cultures of *S. griseus* NRRL B8090 catalyzed the oxidation of naphthalene (29) to 4-hydroxy-1-tetralone (30). The latter is a natural product isolated from *Juglans mandshurcia Maximowicz*, which has anti-leishmanial and anti-diabetic activities.[70] Among different cultures, *S. griseus* NRRL B8090 gave the highest conversion, and the subsequent trials with the whole cells for a preparative scale conversion of 150 mg naphthalene resulted in 81 mg of the pure product after 72 h. Furthermore, another derivative, 2-methyl-1,4-naphthoquinone (31), was converted to the corresponding hydroxyl compounds (32) with the whole cells of the same *Streptomyces* strain (Scheme 10).[70]

Gurram *et al.* (2009) reported the use of S. *griseus* NCIM 2622, among five tested *actinomycetes*, to catalyze the oxidation of meloxicam (33), a non-steroidal anti-inflammatory drug, into two metabolites: 5-hydroxymethyl meloxicam (34) and trace amounts of 5-carboxy meloxicam (35) (Scheme 11), with a higher biological activity.[69] A moderate amount of 5-hydroxymethyl meloxicam was obtained at a yield of 41% utilizing growing cultures of *S. griseus* NCIM 2622.

Mori *et al.* conducted a study in 1996 on screening the activity of proline hydroxylases in different microbial cultures.[73] Among 3000 strains isolated from soil, few strains including *Streptomyces* sp. strain TH1 and *Streptomyces canus* ATCC12647 exhibited high specific and regio-hydroxylation. L-Proline (36) was converted to *cis*-3-hydroxy-L-proline (37) with a yield of 63% employing the resting cultures (Scheme 12). Proline 3-hydroxylase activity was preliminarily characterized in cell extracts. Notably, hydroxyl prolines are useful as chiral synthons in organic synthesis.[110]



Scheme 9 Biotransformation of hydrocortisone (25) by coupling the resting cultures of *S. roseochromogenes* ATCC13400 and *Arthrobacter simplex* ATCC31652. This scheme has been adapted, with amendments, with permission from Restaino *et al.*,[28] Molecules; published by MDPI, 2020.

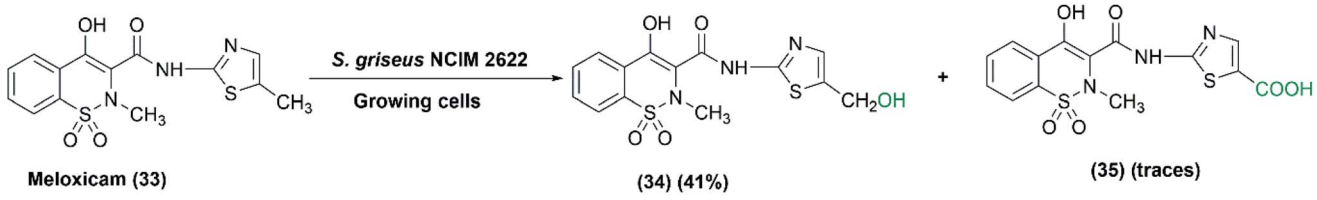


Scheme 10 Biotransformation of naphthalene (29) and 2-methyl-1,4-naphthoquinone (31) to 4-hydroxy-1-tetralone (30) and 2-methyl-4-hydroxy-1-tetralone (32), respectively, with the growing cells of *S. griseus* NRRL B8090. This scheme has been adapted, with minor modifications, from Gopishetty *et al.*;[70] with permission from Elsevier, copyright 2006.

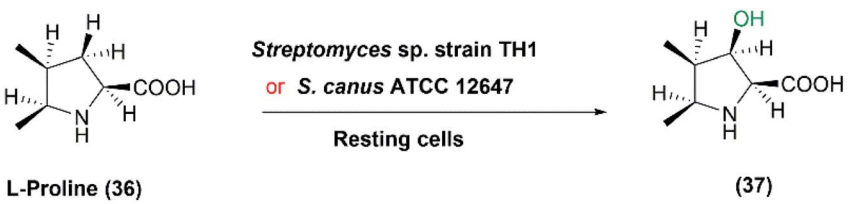
In 1991, the first study was conducted on the microbial hydroxylation of vitamin D_3 derivatives.[36] Among 300 *Streptomyces* species strains examined, *S. sclerotialus* FERM BP-1370 and *S. roseosporus* FERM BP-1574 could convert 25-hydroxyvitamin D3 (38) and l α-hydroxyvitamin D3 (39), respectively, to l-α,25-dihydroxyvitamin D3 (40) with a productivity of approximately 7 μg L^{-1} min^{-1} (Scheme 13). Cytochrome P450 inhibitors, including carbon monoxide and metyrapone influenced the hydroxylation process, indicating that P450 oxidizing enzyme system was responsible for mediating the reaction in the whole-cell suspensions.[36]

Hydroxylated adamantane, pharmaceutical intermediates, polymers, and materials for electronic industry were obtained upon conjugation of admantane (41) with *S. griseoplanus* AC122 cells. Among 470 examined strains, *S. griseoplanus* was found to be highly regioselective to give 1-adamantanol (42) from adamantane with up to 32% conversion yield (Scheme 14). Eventually, the products, 1-adamantanol (42) and 2-adamantanol were separated by silica gel chromatography, and obtained in purified forms.[43]

In another study aimed at screening actinomycetes displaying high hydroxylation activity with high regio-selectivity, *Streptomyces* sp. SA8 was one of the identified strains possessing the highest 1,3-admantanediol (43) producer from 1-adamantanol (42).[37] The resting culture produced 2.3 g L^{-1} of (43) at 69% conversion after 96 hours of incubation. Cycloketones such as adamantane, 1-admantanol, cyclohexane, cyclohexanol, or cyclooctane were utilized as inducers of hydroxylation activity during cultivation. 1-Admantanol at a concentration of 0.3% (w/v) exhibited the highest level of induction, and in combination with 1% (v/v) Tween 20, was most effective for preparing high activity resting cells. Also, glucose or glycerol was added at 100 mM acting as carbon sources for coenzyme-recycling systems. The growing cells of *Streptomyces* sp. SA8 produced 5.9 g L^{-1} of 1,3-admantane diol (43) from 6.2 g L^{-1} 1-admantanol (42) after 120 hours under optimized conditions.



Scheme 11 Biotransformation of meloxicam (33) by the growing cells of *S. griseus* NCIM 2622.



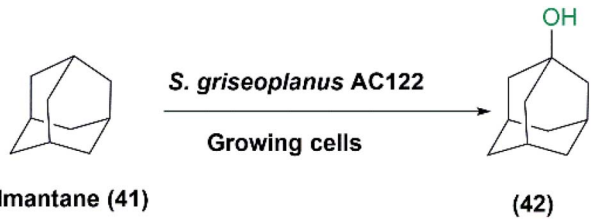
Scheme 12 Biotransformation of L-proline (36) with resting cells of *Streptomyces* sp. strain TH1 or *S. canus* ATCC 12647.

However, purification of the products by silica gel column chromatography was easier from the reaction mixture of the resting cultures than from culture broth owing to higher productivity. The ratio of the product 1,3-admantanediol (43) to the byproduct 1,4-admantanediol (44) was 85 : 15. This made *Streptomyces* sp. SA8 superior to other studied species for the regio-selective hydroxylation of 1-admantol (42) to the corresponding diol. The position, number, and steric effect of the hydroxyl group on the adamantane skeleton could influence the activity of the oxidizing enzymes of *Streptomyces* sp. SA8. meso-2,4-Admantanediol (46) and 1,4ax-admantanediol (44) were produced from 2-admantanol (45) at a ratio of 76 : 24 at 32% conversion rate. Also, 2-Me-2-adOH (46) was transformed into meso-2,4-ad (OH)2 (45) and a diastereomeric mixture of 2-Me-2,4-ad(OH)2 (47) at a ratio of 63 : 37, and at a 22% conversion rate (Scheme 15).[37]

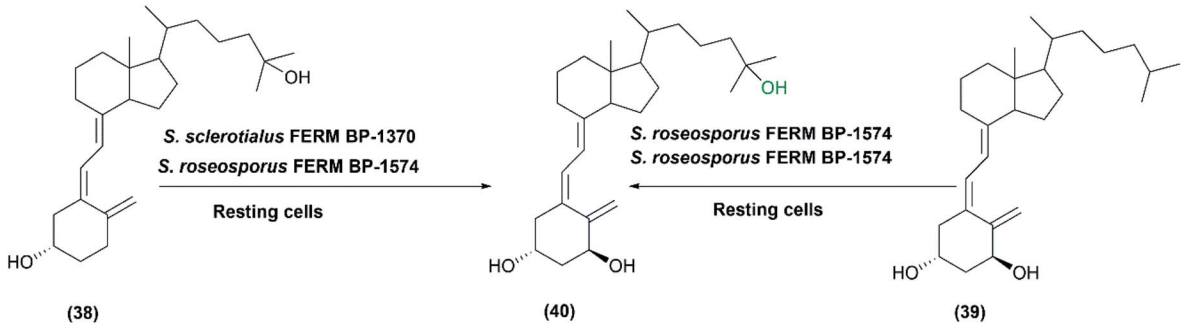
Daidzein and genistein are the major isoflavone compounds of phytoestrogen found in plants and soybeans. They act as antioxidants, antimicrobials, free radical scavengers, and metal chelators. Roh *et al.* reported that *Streptomyces avermitilis* MA-4680, among different screened microorganisms, exhibited regiospecific hydroxylation of the two isoflavones.[44] Daidzein (5) and genistein (48) were converted at highest rates to 3′,4′,7-trihydroxyisoflavone (7) and 3′,4′,5,7-tetrahydroxyisoflavone (49), respectively (Scheme 16). It is noteworthy that the detected antioxidant activities of the hydroxylated products are higher than the original isoflavones.[111] Upon using 100 g L^{-1} of *S. avermitilis* wet cell mass, *ortho*-dihydroxylated isoflavones, more potent antioxidants, were produced at a yield of 2.03 mg L^{-1} from daidzein and genistein as substrates. Strikingly, the *ortho*–dihydroxylation activity for isoflavones was approximately 300–500 times higher for the wild type *S. avermitilis* MA-4680 than the *E. coli* recombinant system expressing P450 genes from various microorganisms.[44]

