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Microbial Transformations of Plant Origin Compounds as a Step in Preparation of Highly Valuable Pharmaceuticals

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Abstract

The aim of the paper is to present microbial transformation reactions as a step in the preparation of drugs or their key intermediates from plant derived compounds. Described are some successful applications of microbial transformation processes for preparation of steroid drugs and/or their important intermediates as well as some microbial transformations of terpenes, alkaloids, flavonoids and poly(phenols) affording derivatives with improved biological activities.

Keywords: Microbial transformation; Phytosterols; Steroids; Terpenes; Alkaloids; Flavonoids; (Poly)Phenols

Introduction

From ancient times to recent days plants have been subjected to different manipulations to obtain biologically active compounds for medical purposes. Methods applied range from traditional extractions to environmental friendly techniques including extraction with ionic liquids, microwave-assisted and ultrasound-assisted extraction, solid phase extraction and supercritical fluid extraction [1-3]. Additionally, acid or enzymatic hydrolysis may be applied in order to make the active ingredients available for the intended clinical applications [4,5]. The plant derived compounds are also subjected to processes of microbial transformation which are nowadays considered as promising technologies for drug development and improvement [6-9]. The microbial transformations are carried out in mild conditions, proceed with high regio- and stereo-specificity and give rise to derivatives which are either difficult to be prepared by chemical means or not economically reasonable. In the recent years the interest in microbial transformations of plant derived biologically active compounds affected even the alternative medicine. Some traditional Chinese medicinal herbs and their ingredients were subjected to biotransformations as well [10,11].

The microbial transformation process

Klaus Kieslich defined the biotransformation processes as "chemical reactions by microorganisms or enzymes" [12]. Several requirements were further on added to this definition aiming to distinguish the microbial transformation processes from these of bioconversion and biodegradation, i.e. the substrate should be a foreign to the microbial cell compound as well as at least one of the products should keep the structure of the substrate unchanged [13]. Actually, the definition of the microbial transformation process was refined primarily based on studies dealing with microbial transformations of steroid compounds and these processes gained their recent importance due to their successful application in steroid drug manufacturing [14,15].

Microbial transformation reactions and the steroid drug story

Microbial transformation reactions of phytosterols include as a first step their side-chain cleavage as well as processes of hydroxylation and dehydrogenation of the steroid ring structure and isomerization of the double bonds. It is important to highlight that any of the positions of the steroid ring is prone to microbial attack, either bacterial or fungal. In general, bacteria are more active in complete steroid structure degradation while fungi are much more active in multiple steroid hydroxylations. However, despite of the attempts that have been made to find out which microorganisms are best in performing each type of the transformation reaction, no proper correlation between the type of substrate, taxonomic position of the microorganism and the reaction performed has been established. It is worth mentioning the Akhrem and Titov's classical book "Steroids and microorganisms" in which the authors returned back to 1913 when the first studies on microbial *(Mycobacterium)* cleavage of the cholesterol molecule were performed. The same investigation was marked as a starting point in studies on a large group of microbial transformation reactions proceeding with cleavage of carbon-carbon bonds and leading to partial or full splitting of the steroid molecule [16]. Later on huge amounts of data on diversity of microorganisms revealing steroid transformations activities were collected and made available [17-25].

The era of practical application of microorganisms in the large-scale process of manufacturing of steroid drugs and/or their key intermediates began with the discovery of the ability of a *Rhizopus* strain to introduce oxygen in the molecule of progesterone made by Peterson and Murrey [26]. In the Proceeding of The International Symposium on the History of Steroid Chemistry held in New York City in 1991, representatives of the pharmaceutical companies involved in the early stages of steroid drug manufacturing like Upjohn [27], Merck [28], Schering [29], Searle [30], Squibb [31], Syntex [32] presented

their point of view. As seen from what participants in development of the steroid drug story told firsthand, a lot of knowledge had been accumulated and lot of specialists had been involved as the steroid drug manufacturing to become possible. "From 1927 to 1984 fifteen Nobel prizes were awarded out of research work involving sterols" [33]. Among these Nobel Prizes, the one in Medicine and Physiology from 1950 was awarded for discoveries regarding the hormones of the adrenal cortex, their structure, and biological effects [34]. Thus, through discovery of cortisone and its activity against the rheumatoid arthritis, the chemist Edward Kendall, the clinic physician Philip Hench and the professor of pharmaceutical chemistry Tadeusz Reichstein paved the way towards the forthcoming successful steroid drug story. Of key importance in this story was to find out an available and cheap raw material to serve as precursor in large scale production.

The breakthrough was made by Russel Marker, a brilliant chemistry professor conducting research on sapogenins who concentrated his attention on the chemistry of the steroid sapogenin diosgenin, present in certain inedible yams growing wild in Mexico. His efforts in the area led to the formation in 1944 of the Syntex Company to produce progesterone from diosgenin in Mexican yams by a four-step process now known as Marker degradation [35]. Almost at the same time, in the early 1950s Glaxo started in UK production of cortisone from hecogenin from *Agave* family plants [36] while Upjohn concentrated on production of progesterone from sitosterol [37].

