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MINI-REVIEW

Fungi as a source of natural coumarins production

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Abstract Natural coumarins and derivatives are compounds that occur naturally in several organisms (plant, bacteria, and fungi) consisting of fused benzene and α-pyrone rings. These compounds show high technological potential applications in agrochemical, food, pharmaceuticals, and cosmetics industries. Therefore, the need for bulk production of coumarins and the advancement of the chemical and pharmaceutical industries led to the development of synthetic coumarin. However, biotransformation process, synthetic bioengineering, metabolic engineering, and bioinformatics have proven effective in the production of natural products. Today, these biological systems are recognized as green chemistry innovation and business strategy. This review article aims to report the potential of fungi for synthesis of coumarin. These microorganisms are described as a source of natural products capable of synthesizing many bioactive metabolites. The features, classification, properties, and industrial applications of natural coumarins as well as new molecules obtained by basidiomycetes and ascomycetes fungi are reported in order to explore a topic not yet discussed in the scientific literature.

Keywords Filamentous fungi · Natural coumarins · Biotransformation · Therapeutic agent

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Introduction

The production of chemicals by microbial conversion is becoming an important component of industrial biotechnology. Advances in production of biologically active metabolites have opened up new possibilities for more extensive industrial scale-up applications (Vannelli et al. 2007). Physiologically active compounds such as antibiotics, antitumor drugs, antibodies, hypocholesterolemic drugs, anticancer drugs, immunosuppressants, plant hormones, growth promoters for livestock, alkaloids, statins (Vieira et al. 2008; Demain 2014; Khan et al. 2014), phenolic compounds, terpenes, steroids (Hasnat et al. 2015), and antioxidants (Borderes et al. 2011) are obtained by fungal culture in bioprocess. Fungi are also recognized by their wide potential in enzyme production of commercial scale (Demain 2014; Hansen et al. 2015), biotransformation process (Aguirre-Pranzoni et al. 2011), and synthesis of organic acids (Liaud et al. 2014).

Coumarins and derivatives are structural units present in several natural products (Daru and Stirling 2011; Kalita and Kumar 2012), obtained by plant extraction (Bertin et al. 2014) or by microbial synthesis. More than 1300 coumarins have been identified as secondary metabolites from plants, bacteria, and fungi (Hwang et al. 2013; Venugopala et al. 2013; Wang et al. 2013; Islam et al. 2015). The name coumarin comes from a French term for the Tonka bean, *coumarou* (*Dipteryx odorata*), isolated by Vogel, 1820 (Venugopala et al. 2013; Rohini and Srikumar 2014; Matos et al. 2015). Due to its sweet odor, similar to vanilla, coumarin has been used in perfumes since 1882 (Aslam et al. 2010; Santos et al. 2013; Matos et al. 2015).

Coumarin presence is reported in about 150 plant species, distributed in 30 different families. The most important are *Rutaceae*, *Umbelliferae*, *Clusiaceae*, *Guttiferae*, *Caprifoliaceae*, *Oleaceae*, *Nyctaginaceae*, and *Apiaceae* (Venugopala et al. 2013). Although present in natural form,

the isolation and purification of coumarins may be difficult, expensive, and oscillatory. There is the possibility that climatic factors can interfere in the accumulation of active principles of the plant (Lin et al. 2013b; Venugopala et al. 2013; Hara et al. 2014).

Coumarins are very attractive targets for combinatorial library synthesis due to their wide range of valuable biological activities (He et al. 2015). They were first synthesized in 1868, and it was used in the pharmaceutical industry as a precursor in the synthesis of a number of anticoagulant pharmaceuticals. The simplicity and versatility of the coumarin molecule makes it an interesting starting point for a wide range of applications (Matos et al. 2015). They are used as technological potential in pharmaceutical, agrochemical, fragrance industries, food, cosmetic industry, and in color technology as optical brightening agents or laser dyes (Daru and Stirling 2011; Santos et al. 2013; Venugopala et al. 2013; Vekariya and Patel 2014). Coumarins constitute the largest class of fluorescent dyes (Martins et al. 2012).

Strategies were developed to chemically synthesize coumarin using petro-derived chemicals. However, increasing concerns on environmental issues have stimulated efforts towards the development of biological processes capable of utilizing renewable resources (Lin et al. 2013a). Several bacteria and fungi are known to synthesize coumarin (Aslam et al. 2010; Nikhil et al. 2012; Rohini and Srikumar 2014), e.g., *Agaricus* sp. (Mogland and Saadabi 2012), *Armillariella tabenscens* (Wang et al. 2007), *Aspergillus* (Rohini and Srikumar 2014), *Fomitopsis officinalis* (Hwang et al. 2013), *Ganoderma lucidum* (Islam et al. 2015), *Macrolepiota mastoidea* (Shirmila and Radhamany 2013), *Penicillium*, *Pestalotiopsis* sp. (Wang et al. 2013), *Phellinus* sp. (Ma et al. 2015), *Streptomyces spheroides*, *Streptomyces niveus* (Steffensky et al. 2000), *Talaromyces flavus* (He et al. 2014), *Trichosporon asahii* (Awe et al. 2009), and *Xylaria* sp. YX-28 (Liu et al. 2008). One of the advantages of microbial fermentation is the provision of inexpensive sources of carbon, nitrogen, trace elements, and energy for growth and product synthesis. Lower temperatures compared to chemical synthesis has also been observed as a positive factor in bioprocesses (Hara et al. 2014).