However, 3′,4′,7-trihydroxyisoflavone (7), the product produced from daidzein biotransformation, was subjected to degradation by the bacterial cells. Genetic manipulation of the producer microorganism is a possible approach to prevent degradation and also to avoid secondary reactions by blocking side pathways.[5,71] CYP105D7, the P450 presumably responsible for the oxidative activity, was overexpressed with its electron transfer proteins in *S. avermitilis*, resulting in higher yield of (7) (112.5 mg L^{-1}), however degradation was observed within only 15 hours, which limited its industrial scale application.[108,112] The enzyme tyrosinase catalyzes oxidation of phenolic compounds into corresponding quinone forms. An extracellular tyrosinase, involved in melanin production in *S. avermitilis*, was thought to contribute to the modification of 3′,4′,7-trihydroxyisoflavone (7).[71] Tyrosinase deletion mutant (ΔmelC2), constructed to evaluate the effect on the production of 3′,4′,7-trihydroxyisoflavone (7), and regulation of CYP105D7 and its redox partners, prevented the degradation of 3′,4′,7-trihydroxyisoflavone (7), but the hydroxylation activity was decreased by 40% compared to the wild-type strain. This was due to a decrease in the expression level of the responsible P450 enzyme, CYP105D7, and its electron transfer protein counterparts.

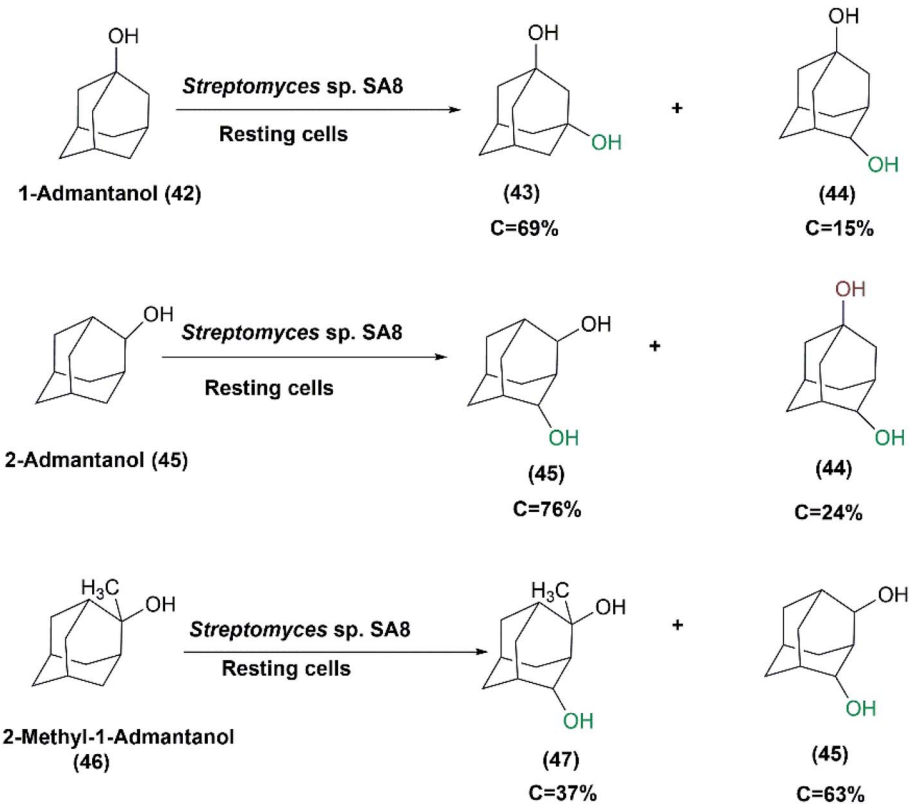
In a study reported in 2012,[72] the melanin-producing *S. avermitilis* MA4680 was utilized to produce piceatannol from *trans*-resveratrol (50) (Scheme 17). By generating deletion mutants, extracellular tyrosinase (MelC2) was detected as the enzyme responsible for the *ortho*-specific hydroxylation. Phenolic compounds such as catechol and hydroquinone were crucial additives for production of piceatannol in high yield. Catechol acted as a competitive inhibitor of the dioxygenase pathway involved in tyrosinase functioning for melanin production, which blocked subsequent oxidation, hence preventing piceatannol degradation. As a result, piceatannnol (51) was produced, with a yield of 78%, *via* a single monooxygenation pathway using catechol at the optimal concentration of 1 mM for inhibition.[72]



Scheme 14 Biotransformation of adamantane (41) with the growing cells of *S. griseoplanus* AC122 to 1-adamantanol.



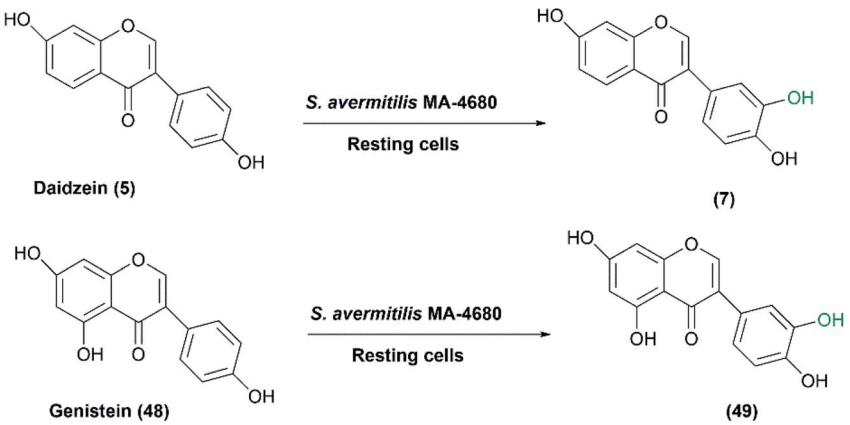
Scheme 13 Biotransformation of 25-hydroxyvitamin D3 (38) and l α-hydroxyvitamin D3 (39) with the resting cells of *S. sclerotialus* FERM BP-1370 and *S. roseosporus* FERM BP-1574. This scheme has been adapted, with minor modifications, from Sasaki *et al.*, Applied and environmental microbiology;[36] with permission from America Society for Microbiology, copyright 1991.



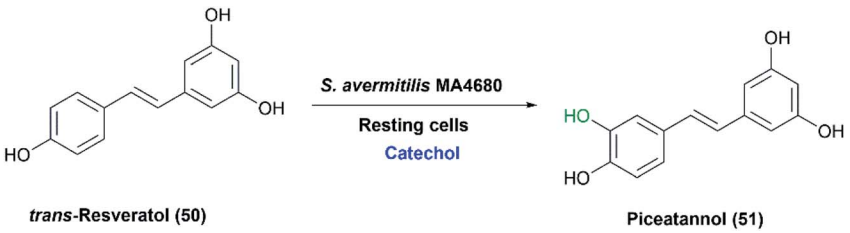
Scheme 15 Biotransformation of 1-admantanol derivatives with resting cells of *Streptomyces* sp. SA8.

In another study, three P450s, CYP107Y1, CYP125A2 and CYP107P2, from *S. avermitilis* MA4680 exhibited good hydroxylation activity.[66] The strain was developed and utilized as expression host for these enzymes using a self-cloning strategy employing genistein (48), chrysin (52) and apigenin (54), respectively, as substrates (Scheme 18). It is noteworthy that the *E. coli* host system did not attain enough quantity of products to well determine the entire structure of the hydroxylated products. In the case of *S. avermitilis*, all the electron transfer proteins (*i.e.*, ferredoxins and ferredoxin reductases) were not well expressed in the *E. coli* system due to the high GC-content of *Streptomyces* species. So, *S. avermitilis* host system was convenient for efficient expression of its own P450s.[66]

Recently, *S. avermitilis* MA4680 was used for regiospecific hydroxylation of phloretin (54), a dihydrochalcone found in apple bark.[45] Selective hydroxylated products of phloretin in B ring were obtained utilizing the resting cultures with a maximal conversion of 6.7% (Scheme 19). Remarkably, phloretin and its hydroxylated derivatives (57) and (58) are most known for their



Scheme 16 Biotransformation of daidzein and genistein to the corresponding selective hydroxylated compounds with the resting cells of *S. avermitilis* MA-4680. This scheme has been adapted, with minor modifications, from Roh *et al.*;[44] with permission from Elsevier, copyright 2009.