Simultaneously, the enzymatic transformations of steroids evolved as a particularly important new dimension in the steroid research at Upjohn during 1949 [27] where the microbiologist Herbert C. Murrey and the biochemist Durey H. Peterson made together a history-making discovery: progesterone was the first steroid transformed microbially by *Rhizopus nigricans* strain [26]. On this occasion Carl Djerassi, the inventor of the anti-baby pill, named also Father of the Pill, confessed that "what we chemists had accomplished laboriously through a series of complicated chemical conversions, Upjohn's microorganism with its own enzymes did in a single step" [35]. It is worth reading the Fifth David Perlman Memorial Lecture presented by William Maxon [37] as well as John Hogg's vision on Upjohn steroid [27] to get further details on the intriguing events happened within the Upjohn steroid community during the golden age of steroids.

Microbial transformation reactions of importance in steroid drugs manufacturing comprise phytosterols side-chain cleavage, 9α-steroid hydroxylation, hydroxylations at 11 and 16 position and dehydrogenation at 1-2 position.

The microbial process of β-sitosterol side-chain cleavage

This process leads to formation of androstenedione (AD) and androstadienedione (ADD) which are key intermediates in steroid drug manufacturing [38,39]. The process has been carried out with immobilized cells [40-42], with micronized substrate [43], in organic medium [44,45], in the presence of water-miscible solvents [46], in microemulsions [47], in liquid polymer medium, e.g. silicone oil [48], in two-phase aqueous-organic solvent media [49,50], in two-phase aqueous-vegetable oils medium [51], in two-phase aqueous-liquid polymer, e.g. silicone oil, polypropylene glycol, polyethylene glycol [43,52-54], with phytosterols encapsulated in cyclodextrins [55-59] and in cloud point systems, which involve the use of nonionic surfactants [60].

9α-hydroxylation of steroids

The importance of the 9α-steroid hydroxylation reaction is due to fact that it opens the way for the fluorination at the same position which can be achieved chemically and which further increases the anti-inflammatory potency of the preparations [61]. The introduction of hydroxyl function at C9 unlocks the way to opening the ring B of the steroid structure. Best studied is the process of 9α-hydroxylation of androstenedione by *Rhodococcus* sp. which is also capable of introducing hydroxyl function at C9 position of 5α-*H*-steroids of the 5α-*H*-androstane and 5α-*H*-pregnane series despite the lack of Δ^4-3-keto- configuration [14,20,21,62,63]. Detailed investigations on the enzymes have shown that 9α hydroxylase consisted of a multimeric two component Rieske type non-heme oxygenase [64]. The androstenedione might be attacked either by 9α-steroid hydroxylase with formation of 9α-hydroxy-androstenedione or by Δ^1-steroid dehydrogenase with formation of androstadienedione. Both enzymes normally exist in bacteria and due to their activity steroid ring is cleaved and further degraded. As usually one of these activities prevails, this leads to accumulation of one of the products, 9α-hydroxy-androstenedione [65] or androstadienedione [66] which are further used for synthesis of diuretics, anabolics, estrogens and anticancer drugs [14,38].

The consecutive induction of 9α-steroid hydroxylase and Δ^1-steroid dehydrogenase in resting *Rhodococcus* sp. cells was used to prevent the degradation of the accumulated in the reaction medium 9α-hydroxy-androstenedione [67]. The process of 9α-hydroxylation of androstenedione was successfully performed in the presence of Tween 80 [68] as well as in an organic solvent media [69].

Hydrocortisone and the ways to it

There are two possibilities to get to hydrocortisone employing microbial steps– directly through microbial 11β-hydroxylation of the Reichstein's compounds "S" (cortexolone) which on its turn is prepared by chemical means from progesterone; and indirectly through microbial 11α-hydroxylation of progesterone followed by six chemical steps. As in any of the cases both chemical and microbial steps are involved, the decision on which way to choose depends on a large extent on the activity of microorganisms.

For progesterone 11α-hydroxylation employed are *Rhizopus nigricans*, *Rhizopus arrhizus* and *Aspergillus ochraceus* [70,71]. To underline the importance of the conditions at which microbial transformation processes are performed (pH, temperature, aeration etc.) it is interesting to mention that the discovery of Murray and Peterson might have been put off for some time, if they were applied more rapid stirring as it was shown later that at higher aeration *Rhizopus nigricans* transforms progesterone in dihydroxyprogesterone as a single product [27]. For 11β-hydroxylation of cortexolone *Curvularia lunata* is usually the preferred choice, although *Cunninghamella blakesleeana*, *C. echinulata* and *C. elegans* have also been used [72-77].