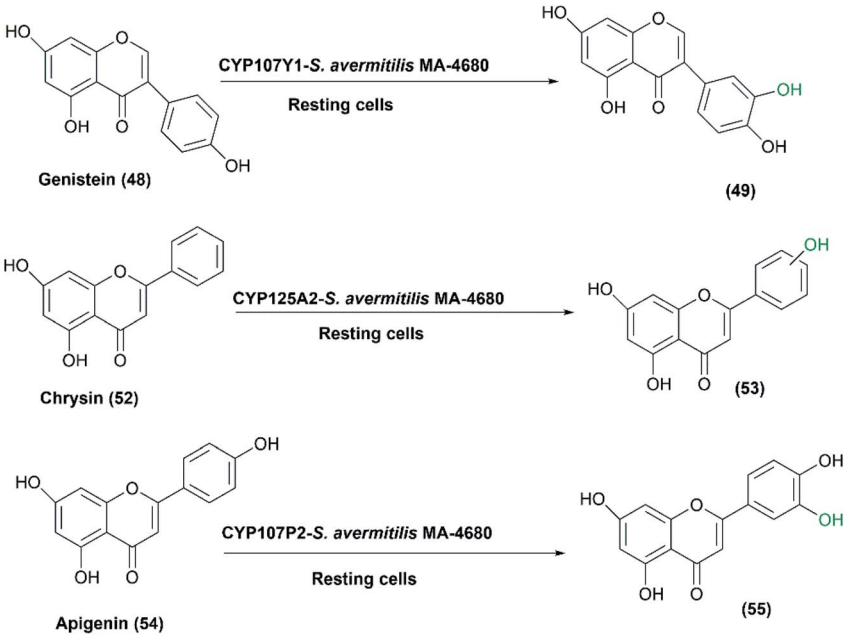
Scheme 17 Biotransformation of *trans*-resveratrol with the resting cells of *S. avermitilis* MA4680 in the presence of catechol as an inhibitor for the potential dioxygenase activity. This scheme has been adapted with amendments; with permission from Nahum Lee, Eun Jung Kim and Byung-Gee Kim, *ACS Chemical Biology*, 2012 **7** (*10*), 1687–1692, DOI: 10.1021/cb300222b.[72] Copyright 2012 American Chemical Society.

therapeutic properties as anti-amyloid, antiarthritic, and anti-inflammatory effects.[45,111]

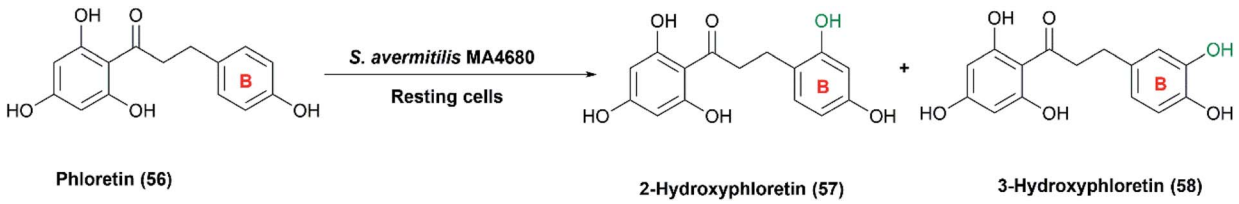
(3) Whole-cell asymmetric oxidation of prochiral sulfides catalyzed by *Streptomycetes*

The organo-sulfur compounds are crucial biological groups involved in metabolism, and also constitute amino acids and their derivatives *i.e.*, cysteine and methionine. The largest group of sulfur-based compounds are sulfoxides for which several attempts have been established to develop biotechnological synthesis.[113,114] Chiral sulfoxides possess many applications; they are used as chiral ligands, chiral auxiliaries, and intermediates in asymmetric synthesis.[79,115–117] They are used in the pharmaceutical industry *e.g.* modafinil and esomeprazole.[118] Remarkably, the use of microorganisms in the asymmetric sulfoxidation has recently received significant attention.[114]

Microbial asymmetric sulfoxidation reactions have been successfully performed during the last 50 years. In 1982, Holland and Carter presented results of a study involving the use of *Mortierella isabellina* to oxidize aryl-substituted phenyl and benzyl-methyl sulfides to the corresponding sulfoxides.[119] In 2013, Mascotti *et al.* examined several bacterial strains to explore their abilities to perform the oxidation of cyclohexyl methyl sulfide (CMS) (59) as a model prochiral sulfide. *S. hiroshimensis* ATCC27429, *S. flavogriseus* ATCC33331 and *S. phaeochromogenes* NCIMB 11741, displayed sulfoxidation of the compound with different enantioselectivities. *S. phaeochromogenes* showed a characteristic biotransformation profile as the stereochemistry of the process was inverted over the time-course of the experiments as shown in Scheme 20. This was explained by the expression of at least two enzymes that may reach their highest rates at different times. The three *Streptomyces* strains conducted the sulfoxidation of cyclohexyl methyl sulfide (59), in an enantio-complementary style, to the corresponding sulfoxide, cyclohexyl methyl sulfoxide (CHMO), (60) and (61).[41] *S. flavogriseus* was the only strain that exhibited a constant S enantiopreference with maximal conversion of 73% despite poor optical purity. The effect of solvents like isopropyl alcohol (IPA) and dimethyl sulfoxide (DMSO), as



Scheme 18 Biotransformation of genistein, chrysin and apigenin with the resting cells of *S. avermitilis* MA4680 overexpressing three P450 responsible genes.



Scheme 19 Selective hydroxylation of phloretin in the B ring with the resting cells of *S. avermitilis* MA4680.

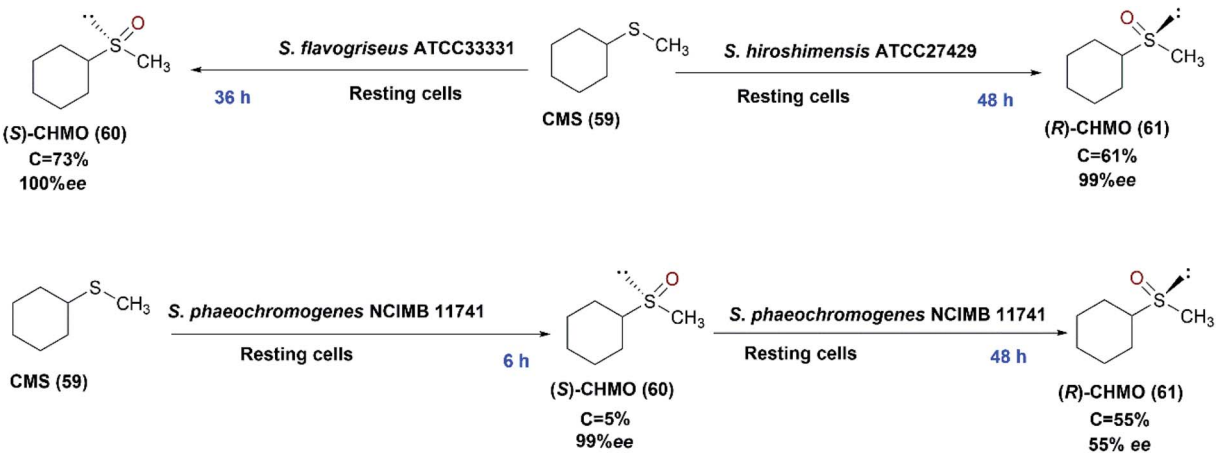
recommended solvents,[120,121] on enhancing the biotransformation process catalyzed by *S. hiroshimensis* ATCC 27429 was tested; 2% (v/v) DMSO caused a decrease of conversion to 18%, while IPA dropped the enantiomeric excess to 66%. This was probably due to inhibition of the enzymes by the co-solvents.

We have recently checked the capability of *S. glaucescens* GLA.0 for asymmetric sulfoxidation using thioanisole (62) as a prochiral aromatic sulfide model.[32] The substrate was converted to the pure isomer of phenyl methyl sulfoxide (*R*-PMSO) (63) at a high yield of 90% and excellent *ee* exceeding 99% employing the growing bacterial culture. Different parameters were varied to optimize the reaction conditions reaching higher yields of the product and smaller amounts of phenyl methyl sulfone (64) as a byproduct. Lower concentrations (0.2–1%) of IPA as a co-solvent were used to increase substrate solubility (Scheme 21), which contributed to higher product yields compared to the analogous reactions utilizing the sparingly soluble substrate. Moreover, it had negligible effect on the selectivity of the bio-oxidation reaction. Furthermore, other parameters helped in enhancing the biotransformation including the use of higher yeast extract-containing medium, higher rotation speed (180 RPM), and addition of the substrate at the time of the inoculation (zero time) of the biotransformation reaction medium (BRM).

The enantioselective sulfoxidation is a typical Baeyer–Villiger reaction.[114] So, we hypothesized a putative Baeyer–Villiger monooxygenase (BVMO), identified *via* an *in-silico* analysis of the genome of *S. glaucescens* GLA.0, to be responsible for the activity. Notably, BVMOs are part of class B flavoprotein monooxygenases that are among the major enzymes catalyzing the formation of chiral sulfoxides such as albendazole.[7,122,123] *S. glaucescens* GLA.0 cells were primarily screened for BVMO activity using cyclohexanone as a model substrate. Qualitative analysis of cyclohexanone consumption by the resting bacterial cells, by a method based on the reaction of an enolizable ketone (*i.e.*, cyclohexanone) with dinitrobenzoic acid in an alkaline solution leading to the formation of a purple coloured complex,[124] showed total disappearance of color within 24–48 hours. Monitoring the bioconversion of cyclohexanone by the bacterial cells over time revealed 6-carbon and 7-carbon esters and traces of ε-caprolactone by GC-MS analysis. This may be due to the action of esterases, lactonases, and/or hydrolases, the presence of which is documented for different *Streptomyces* strains.[125] Such enzymes might be produced by the bacterial cells upon incubation with cyclohexanol (65) or cyclohexanone (Scheme 22) as detected before in the case of cyclohexanol-grown *Acinetobacter calcoaceticus*.[126] This would affect the quantity of the produced caprolactone. As revealed, the putative monooxygenase (BVMO) from *S. glaucescens* GLA.0 shares 26% identity with cyclohexanone monooxygenase from *A. calcoaceticus* NCIMB 9871.

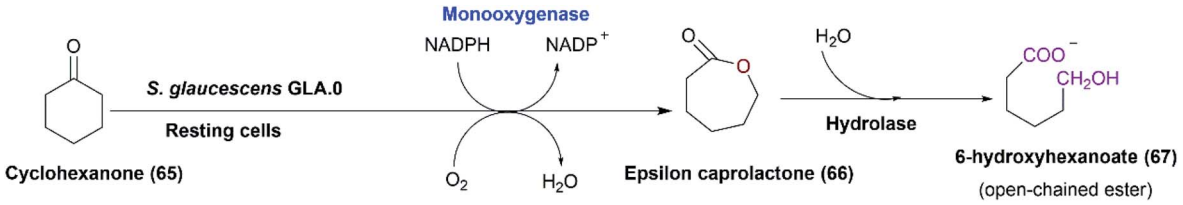
(4) Whole-cell oxidation of aldehydes catalyzed by *Streptomycetes*

Although traditional chemical oxidations of aldehydes to carboxylic acids by oxidants like oxone are highly efficient and



Scheme 20 Biotransformation of cyclohexyl methyl sulfide (59) with the resting cells of three different *Streptomyces* strains (*S. hiroshimensis* ATCC27429, *S. flavogriseus* ATCC33331 and *S. phaeochromogenes* NCIMB 11741) indicating the time detected for each product in blue font. This scheme has been adapted, with amendments, from Mascotti *et al.*[41] with permission from Elsevier, copyright 2013.

Scheme 21 Biotransformation of thioanisole (62) with the growing cells of *S. glaucescens* GLA.0 under optimized conditions.



Scheme 22 Putative pathway of cyclohexanol and/or cyclohexanone catalyzed by the whole cells of S. glaucescens GLA.0. This scheme has been adapted, with amendments, from Donoghue *et al.*;[127] with permission from John Wiley and Sons, copyright 2008.

straightforward, microbial selective oxidation of aldehydes are still of interest.[4] Various examples of aromatic aldehydes biooxidation to the respective carboxylic acids employing the whole cells of *Streptomyces* species were reported. *S. viridosporus* T7A catalyzed the oxidation of vanillin (68) to the more valuable, vanillic acid (69) in a two-stage fermentation process.[38] Vanillin (68) was used as inducer in the first stage of the bacterial cultivation, to help the production of aromatic aldehyde oxidase *in vivo* and make the growing cells active for a longer time (Table 1). The product was recovered with high purity and yield exceeding 96% of the initial amount of vanillin, and concentration of 1.6 g L^{-1} (Scheme 23).

In a study published in 2001, *S. cattleya* NRRL 8057 was screened for the enzyme involved in fluorometabolite biosynthesis.[77] The resting cultures transformed fluoroacetaldehyde (70) to the corresponding acid, fluoroacetate (71) (Scheme 24). The enzyme was produced mainly in the late exponential to the stationary phase after around 6 days of growth. Determination of the substrate specificity of the isolated fluoroacetaldehyde dehydrogenase indicated fluoroacetaldehyde and glycoaldehyde to be good substrates.[77]

(5) Multi-step and/or concurrent oxidations catalyzed by the whole cells of *Streptomyces*

One-pot multi-step reactions catalyzed by microbial cells represent an alternative pathway to others consuming time and materials.[1,128] *S. fulvissimus* NRRL B1453 was reported to catalyze multi-step oxidation reactions;[83] the bacteria transformed 5-hydroxyflavone (72) first to 5,4′-dihydroxy- (73) and 5,3′,4′-trihydroxyflavone (74), and subsequently sulfation at the 4′ position of (73), resulting in the formation of 5,4′-dihydroxyflavone-4′-sulfate (75) as shown in Scheme 25.

Mazier *et al.* reported the biooxidation of terfenadine (76) utilizing the whole cells of *S. platensis* NRRL 2364.[31] The formation of the alcohol, hydroxyl terfenadine (77), and the acid, fexofenadine (78) depended on the culture conditions. Fexofenadine (78) was formed together with hydroxy terfenadine (77) in a yeast extract-malt medium containing soybean peptone (YMS), depending mainly on the age of the culture. The highest yield of fexofenadine (78) (25%) was achieved just in 48-hour-old culture rather than older cultures. In contrast, hydroxy terfenadine (77) was solely obtained at a yield up to 51% in a medium devoid of soybean peptone (YM) (Scheme 26). Moreover, the oxygen dependency of the process was studied through performing transformation in aerobic or argon atmospheres. No oxidation reaction occurred under argon and neither upon using terfenadine nor hydroxyterfenadine as substrates, which indicated that the oxidizing activity was due to an oxygenase not a dehydrogenase. Different ratios of hydroxy terfenadine (77) and fexofenadine (78) were observed in the biotransformation process due to the instability of the oxidative enzyme system induced by soybean peptone. Later the purified P450 dependent monooxygenase, CYP107L was found to be responsible for the hydroxylation of the *tertiary*-butyl group of terfenadine.[95,129] *S. platensis* CYP107L was cloned and expressed in *E. coli*, and examined for whole-cell conversions of amodiaquine, ritonavir, amitriptyline, and thioridazine. Its behavior resembled that of human P450s involved in the metabolism of these drugs, which may contribute to preparative synthesis of the metabolites.[95]

Streptomyces griseus has several applications in oxidative transformation of xenobiotics, which could be catalyzed by the same enzyme system. *S. griseus* (UI 1158, NRRL 8090) was screened for the transformation of 7-ethoxycoumarin (79), a sensitive analytical substrate, to detect and characterize such type of enzymatic activity.[84] Conversions included *O*-deethylation of 7-ethoxycoumarin to 7-hydroxycoumarin (80), followed by hydroxylation of 7-hydroxycoumarin to 6,7-dihydroxycoumarin (81), and finally the latter was further methylated to form 7-hydroxy-6-methoxycoumarin (82) (Scheme 27). The products were isolated at a yield of up to 30%, but trials to obtain an isolated enzyme from the cell-free extract were unsuccessful. However, the findings indicated that the strain has biosynthetic machinery for coumarin derivatives almost

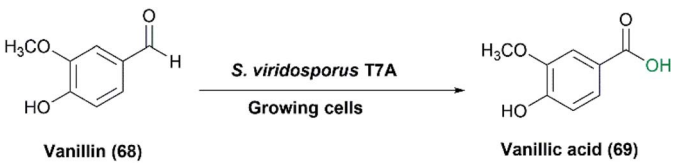
Table 1 Selected examples of oxidation reactions catalyzed by different Streptomyces species[a]

No.	*Streptomyces* species	Biological application	Type of reaction	Substrate	Scheme number	Reference
1	*Streptomyces lividans* TK54	Recombinant whole cells	Regiospecific hydroxylation	Avermectin	1	65
2	*Streptomyces avermitilis* MA4680	Recombinant whole cells	Regiospecific hydroxylation	Genistein	17	66
3	*S. avermitilis* ΔSaOMT2	Recombinant whole cells	Regioselective hydroxylation	Daidzein	3	40
4	*Streptomyces lividans* TK24	Recombinant whole cells	Oxidative dealkylation	7-Ethoxycoumarin	2	67
5	*Streptomyces albus* J1074	Recombinant whole cells	Carboxylation	12-Desnitrile-12-methyl-borrelidin	4	68
6	*Streptomyces griseus* NCIM 2622	Wild type whole cells	Hydroxylation	Meloxicam	11	69
7	*Streptomyces griseus* NRRL B8090	Wild type whole cells	Hydroxylation	Naphthalene	10	70
8	*Streptomyces sclerotialus* FERM BP-1370	Wild type whole cells	Hydroxylation	25-Hydroxyvitamin D3	13	36
9	*Streptomyces griseoplanus* AC122	Wild type whole cells	Regioselective hydroxylation	Adamantane	14	43
10	*Streptomyces* sp. SA8	Wild type whole cells	Regioselective hydroxylation	1-Adamantanol	15	37
11	*Streptomyces avermitilis* MA-4680	Wild type whole cells	Regiospecific hydroxylation	Daidzein	16	44 and 71
12	*Streptomyces avermitilis* MA4680	Wild type whole cells	Regioselective hydroxylation	*Trans*-resveratrol	17	72
13	*Streptomyces canus* ATCC 12647	Wild type whole cells	Stereospecific hydroxylation	L-Proline	12	73
14	*Streptomyces roseochromogenes* NCIB 10984	Wild type whole cells	Hydroxylation	Progesterone	6	74
15	*Streptomyces roseochromogenes* ATCC 13400	Wild type whole cells	Hydroxylation	Hydrocortisone	8	75
16	*Streptomyces roseochromogenes*	Wild type whole cells	Hydroxylation	Hydrocortisone	9	28
17	*Streptomyces avermitilis* MA4680	Wild type whole cells	Regioselective hydroxylation	Phloretin	19	45
18	*Streptomyces* sp. W2233-SM	Wild type whole cells	Hydroxylation	Progesterone	7	76
19	*Streptomyces phaeochromogenes* NCIMB 11741	Wild type whole cells	Asymmetric sulfoxidation	Cyclohexyl methyl sulfide	20	41
20	*Streptomyces glaucescens* GLA.0	Wild type whole cells	Enantioselective sulfoxidation	Thioanisole*	21	32
21	*Streptomyces cattleya*	Wild type whole cells	Carboxylation	Fluoroacetaldehyde	24	77
22	*Streptomyces viridosporus* T7A	Wild type whole cells	Carboxylation	Vanillin *	23	38
23	*Streptomyces griseus* ATCC 13273	Wild type whole cells	Carboxylation followed by hydroxylation (site-selective oxidation)	Ursane triterpenes [ex; ursolic acid]	31	78
24	*Streptomyces griseus* ATCC 13273	Wild type whole cells	Carboxylation & glycosylation	3-Oxo oleanolic acid	30	79
25	*Streptomyces griseus* ATCC 13273	Wild type whole cells	Hydroxylation & carboxylation (site-selective oxidation)	Oleanane triterpenes [ex; oleanolic acid]	32	80
26	*Streptomyces platensis*	Wild type whole cells	Hydroxylation followed by two-step oxidation	Terfenadine	26	31
27	*Streptomyces virginiae* IBL-14	Wild type whole cells	Hydroxylation	Diosgenone	5	81 and 82
28	Streptomyces fulvissimus NRRL B1453	Wild type whole cells	Hydroxylation & sulfation	5-Hydroxyflavone	25	83
29	*Streptomyces griseus* (UT 1158, NRRL 8090)	Wild type whole cells	*O*-Deethylation & hydroxylation	7-Ethoxycoumarin	27	84