There are a lot of studies devoted to the processes of 11α-hydroxylation of progesterone and 11β-hydroxylation of cortexolone or their derivatives. Employed are free and immobilised [78-82], growing and resting cells [75-77,83], reactions are performed in microchannels [84] etc. Reported in the literature are details regarding inducibility of steroid hydroxylases [85], application of cyclodextrins [55] and crystalline substrates [86] in processes aimed at improving their effectiveness.

The importance of the oxygen function at 11position is also due to the possibility which it opens for introducing by chemical means of fluorine in the ring B which further enhances anti-inflammatory activity of the obtained steroid [12].

Δ^1-steroid dehydrogenation

The introduction of 1-2 double bond in ring A of hydrocortisone and cortisone creates derivatives with improved anti-inflammatory properties and reduced undesirable side effects. Arthur Nobile and his team from Schering Corporation discovered that cortisone can be oxidized to prednisone by the bacterium *Corynebacterium simplex* [87]. Prednisolone was also synthesized at Schering. Both, prednisone and prenisolone revealed antiarthritic activity and absence of significant associated salt retention [29]. Elegant chemical research at Upjohn led also to 6α-methylprednisolone, the clinically important Medrol [27].

16-substituted steroid drugs

16α-hydroxylated compounds retain glucocorticoid activity without concomitant salt and fluid retention while 16β-methylation further increases the anti-inflammatory activity of steroid drugs [61]. The 16α-hydroxylation of progesterone was accomplished at Squibb by an unidentified Actinomycete strain in the same year of the phenomenal success of the Upjohn chemists Murrey and Peterson [88].

Leader in studying and development of 16-substituted corticoids was Lederle with its research on 16-oxygenated steroids culminating in the synthesis and therapeutic use of triamcinolone and related compounds [89]. The Squibb process for the production of triamcinolone is based on microbial 16α-hydroxylation of 9α-fluorohydrocortisone and 9α-fluoroprednisolone performed by *Streptomyces roseochromogenus* [90].

16β-methylated steroid drugs were almost simultaneously reported by Merck and Schering, betamethasone being another highly potent glucocorticoid devoid of salt retention [28].

Microbial 16-hydroxylation activity was reported in *Aspergillus niger* by introducing the hydroxyl function directly at 16β-position of 17-oxo-steroids [91] and in *Streptomyces roseochromogenus* at 16α-position of progesterone [92]. *Nocardia farcinica* IFM 10152 displayed 16α-hydroxylation activity, a feature ascribed to the bacterial P450 monooxygenase CYP154C5 which in turns can be exploited to obtain 16α-hydroxylated steroids at preparative scale [93].

9α-fluorosteroids and their place in the steroid drug industry

The merit for the discovery of fluorosteroids belongs to Squibb chemists and a patent was issued in 1958 [94]. The recollections of John Fried regarding events leading to first synthesis of 9α-fluorosteroids and how their potential was revealed present an impressive reading. As he mentioned, nobody at Squibb really believed that this would be of great interest, since no fluorine-containing drug had ever reached the market, even more fluoroacetate was a highly toxic enzyme inhibitor. Interestingly, 9α-bromocortisol and 9α-iodocortisol obtained from 11-epicortisol had one third and one tenth of the activity of cortisol, respectively. Most unexpectedly, 9α-chlorocortisol was shown to possess the appreciable (3.5 x Cortisol) activity while the 9α-fluorocortisol turned out to be a superglucocorticoid with 10 times higher activity of cortisol, possessing as well activity equal to the mineralcorticoid hormone aldosterone, at that time a laboratory curiosity [31].

Combinations of steroid transforming reactions

Combining processes of 9α- and 11-hydroxylation, 1-2 dehydrogenation and 16α-methylation with fluorination led to further development of powerful not-salt retaining anti-inflammatory chemical analogues hydrocortisone, prednisolone, dexamethasone, betamethasone etc., presented on Figure 1.

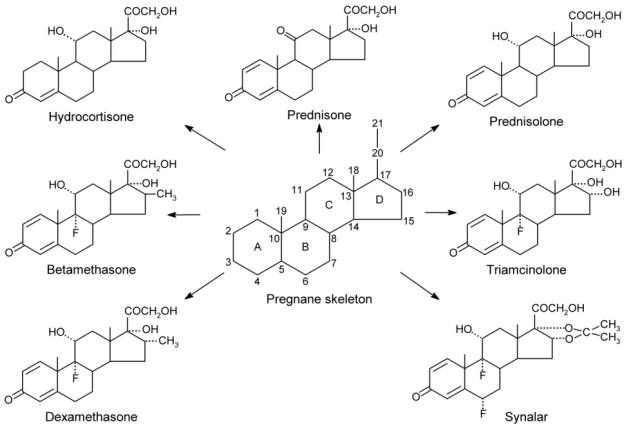

Figure 1: Pregnane skeleton and some steroid drugs which manufacturing involves microbial steps like hydroxylation at C9, C11 and C16, and dehydrogenation at C1-C2.