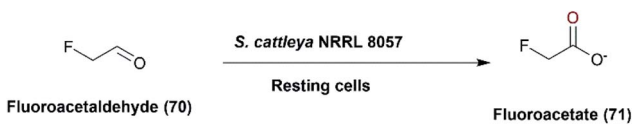
Table 1 (*Contd.*)

No.	*Streptomyces* species	Biological application	Type of reaction	Substrate	Scheme number	Reference
30	*Streptomyces griseus* ATCC 13273	Wild type whole cells	Oxidation	Precocene II	28	30 and 85
31	*Streptomyces griseus* (SC 1754 and SC 13971)	Wild type whole cells	Selective hydroxylation	Mutilin	29	86
32	*Streptomyces cinnamonensis*	Wild type whole cells	Oxidative cleavage	1,4-Naphthoquinone (INO5042)	33	87
33	*S. griseus* CECT 3116	Wild type whole cells	*N*-oxidation	*p*-Aminobenzoic acid	34	88

[a] Key points related to the biooxidation reaction conditions are presented, the asterisk mark '*', beside the substrate of the entry 20 and 22, indicates that low concentrations of substrates were used in the first stage of fermentations.



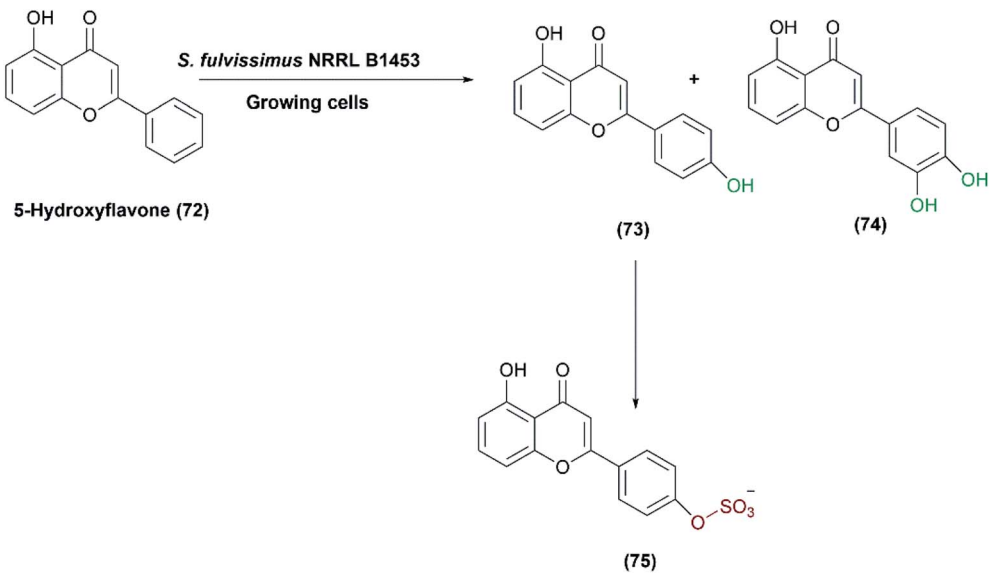
Scheme 23 Biotransformation of vanillin with the growing cells of *S. viridosporus* T7A.



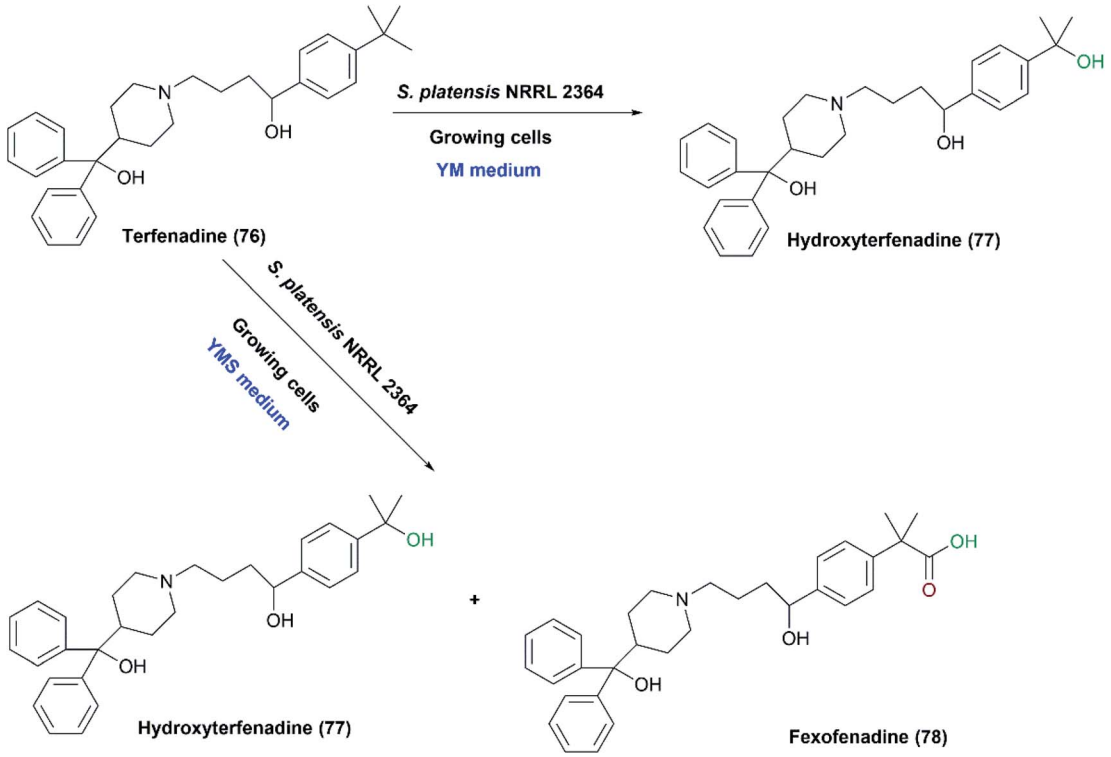
Scheme 24 Biotransformation of fluoroacetaldehyde (70) with the resting cells of *S. cattleya* NRRL 8057.

resembling eukaryotic systems.[84] Later, the enzyme, CYP105D1, was cloned and heterologously expressed, and examined to convert a variety of xenobiotics to equivalent mammalian metabolites.[96,130]

S. griseus ATCC 13273 was found to possess a similar metabolism for precocene II (84), a fourth-generation pesticide, as in eukaryotic systems.[85] On incubation with the growing culture, three oxidized products were obtained, identified, and isolated. An inducible cytochrome P-450 system enzyme was considered responsible for the mono oxygenation pathway as reported in a subsequent study performed by the same authors.[30] The high levels of the enzyme were produced in bacterial cultures grown on an enriched medium containing soybean flour, leading to rapid oxidation of precocene II, compared to other media compositions. Furthermore, aerial mycelium-negative variant of *S. griseus* (AMV cells), that lacked to cytochrome P-450 after growth in the same medium containing soybean flour, failed to oxidize precocene II, which

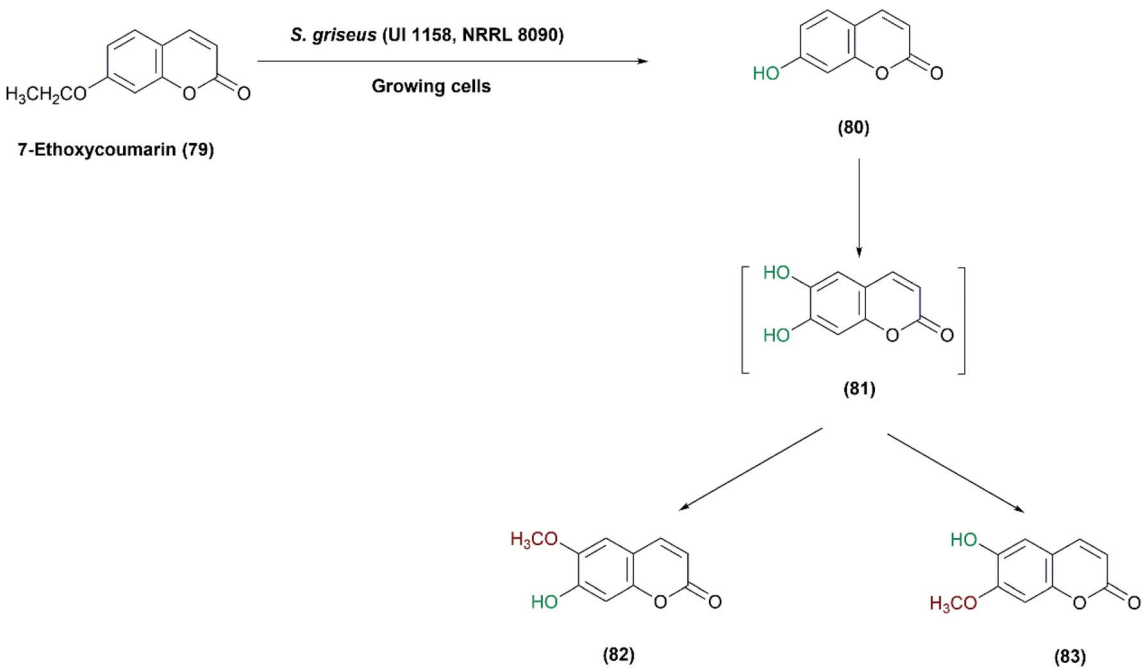


Scheme 25 Biotransformation of 5-hydroxyflavone (72) with the growing cells of *S. fulvissimus* NRRL B1453 in which one of the two products undergoes sulfation at the 4′ position forming 5,4′-dihydroxyflavone-4′-sulfate (75).