It is important to notice that they are still irreplaceable in medicine despite of being denied and blamed for different reasons.

Microbial transformation of terpenes

Terpenes have always been in the focus of investigations due to their wide applications in the flavor and fragrance industry, as well as because of their potential for further biotechnological developments as pharmaceutical agents and insecticides [95]. Terpenes have a variety of roles in mediating antagonistic and beneficial interactions among organisms [96]. There are a lot of data in the literature describing the ability of specific microorganisms to perform microbial transformations of terpenes like *Mucor* sp. [97], *Aspergillus niger* cultures [98], etc. There are reviews on microbial transformation of monoterpenes [99] and triterpenes [100-102]. The microbial transformations of terpenoids applied in folk medicine and of interest for pharmacy are also reviewed in the literature like antimalarial ones [103,104], *ent*-kaurane diterpenes [105], *Schisandraceae* [106] and oleanane triterpenoids [107], taxanes [108], etc.

Here we will discuss microbial transformations of terpenes which afford compounds with improved biological activity.

Taxol is a naturally occurring diterpenoid widely applied as a powerful anti-cancer drug. More than 500 microorganisms were screened for their ability to achieve useful biotransformation of taxol/cephalomannine and *Streptomyces* sp. MA 7065 was selected due to its ability for formation of hydroxy-derivatives with significantly enhanced characteristics against human tumor cell lines than the respective substrates [109].

Two taxadienes obtained from sinenxan A by chemical synthesis were transformed by filamentous fungi (*Cunninghamella echinulata* and *Aspergillus niger*) and actinomycete strains (*Streptomyces griseus* and *Nocardia purpurea*) into twenty one derivatives. Two of these derivatives (2α-hydroxy-5α,10β,14β-triacetoxytaxa-4(20),11(12)-diene and 2α,5α,10β,14β-tetraacetoxytaxa-4β,20-epoxy-11(12)-ene) are considered promising lead compounds for reversal agents against A549/taxol tumor MDR cells [110].

When subjected oleanoic acid to transformation with the filamentous fungus *Fusarium lini*, Choudhary et al. obtained two metabolites, one with a hydroxyl group at C2 and one with hydroxyl groups at C2 and C11. Both metabolites showed more potent inhibitory activities against the enzyme α-glucosidase than the clinically used drug acarbose and comparable activities with the standard drug deoxynojirimycin [111]. In 2013, Martinez et al. reported on the antitumor properties of the 30-hydroxyderivative of the oleanoic acid (queretaroic acid) derived from the transformation of the oleanoic with *Rhizomucor miehei* [112].

Cycloastragenol, which is the main aglycon of many cycloartane-type glycosides found in *Astragalus* genus, has been recently introduced to the dietary supplement market as TA-65®, a new generation anti-aging molecule. Subjected to transformation by *Cunninghamella blakesleeana* cycloastragenol gave a metabolite with an interesting triterpenic skeleton derived due to an exceptional transformation involving ring cleavage and methyl group migration [113].

Steviol is an aglycone of stevioside, the major sweet component isolated from leaves of *Stevia rebaudiana* (Bertoni) Bertoni (*Compositae*). The group of de Olivera et al. performed continuous work for obtaining new derivatives of steviol. They functionalized rings B and C of isosteviol (a beyerane-type diterpenoid) by *Fusarium verticilloides* affording 7α-hydroxy- and 12β-hydroxy-derivatives [114]. The 7β-hydroxylation of isosteviol was achieved by *Aspergillus niger* and *Rhizopus arrhizus*, the 1α-hydroxylation by *Aspergillus niger* and the 17-hydroxylation by *Penicillium chrysogenum* [115].

Akihisa et al. also obtained 7β-hydroxyisosteviol from isosteviol by *Aspergillus niger* accompanied by 11β-hydroxyisosteviol and 12β-hydroxyisosteviol as well. The transformation of isosteviol by *Glomerella cingulata* afforded 17-hydroxyisosteviol and resulted in 7-oxoisosteviol when *Mortierella elongate* was employed. Importantly, all five hydroxylated metabolites exhibited more potent inhibitory effects on tumor promoters than parent diterpenes [116].

Mucor recurvatus was found to transform steviol-16α,17-epoxide into ent-13,16β,17-trihydroxykauran-19-oic acid, derivative with higher antihyperglycemic activity than steviol. Importantly, the amounts of the derivative were enough to provide a technical basis for studying its mechanism of action as well as its pharmacological and toxicological effects [117].

Aspergillus niger and *Fusarium moniliforme* are found capable of increasing polarity of *Isodon* and *Rabdosia* diterpenoids, known as antimicrobial and antitumor compounds, by hydroxylating non-activated positions. The activity of the polyhydroxylated derivatives is dependent on the number and the position of hydroxyl groups in the molecule [118].

Ent-8(14),15-primaradien-19-ol (pimarane-type diterpene) was obtained by fungal transformation and showed very promising minimal inhibitory concentration value against the main microorganisms responsible for dental caries [119].