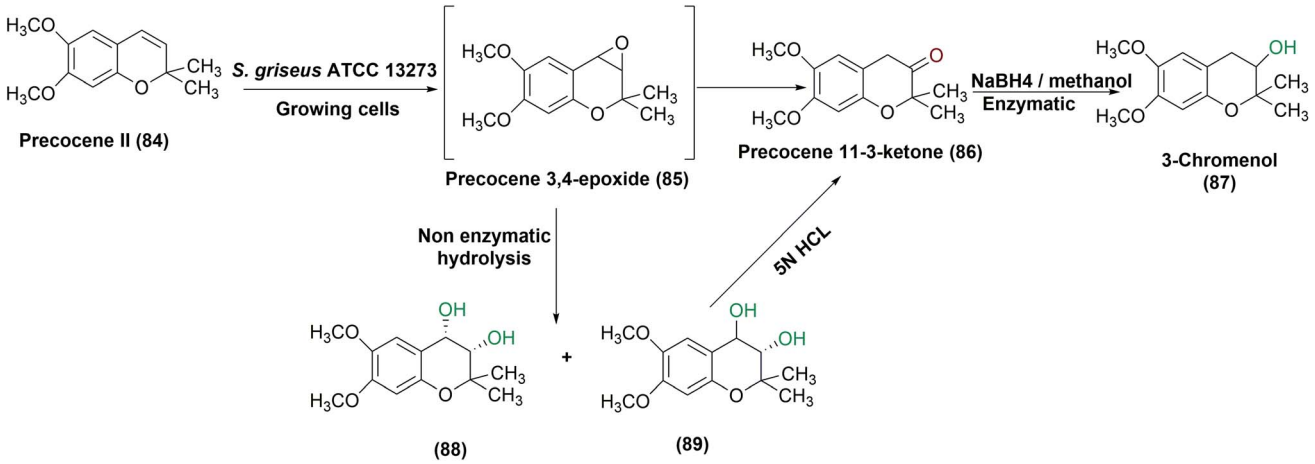


Scheme 26 Biotransformation of terfenadine (76) with the growing cells of *S. platensis* NRRL 2364; only hydroxyterfenadine (77) was formed in the case of YM medium while hydroxyterfenadine (77) was formed together with fexofenadine (78) upon utilizing YMS medium.

proved that the P-450 system was responsible for the reaction. *S. griseus* growing cells catalyzed the production of a highly reactive epoxide (85) which acted as an initial intermediate in the process. (−) *Cis*- and (+) *trans*-Precocene II 3,4-dihydrodiols (88), (89) and (+)-3-chromenol (87) were then detected as final products (Scheme 28).[85]



Scheme 27 Multistep oxidation of 7-ethoxycoumarin (79) with the growing cells of *S. griseus* (UI 1158, NRRL 8090). This scheme has been adapted, with minor modifications, from Sariaslani *et al.*;[84] with permission from American Society for Microbiology, copyright 1983.

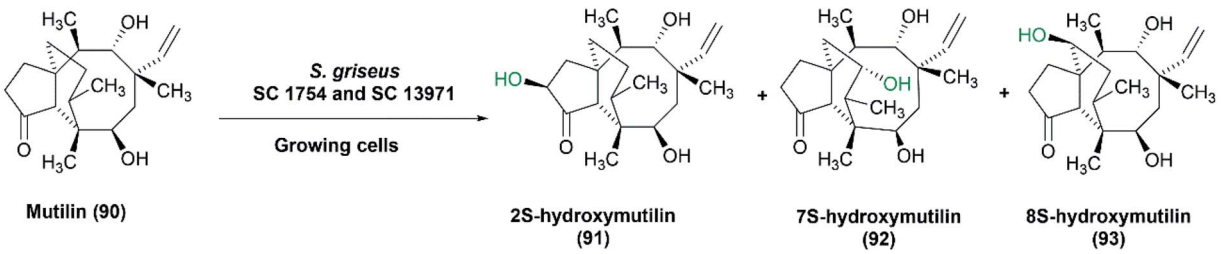


Scheme 28 Multistep oxidation of precocene II (84) with the growing cells of *S. griseus* ATCC 13273 under optimized conditions of growth. This scheme has been adapted, with minor modifications, from Sasaki *et al.*, Applied and environmental microbiology;[85] with permission from American Society for Microbiology, copyright 1987.

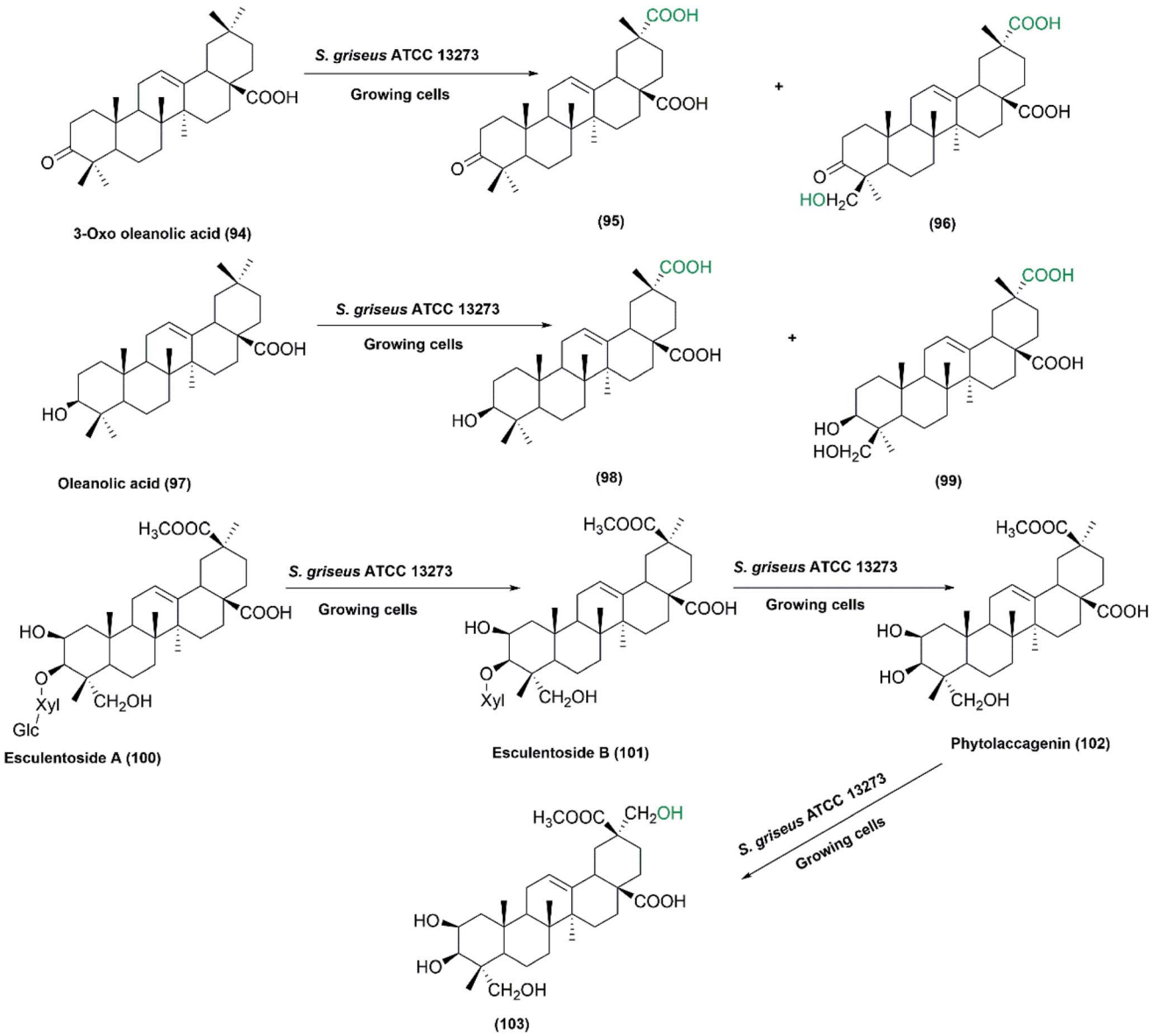
Hanson *et al.* checked the ability of *S. griseus* to hydroxylate mutilin, an antibiotic for poultry and animals.[86] Hydroxylated derivatives of mutilin prevent its metabolism (*i.e.*, hydroxylation at definite positions), hence inactivation. Furthermore, their biological activities do not change, and can be the same as the parent molecule, mutilin. Two strains of *S. griseus*, SC 1754 and SC 13971 (ATCC 13273) transformed mutilin (90) selectively to 2*S*-hydroxymutilin (91), 7*S*-hydroxymutilin (92) and 8*S*-hydroxymutilin (93) (Scheme 29). The process was performed using bacterial cultures grown on media containing soy flour. A typical medium, including 2% soy and 0.5% glucose gave the highest rates of hydroxylation in shake flasks. Glycerol as a carbon source produced lower yields compared to glucose. The reaction was scaled up to 15, 60 and 100 L and larger amounts of hydroxy products, 16–100 mg, were extracted.[86]

Zhu *et al.* set up a study on biotransformation of olean-type pentacyclic triterpenes, the natural products possessing various pharmacological applications, by employing whole cells of *S. griseus* ATCC 13273, among other microorganisms.[79] Four substrates, including 3-oxo oleanolic acid, 3-acetyl oleanolic acid, oleanolic acid, and esculentoside A were used and underwent regio-selective methyl oxidation and glycosylation. The corresponding oxidized products as depicted in Scheme 30, were isolated and structurally elucidated by ESI-MS, 1H NMR, 13C NMR, and 2D-NMR spectroscopy. Such microbial conversion provides an alternative approach to enrich the structural diversity of olean-type pentacyclic triterpenes.[79]

The same authors recently reported a study on the capability of *S. griseus* in transforming a number of ursane triterpenes.[78] Ursolic acid (104), 3-oxo ursolic acid (107), corosolic acid (110), asiatic acid (113) and madasiatic acid (115), as substrates, were converted by the growing cells of *S. griseus* ATCC 13273 to give moderate yields of hydroxylated and/or carboxylated products *via* site selective oxidation of the C–H group (Scheme 31). It is essential to make use of readily available approaches for expanding the structural diversity of such natural products to obtain more effective and less toxic derivatives with regard to biological activities. Among the compounds produced, eight metabolites were isolated and structurally elucidated for the first time. The biological processes allowed the production of novel metabolites in a single step under environmentally benign conditions, which was difficult to achieve by chemical methods. The strain selectively catalyzed both carboxylation of the C-30 methyl group and hydroxylation of the C-24 methyl



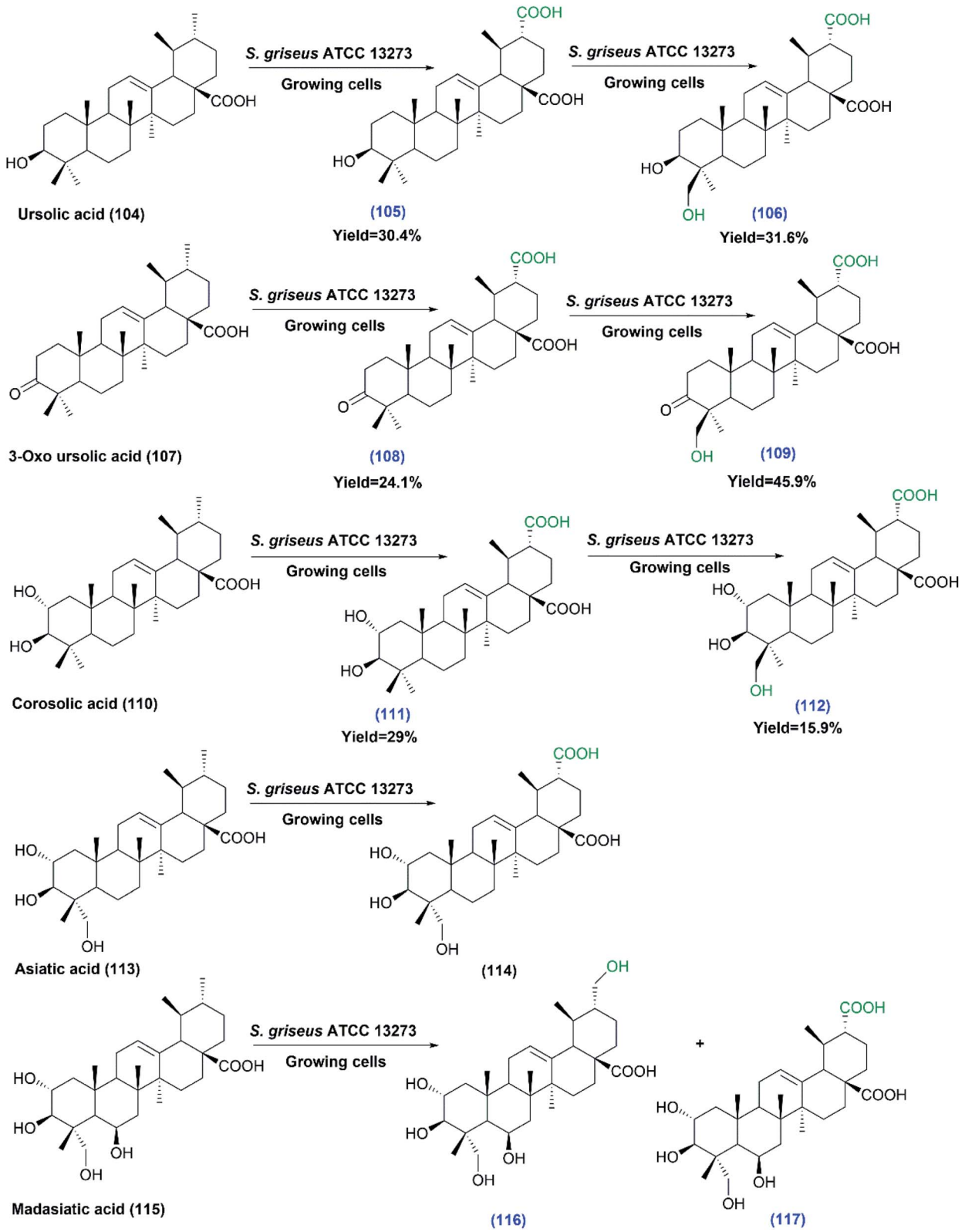
Scheme 29 Biotransformation of mutilin (90) with the growing cells of *S. griseus* SC 1754 and SC 13971 to the corresponding hydroxylated products (91–93). This scheme has been adapted with permission from Ronald L. Hanson, James A. Matson, David B. Brzozowski, Thomas L. LaPorte, Dane M. Springer and Ramesh N. Patel, Organic Process Research & Development, 2002, **6** (*4*), 482–487, DOI: 10.1021/op020028q.[86] Copyright 2002 American Chemical Society.



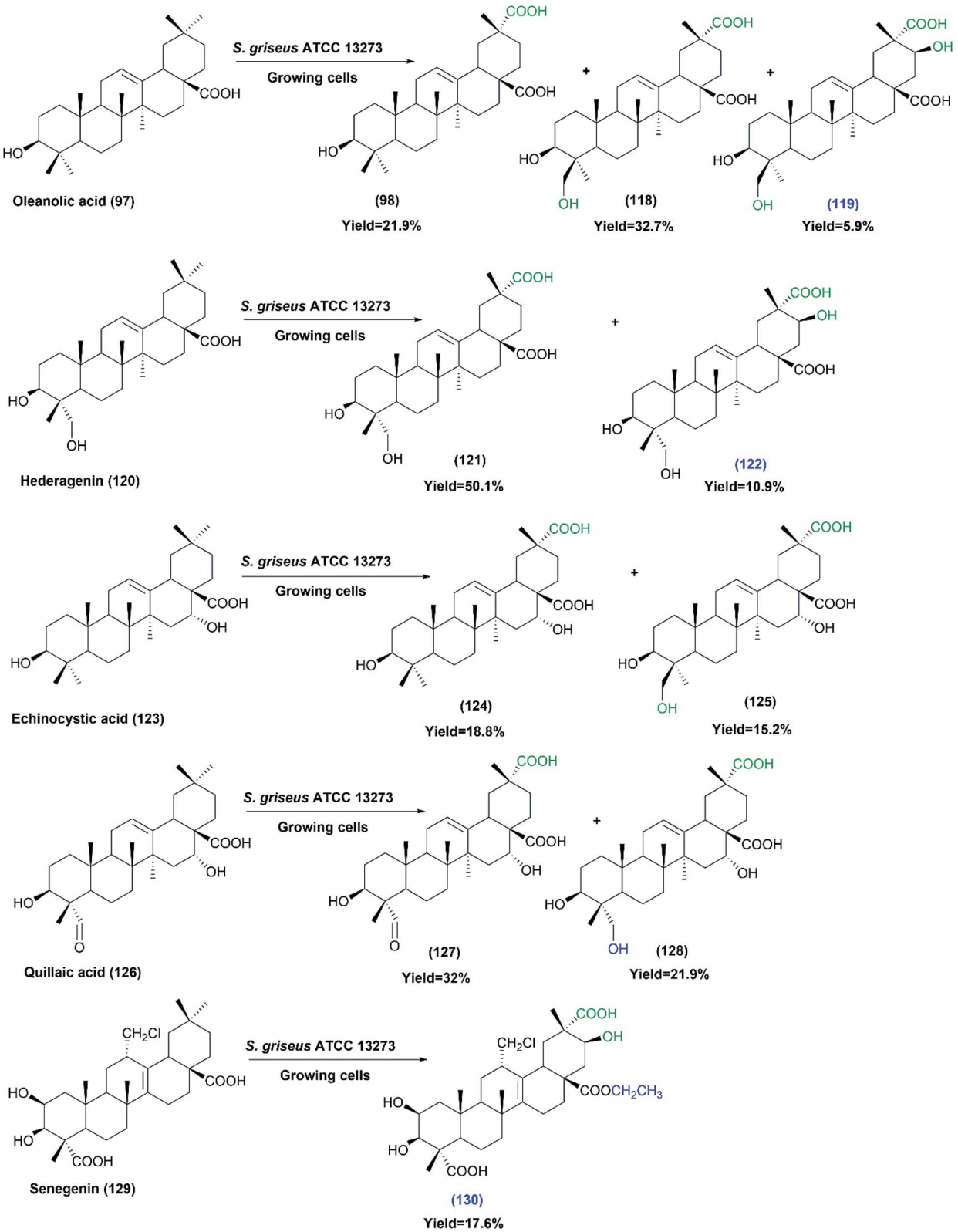
Scheme 30 Biotransformation of 3-oxo oleanolic acid (94), oleanolic acid (97), and esculentoside A (100), respectively, with the growing cells of *S. griseus* ATCC 13273 to the corresponding hydroxylated and carboxylated products. This scheme has been adapted, with minor modifications, from Zhu *et al.*[79] with permission from Elsevier, copyright 2011.

group in (104), (107), and (110), while the hydroxylation of C-24 was blocked in case of asiatic acid (113) and madasiatic acid (115), which bear a hydroxyl group on C-23. The new products (105, 106, 108, 109, 111, 112, 116 and 117) were isolated and structurally elucidated based on 1D and 2D NMR and HR-MS data.[78]

Oleanolic triterpenes have also been recently tested as substrates for the growing cultures of the same strain (*S. griseus* ATCC 13273).[80] The substrates including oleanolic acid (97), hederagenin (120), echinocystic acid (123), quillaic acid (126) and senegenin (129), were selectively carboxylated at the C-29 methyl group. In addition, the hydroxylation of C-24 and C-21 were also detected on some substrates, as indicated by Scheme 32. Most of the produced metabolites were isolated and identified by MS and NMR analyses. All the products did not exhibit any cytotoxicity, in contrast to their corresponding substrates that exhibited cytotoxic activities. Moreover, testing the anti-inflammatory activities of all substrates and products against lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophages showed the products (124), (127) and (128) to have moderate inhibitory effects, while the products (119), (122) and (130) showed enhanced inhibitory activities. The findings from the two studies suggest that *S. griseus* ATCC 13273 could be a useful biocatalyst to enrich the structural diversity of pentacyclic triterpenes, which will have great impact on medicinal chemistry research.