Diterpenes stemodin, stemodinone, stemarin are isolated from the shrub *Stemodia maritime*. All three compounds were transformed by *Aspergillus niger* ATCC 9142 and gave three hydroxylated compounds, two known analogues and one novel metabolite. Stemodione was hydroxylated to two known analogues while stemarin gave four new compounds [120]. When transformed by *Phanerochaete chrysosporium* three three-hydroxylated products of stemodin and one dihydroxylated product of stemodin were produced [121]. The transformation with *Cunninghamella echinulata* resulted in three three-hydroxylated products of stemodin: two dihydroxylated products of stemodinone, one of which new, and in one novel metabolite from stemarin as a sole metabolite [121]. *Beauveria bassiana* ATCC 7159 transformed stemodin and stemodinone exclusively into hydroxylated derivatives 2α,13,18-trihydroxystemodane and 13,18-dihydroxystemodan-2-one, respectively. Stemarin was converted to the novel 1β,13,19-trihydroxystemarane and 13-hydroxystemarane-19-carboxylic acid [122].

Jatrophone was transformed by *Aspergillus niger* ATCC 16404 and afforded the new diterpene 9β-hydroxyisabellinone which revealed strongly reduced cytotoxicity and enhanced selectivity assessed on a permanent human epithelial gastric cell line (AGS) (ATCC CRL-1739) [123].

The transformation of the imbricatoic acid by *Aspergillus niger* afforded a main compound identified as 1α-hydroxylabdan-19-oic acid. *Rhizopus nigricans* gave rise to 15-hydroxy-8,17-epoxyderivative. The main products obtained by transformation with *Cunninghamella echinulata* were identified as mycophenolic acid and its 3-hydroxy derivative. The last two compounds showed low toxicity towards human lung fibroblasts and AGS cells while the cytotoxicity of 1α-hydroxyimbricoic acid was a moderate one [124].

Microbial transformation of the two 8,9-unsaturated lactonic drimane derivatives confertifolin and isodrimenin (isolated from the bark of *Drymus winteri* Forst, *Winteraceae*, a South American tree commonly found in Chile and Argentina) with *Mucor plumbeus*, *Aspergillus niger* and *Rhizopus arrhizus* has been reported. It was shown that process easily provides 3β-hydroxyderivatives in high yield. In the case of incubation of isodrimenin with *R. arrhizus*, an additional product hydroxylated at C7 could be obtained. Such regio- and stereo-selectively functionalized compounds are of interest because they often correspond to minor natural products usually isolated in very small amounts [125].

Limonoids, chemically classified as tetranortriterpenoids, have been found to possess anti-cancer, anti-malarial, anti-HIV, antimicrobial and several other pharmacological activities. Haldar et al. reported 12β- and 17β-hydroxylation on the basic limonoid skeleton using *Mucor*-mediated microbial transformation. 12β-hydroxy products are rare in nature and therefore this report is important providing way to production of 12β-hydroxy limonoids and further evaluation of their bioactivities [126].

Garcia-Granados et al. attempted microbial transformation of *ent*-13-*epi*-manoyl oxides - labdane-type diterpenoids - to introduce hydroxyl groups at positions difficult to achieve by chemical means as to produce new bioactive, highly hydroxylated analogues of *ent*-forscolin [127] which is naturally produced by the Indian plant *Coleus forskohlii* and is commonly used to raise levels of cyclic AMP in the study and research of cell physiology.

The maslinic acid was transformed by *Cunninghamella blakesleeana*. The obtained four new compounds (7β-hydroxy-, 15α-hydroxy-, 7β, 15α-dihydroxy- and 13β-hydroxy-derivatives) were more polar than the parent one [128]. The action of *Rhizomucor miehei* on the maslinic acid resulted in formation of five derivatives, an olean-11-en-28,13β-olide derivative, a metabolite hydroxylated at C30, an 11-oxo-derivtive and two metabolites with an 11α,12α-epoxy group, hydroxylated or not at C30 [112].

Licorice (*Glycyrrhiza grabra*) has well known pharmacological properties [129]. When transformed by *Cunninghamella blakesleeana*, its active component, the glycyrrhetinic acid, affords six metabolites, two of them being major ones and revealing considerable activities against the drug-resistant *Enterococcus faecalis* [130].

Betulin (lupane-type triterpene obtained from the bark extract of white birch, *Betula platyphylla Sukatshev* var. *japonica*) was transformed by *Chaetomium longirostre* into 4,28-dihydroxy-3,4-seco-lup-20(29)-en-3-oic acid and 4-hydroxy-3,4-seco-lup-20(29)-ene-3,28-dioic acid. Betulonic acid, a chemical oxidation product of betulin, transformed by the same fungus gave rise to 4,7β,17-trihydroxy-3,4-seco-28-norlup-20(29)-en-3-oic acid and 7β,15α-dihydoxy-3-oxolup-20(29)-en-28-oic acid. All compounds (betulin, betulonic acid and their metabolites) showed potent inhibitory effects on tumor promotion. Biotransformation products that underwent ring-opening and hydroxylation exhibited more potent activity than their corresponding precursors [131].

Betulin was transformed also by *Cunninghamella blakesleeana* cells with formation of at least five products among which betulinic acid was the most important one due to its antiretroviral, antimalarial and anti-inflammatory properties. Recently, betulinic acid was described as potential anticancer agent as well. This transformation reaction provides an attractive alternative approach to chemical synthesis, because is less time-consuming and more environmentally friendly [132].

Gentiopicroside is a principal bitter substance found in many gentianaceous plants which are widely used as medicinal herbs in China and Europe due to the variety of pharmacological activities they exhibit. When transformed by the endophytic fungus *Penicillium crustosum* it gave several metabolites, three of them showing potent protective effects against HL-7702 cell injury induced by hydrogen peroxide in the *in vitro* bioassay, while the substrate exhibited no activity at the tested concentrations [133].

β-lapachone is an ortho-naphthoquinone found as a minor constituent in the heartwood of the *Tabebuia* species and considered as promising anticancer agent. It has been included in clinical trials as mono therapy and in combination with other cytotoxic drugs. β-lapachone and one of its derivatives obtained by microbial transformation with *Cunninghamella elegans* containing β-D-glucose moiety attached to position 6 of ring B were subjected to cell toxicity assays. Results displayed lower activity of the derivative against breast cancer line SKBR-3 in comparison with β-lapachone, but did not show cytotoxicity against normal fibroblasts cell line GM07492-A, whereas β-lapachone was highly toxic [134].

Microbial transformation of alkaloids

Alkaloids represent a diverse group of plant natural products with variable chemical structure. They are used for centuries because of the wide variety of their physiological effects [135]. The interactions of microorganisms with alkaloids are of special interest. The data on microbial transformations of alkaloids accumulated up to 2000-2001 were reviewed by Abraham and Spassov [136] while at the same time Rathborne and Bruce emphasized in their review paper on the engineering biocatalytic routes for production of semisynthetic opiate drugs [137]. Morphine and codeine were transformed into potent analgesic hydromorphone and the mild analgesic/antitussive hydrocodone, respectively, by recombinant *E. coli* [138]. Demethylations, oxidations and reductions of morphine alkaloids were performed with different fungal strains, *Cunninghamella echinulata* being the most effective one [139]. *Rhizobium radiobacter* was reported to hydroxylate codeine to its C-14 derivative. This transformation reaction is of importance for the production of drugs displaying analgesic, antitussive and narcotic antagonist characteristics like oxycodone [140].

Veratrum alkaloids are a group of potent hypotensive agents that lower blood pressure by reflex suppression of the cardiovascular system. Lü et al. reported biotransformation of vermitaline (verazine type steroidal alkaloid isolated from the roots of *Veratrum dahuricum* and one of the most extensively studied) by *Cunninghamella echinulata* into four metabolites, three of which being new compounds [141].

The steroidal alkaloid dictyophlebine (potent cholinesterase inhibitor) from the plant *Sarcococca hookeriana* BAILL was transformed by *Rhizopus stolonifer* into three polar derivatives, one of which revealed higher inhibitory activity than that of the parent compound [142].

The antimalarial property of cinchona bark and the subsequent isolation of its active compound, quinine, have played a pivotal medicinal role in human society for over 300 years [143]. The incubation of cinchona alkaloids with the endophytic *Xylaria* sp. isolated from *Cinchona pubescens* (*Rubiaceae*) led to formation of three derivatives, quinine 1-*N*-oxide, quinidine 1-*N*-oxide and cinchonine 1-*N*-oxide, which revealed weakly inhibiting effect on the proliferation of the malaria pathogen *Plasmodium falciparum*, a chloroquine-resistant strain [144].

Ruscogenin is a steroidal glycoside extracted from ruscus roots. In Europe, the roots and stems of the *Ruscus aculeatus* (Butcher's Broom or thorny ruscus) have been used for centuries. Recent clinical observations reveal the vasculoprotective and phlebotonic properties of butcher's broom-based preparations. Ruscogenin is low water soluble which restricts its application, but a derivative with higher water solubility has been obtained via microbial transformation [145].

Microbial transformations of flavonoids

Flavonoids are plant metabolites with biological functions ranging from coloration of flowers as a visual signal that attracts pollinators and protection from ultraviolet radiation and phytopathogens to participation in stress responses [146]. According to Ren et al., flavonoids are a group of more than 4000 polyphenolic compounds which possess a common phenylbenzopyrone structure (C6-C3-C6) and are categorized according to the saturation level and opening of the central pyran ring [147]. The microbial transformation strategies for production of flavonoids have attracted considerable interest because they allow yielding of novel flavonoids, which do not exist in the nature [148]. The achievement of microbial glycosylation led to significant advance in biotechnological glycosylation of flavonoids [149]. The main function of glycosylation processes are stabilization, detoxification and solubilization of substrates [150]. The substituent

groups in flavonoids affect their properties. Thus, the hydroxyl groups are both important for the antioxidizing capacity and key points for further modification like *O*-methylation and C-glycosilation. The *O*-methylation of flavonoids changes chemical reactivity of the phenolic hydroxyl groups and increases lipophilic properties of the compound which is significant for retaining optimal hydrophilic- lipophilic properties of newly formed flavones [151].