Scheme 31 Biotransformation of ursolic acid (104), 3-oxo ursolic acid (107), corosolic acid (110), asiatic acid (113) and madasiatic acid (115) with growing cells of *S. griseus* ATCC 13273, the first three substrates undergo multistep oxidations. The products' numbers in blue referred to the newly reported compounds. This scheme has been adapted, with minor modifications, from Xu *et al.*;[78] with permission from the Royal Society of Chemistry.



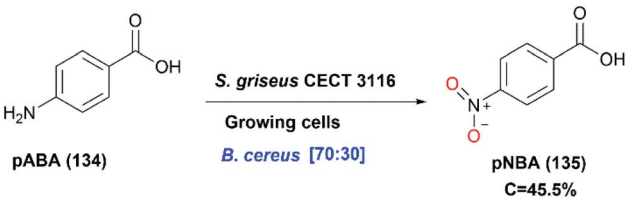
Scheme 32 Biotransformation of oleanolic acid (97), hederagenin (120), echinocystic acid (123), quillaic acid (126) and senegenin (129) with the growing cells of *S. griseus* ATCC 13273. The products' numbers in blue refer to the newly reported compounds. This scheme has been adapted, with minor modifications, from Xu *et al.*;[80] with permission from Elsevier, copyright 2017.

(6) Oxidative cleavage catalyzed by the whole cells of *Streptomyces*

Fosse *et al.* studied the oxidative cleavage of heterocyclic naphthoquinone model in a report published in 2004.[87] Heterocyclic naphthoquinones are known to have potential pharmacological activities against venous alterations and inflammatory edema, but they are poorly soluble and bioavailable, which hampers their testing. Thiazole fused 1,4-naphthoquinone, INO5042 (131), was transformed by the whole cells of either *S. platensis* or *S. cinnamonensis* to the corresponding isomeric phenol-carboxylic acids, (132) and (133), as indicated in Scheme 33. Among other *Streptomyces* and fungal species, both *S. platensis* and *S. cinnamonensis* produced one of the respective oxidized cleaved products in moderate to high yields, which allowed easy purification and further characterization. Optimal production was achieved at pH 6 and 35 °C. Investigation of the mechanism of the bioconversions *via* oxygen isotope incorporation revealed a dioxygenase pathway related to the hydroquinone-epoxidase system, oxygenating enzymes involved in the polyketide antibiotics' synthesis in *Streptomyces*.[87]

(7) *N*-Oxidations catalyzed by the whole cells of *Streptomyces*

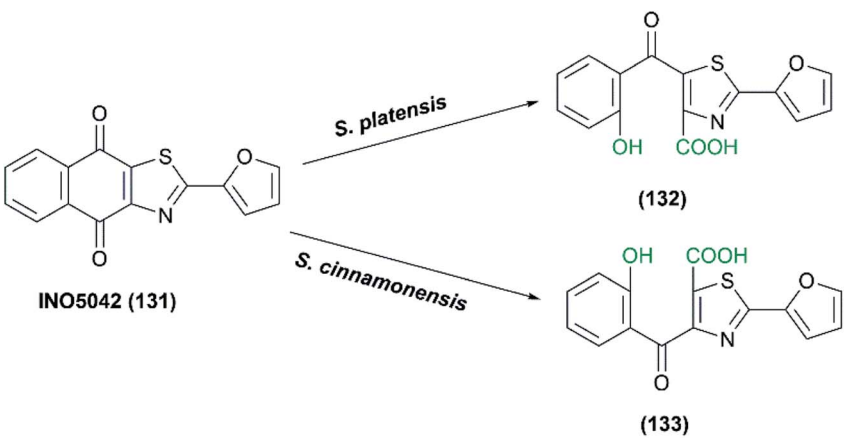
Streptomyces species can convert the amino group to the corresponding nitro groups contributing to the production of nitroaromatic compounds such as nitroaromatic antibiotics like everninomycin.[88,131] A recently published paper has detected an *N*-oxygenase activity displayed by the whole cells of *S. griseus*.[88] Based on bioinformatics and experimental analyses, the authors have been able to identify a diiron monooxygenase gene, AurF, from several *Streptomyces* strains examined. The AurF enzyme was previously characterized from another *Streptomyces* species, *S. thioletus*. The enzyme is responsible for the oxidation of *p*-aminobenzoic acid (pABA) to *p*-nitrobenzoic acid (pNBA), a building block of several drugs such as aureothin.[132] The substrate (pABA) was used to induce the *N*-oxygenase activity, but all trials lead to growth inhibition and/or low conversion. Alternatively, stressful conditions were applied to induce the responsible enzyme (*N*-oxygenase) through the activation of secondary metabolism, which helped optimize the biotransformation process. Conducting the process during the stationary phase of growth (starvation conditions) raised the conversion percentage to the product (pNBA) about 8 times. It was thought that the decrease in nutrients that occurred in the stationary phases can make various secondary metabolites increase their concentrations. Another stressful condition strategy was adopted using co-cultures with other bacterial strains such as *Agrobacterium tumefaciens* CECT 4017, *Arthrobacter oxydans* CECT 387, and *Bacillus cereus*. Those organisms occupy the natural habitat of *S. griseus* and therefore act as natural competitors for nutrients, which in turn affect the production of secondary metabolites.[88,133] Co-cultivation of *S. griseus* with *Bacillus cereus*, as a stressful organism, at an optimized biomass mixture ratio of 70 : 30 improved the conversion to 45.5% within 4 hours (Scheme 34).[88]



Scheme 34 *N*-Oxidation of *p*-aminobenzoic acid to *p*-nitrobenzoic acid with the growing cells of *S. griseus* CECT 3116 under an optimized stimulation strategy of co-culturing with *B. cereus* in 70 : 30 ratios. This scheme has been adapted, with minor modifications, from Nóbile *et al.*;[88] with permission from Elsevier, copyright 2021.

Conclusion

This review has highlighted several examples of *Streptomyces* species catalyzing diverse oxidative reactions, including both



Scheme 33 Oxidative cleavage of thiazole fused 1,4-naphthoquinone (INO5042) with the whole cells (resting cells or growing cells) *S. platensis* or *S. cinnamonensis*. This scheme has been adapted, with minor modifications, by permission from Springer, Applied Microbiology and Biotechnology; Fosse, C., Le Texier, L., Roy, S. *et al.* Parameters and mechanistic studies on the oxidative ring cleavage of synthetic heterocyclic naphthoquinones by Streptomyces strains,[87] Copyright 2004.

single step and multistep reactions and leading to the formation of bioactive products that would not have been easy to produce by chemical syntheses. The reports in the literature have shown that optimization of the culture conditions and adding a suitable inducer to the medium can have highly positive effect on the catalytic activity of the whole cells for the desired reaction. There could be scope for further improvement by testing different process-engineering strategies, like cell immobilization, cultivation mode, type of bioreactors, *etc.*, which could have a great impact on the oxidative activity of *Streptomyces* strains. Furthermore, cloning and expression of the enzymes involved in the oxidation reactions will be useful for understanding the enzyme features for activity and stability, and for designing better enzymes. Selective heteroatom oxidations have just been reported for some *Streptomyces* species,[32,88] in addition to specific oxidation of unactivated C–H bonds,[78,80] which opens up the possibilities for further studies on identifying the enzymes involved in such species. Development of expression hosts for the genes from *Streptomyces* species is yet another aspect that would be needed for increasing the utilization of these important biocatalysts. *Streptomyces* species, sources of eco-friendly catalysts for diverse oxidation reaction including selective ones, can replace chemical approaches that need harsh conditions affecting product selectivity.

Abbreviations

ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonate)
AMV	Aerial mycelium-negative variant
BRM	Biotransformation reaction media
BVMO	Baeyer–Villiger monooxygenase
CHMO	Cyclohexyl methyl sulfoxide
CMS	Cyclohexyl methyl sulfide
DMSO	Dimethyl sulfoxide
DO	Dissolved oxygen
FdR18/FdR28	Ferredoxin (Fd68) and ferredoxin reductases
INO5042	Thiazole fused 1,4-naphthoquinone
IPA	Isopropyl alcohol
melC2	Tyrosinase deletion mutant
meso-2,4-ad(OH)2	meso-2,4-Admantanediol
metK	SAM synthetase gene
2-Me-2-adOH	2-Methyl-2-admantanol
2-Me-2,4-ad(OH)2	2-Methyl-2,4-admantanediol
pABA	*p*-Aminobenzoic acid
pNBA	*p*-Nitrobenzoic acid
PMSO	Phenyl methyl sulfoxide
P450	Cytochrome P450 enzymes
SAM	*S*-Adenosyl-L-methionine
YM	Yeast extract-malt medium devoid of soybean peptone
YMS	Yeast extract-malt medium containing soybean peptone

Conflicts of interest

There are no conflicts to declare.

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