The biological activities of baicalin and baicalein (isolated from *Radix Scutellariae*) among which antiallergic, anti-inflammatory, antitrombotic, and anticancerogenic might be changed and/or improved by microbial transformation. Thus, baicalin can afford 4', 5,6,7-tetrahydroxyflavone by *Coryneum betulinum*, *Chaetomium* sp. and *Cryptosporiopsis radicicola*. *Chaetomium* sp. transforms baicalin also into 5,7-dihydroxy-6-methoxyflavone while *Penicillium chrysogenum* gives rise to 5,7-dihydroxy-4',6-dimethoxyflavone. Both reactions, methylation and hydroxylation, proceed with high region-specificity [152].

Puerarin is an isoflavone from *Pueraria lobata* with promising biological activities but limited clinic use due to its low water solubility and poor adsorption after oral administration. It was transformed by *Lysinibacillus fusiformis* into puerarin-7-*O*-fructosid which revealed an increased antioxidant activity combined with improved water solubility [153].

Quercetin is a natural flavonoid distributed in many plants such as green tea, fruits and leaf vegetables. It has displayed a variety of biological activities including anticancer, antihypertensive, anti-inflammatory and antiviral properties [154]. The metabolism of quercetin which involves C-3 glucosylation, C-3' *O*-methylation, and a dehydrogenation was studied by *Cunninghamella elegans* ATCC 9245 thus expanding knowledge on the catalytic repertoire of this filamentous fungus [155]. Recently, a note regarding efficient bioconversion of quercetin into a novel glycoside (quercetin-7 *O*-β-4″-deoxy-hex-4″-enopyranosiduronic acid) by *Streptomyces rimosus* subsp. *Rimosus* ATCC 10970 was published [154]. This is the strain producing the well-known antibiotic oxytetracycline and the polyene antifungal antibiotic rimocidin. Derivatives of rutin (quercetin 8-C-glucoside) are used to increase capillary resistance and are recommended for treatment of circulatory disorders and inflammation [156]. Reported were promising anticancer activities as well as important antioxidant, radical scavenger, antileukemic, vasodilator activities of flavonoids [147,157].

Two of the derivatives of flavones biotransformation by *Aspergillus niger* (2'-hydroxydihydrochalcone and 2'-hydroxyphenylmethylketone) revealed higher antioxidant activity than the substrate as well as antimicrobial activity against *Pseudomonas aeruginosa*, *Aspergillus flavus* and *Candida albicans* [158].

Mucor species were found to perform reactions of deglycosilation, dehydrogenation and *O*-methylation of the flavonoid naringin (compound giving grapefruit its typical bitter flavor and being reported to exhibit a number of biological activities) resulting in formation of eleven products [159]. *Trichoderma harzianum* was capable of naringin hydroxylation affording 3'-hydroxyl naringin and 3'5'-dihyroxyl naringin which revealed 68.6- and 77.9-forld increase in the antioxidant activity, compared to the parent compound [160].

Microbial transformation of (poly)phenols

Natural (poly)phenols are known with their wide range of pharmacological activities and with their applicability as food additives. In the recent years the useful properties of some of the (poly)phenols like resveratrol and curcumin were improved by microbial transformations.

Resveratrol (3,5,4'-trihydroxystilbene) is one of the most widely studied polyphenols produced by plants and presented in red wine. Microbial transformation of *trans*-resveratrol into piceatannol by a wild type *Streptomyces* sp. was reported and the obtained piceatannol was found to have antioxidative effects and to exhibit potential anticancer properties as suggested by its ability to suppress proliferation of a wide variety of tumor cells, including leukemia, lymphoma, and cancers of the breast, prostate, colon, melanoma and apoptosis in colorectal cancer [161]. Resveratrol was transformed by *Geotrichum histeridarum* in *bis*-resveratrol which revealed 1.7 fold increased activity than parent substrate [162]. The preparative scale microbial transformation of resveratrol by *Bacillus cereus* resulted in formation of piceid [163]. Piceid is the main component of the *Polygonum cupsidatum* roots, used in Japanese and Chinese folk medicine for the treatment of some cardiac ailments, including atherosclerosis and inflammation [164].

The polyphenolic compound curcumin has shown a wide range of pharmacological activities and has been widely used as a food additive. However, the clinical use of curcumin is limited to some extent because of its poor water solubility and low bioavailability. To overcome these problems, many approaches have been attempted and structural modification of curcumin and microbial transformation has been proven to be alternative. The transformation of curcumin into its analogue was carried out by the endophytic fungus CL-Bel-5F isolated from *Curcuma longa* L. Studies on the anticancer and hepatoprotective activities of the hexahydrocurcumin are in progress [165]. Microbial transformation of curcumin into four colorless hydroderivative by the endophytyc fungus *Diaporthe* sp. associated with *Curcuma longa* was reported [166]. The newly isolated yeast strain *Pichia kudriavzevii* was found to transform curcumin into hexa- and tetrahydrocurcumin [167].

Alternative medicine and microbial transformations of its active ingredients

Throughout Old Europe, Asia, Middle East, Africa, and the Americas, early people were making and consuming fermented drinks with an amazing variety of plant substances that were indigenous to their area [168]. The therapeutic advantages of medicinal herbs fermented with *Lactobacillus plantarum* in topical application and its activities on atopic dermatitis were shown [169]. The Taiwanese alternative medicine Lu-Doh-Huang was further developed with an application of pyrosequencing and culture methods to assess the microbial diversity of fermented mung beans [170]. Changes in the gingenoside content [171] and preparation of minor gingenosides [172] were achieved via controlled fermentation processes. In the recent years, with advances in microbial fermentation and transformation, the traditional Chinese medicine has become a new way to produce new drugs and get active compounds [11].

The tetracyclic alkaloids tetrahydroprotoberberines (THPBs) are isolated from Chinese herbs due to their unique pharmacological profile as D2 dopamine receptor antagonists and D1 receptor agonists [173]. The ability of the fungal strain *Gliocladium deliquescens* NRRL1086 for regio- and enantio-selective glycosilation of a series of THPBs is very attractive as glycosidic THPBs are very rare in the nature. This finding could provide pure THPB derivatives for bioassays

and more important, prove to be an alternative method for their preparation [174].

The enantiomeric lycodane alkaloid Huperzine A (Hup A) isolated from the club moss, *Huperzia serrata* (Thunb.), *Huperziaceae* is known in China as Qian Ceng Ta and has been marketed there as a new drug for Alzheimer's disease treatment. Its derivative ZT-1 is being developed as anti- Alzheimer's disease new drug candidate both in China and in Europe [175]. The product M3, obtained via transformation of HupA by *Streptomyces griseus* after a two-step procedure and various chromatographic techniques, was identified as Huperzine A 8α,15α-epoxide [176] and found to protects PC12 cells against sodium nitroprusside-induced apoptosis [177]. Recently, Huperzine A was transformed by the fungal endophyte *Ceriporia lacerate* into several derivatives, some of them comprising tremulane sesquiterpenoids-Huperzine A hybrids [178].

Gingenosides are the main chemical constituents of Chinese ginseng (*Panax ginseng* C.A. Mey, *Araliaceae*), they exhibit extensive biological activities and are responsible for the tonic functions of ginseng. 20(S)-protopanaxadiol and its analogues 20(S)-protopanaxatriol are aglycones of gingenosides. Transformation of 20(S)-protopanaxadiol by *Mucor spinosus* resulted in formation of eight derivatives, six of them being new compounds. The 12β-hydroxyl group of all products was specifically dehydrogenated into carbonyl group while some of the products were hydroxylated at novel positions [120]. The 20(S)-protopanaxadiol was transformed also by *Absidia corymbifera* and three of the five derivatives were found to be more potent inhibitors against DU-145 and PC-3 cell lines than the substrate [179]. It was suggested the regulation of the external calcium concentration to be used for manipulation of the gingenoside Rb1 transformation into gingenoside Rd by *Paecilomyces bainier* [180].

Methyl protodioscin is among the active compounds isolated from the rhizome of *Dioscorea collettii* var. *hypoglauca* (*Dioscoreaceae*), a Chinese herbal remedy for the treatment of carcinomas for centuries. It was transformed by *Penicillium melinii* into seven derivatives, most of them revealing considerable cytotoxic activities against HepG2, NCI-H460, MCF-7 and HeLa cell lines [181].

Closing Remarks

Microbial transformations of organic compounds gained their importance with the development of steroid drugs where such processes take an irreplaceable role. Although the application of microorganisms for carrying out chemical reactions was invented four decades before the term Green Chemistry to be officially coined, it remains one of the outstanding applications of Green Chemistry within the pharmaceutical industry [182]. Since then a great variety of plant derived biologically active compounds were subjected to microbial transformations aiming at improvement of their biological activity and administration. Microbial transformations performed by fungal strains are of immense importance as models of mammalian metabolism of the plant origin compounds which are applied in medicine. They give information regarding correlations between structures of the compounds of interest and their specific biological activities simultaneously. Investigations on microbial transformations give an insight in both, chemical diversity of plant derived biologically active compounds and their derivatives and diversity of microorganisms. From this point of view any single report on specific microbial transformation of some biologically active compound performed by some microorganism is of interest.

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