The conditional requirements to fulfill the price advantages for the production of microbial coumarins over synthetic coumarins or plant extraction are high growth rates at high cell density (Hara et al. 2014). This characteristic is often not obtained conventionally. Significant advances in synthetic bioengineering, metabolic engineering, and bioinformatics have promoted the modified biosynthesis of a variety of pharmaceutically important compounds in heterologous microbial host. This allowed wider applications of these compounds on an industrial scale (Vannelli et al. 2007; Lin et al. 2013a; Hara et al. 2014). In this context, we presented an overview of the potential of fungi as coumarin producer and their biological applications besides the properties, characteristics, production, and biosynthesis.

Coumarins: classifications and properties

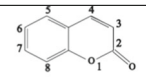
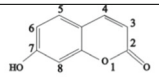
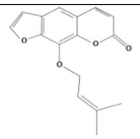
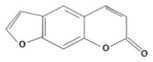
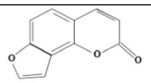
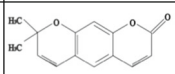
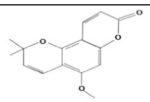
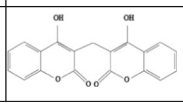
Coumarins (2H-1-benzopyran-2-one) are a group of polyphenolic compounds that occupies an important place in the kingdom of natural products, organic chemistry, and synthetics (Venugopala et al. 2013; Rohini and Srikumar 2014; Vekariya and Patel 2014). They are colorless and crystalline phytochemicals belonging to the family of benzopyrones (Jain and Joshi 2012; Rohini and Srikumar 2014), consisting of fused benzene and α-pyrone rings (Jain and Joshi 2012; Venugopala et al. 2013; Rohini and Srikumar 2014). The numbering of coumarin starts from the ring oxygen, i.e., oxygen receives position-1 and goes round anti-clockwise along with the ring (Keri et al. 2015). Coumarins can be classified into four categories: simple coumarin, furanocoumarin, pyranocoumarin, and coumarin with substituent on the pyrone ring (Jain and Joshi 2012; Lin et al. 2013b; Venugopala et al. 2013; Rohini and Srikumar 2014), as shown in Table 1.

Coumarins and their derivatives have demonstrated a vast array of therapeutic applications including antioxidant, antibacterial, antiviral, anti-inflammatory, antinociceptive, antidepressant, antitumor, antiasthmatic, hepatoprotective, antiallergic, antifungal, and inhibiting serine protease and HIV (Aslam et al. 2010; Kumar et al. 2013; Lin et al. 2013b; Patil et al. 2013; Abdel-Wahab et al. 2014; Vekariya and Patel 2014). They can be used as cholinesterase (ChE) inhibitors for the treatment of Alzheimer's disease and treating Parkinson's disease (Anand et al. 2012; Sandhu et al. 2014). This is what makes coumarins so attractive for screening as a new therapeutic agent (Lacy and O'Kennedy 2004; Rohini and Srikumar 2014).

Extensive research on pharmacological and therapeutic properties of coumarins has resulted in the acknowledgment of their therapeutic role in the treatment of cancer cells (Lin et al. 2013b) and the side effects caused by radiotherapy (Rohini and Srikumar 2014). Many compounds showed cytotoxic activity against several human cancer cell lines (gastric carcinoma, colon carcinoma, hepatoma-derived cell line, and lymphoblastic cell line) in vitro and in xenograft models (Emami and Dadashpour 2015) and the treatment of prostate cancer, renal cell carcinoma, leukemia (Rohini and Srikumar 2014), and pancreatic cancer (Jun et al. 2014).

Far beyond the pharmaceutical importance, coumarins can be used in many industrial processes. Coumarins have a distinctive and accented odor as butter flavorings in processed foods. They can also be used in the production of tobacco, beverages, cleaning products, and cosmetics (deodorants and perfumes) as a fixative or to highlight the fragrance. Besides, coumarins are components of the formulations of hair spray, toiletries, detergents, as well as rubbers, plastics, and paints for masking odors of organic solvents (Egan et al. 1990; Vila Nova et al. 2012). Furanocoumarins (psoralen and bergapten) are used as photodynamism in bronzing, increasing the tanning induced by ultraviolet radiation (Vila Nova et al. 2012).

Table 1 Different structures and applications of coumarins

Molecule	Structure	Example	Applications	Reference
Simple coumarin		Simple coumarin	Perfume fixative; Paint; Spray additive; Food flavoring;	Miyano et al. 2014
		Umbelliferone	Leukemia; Antioxidant; Antimicrobial;	Rohini and Srikumar 2014 Hrobonová et al. 2013
Furanocoumarin linear type		Imperatorin	Anti-inflammatory; Antibacterial; Antifungal; Anticancer; Anticonvulsant;	Venugopala et al. 2013
		Psoralen	Cervical carcinoma; Skin disorders; Psoriasis; Vitiligo;	Rohini and Srikumar 2014
Furanocoumarin angular type		Angelicin	Thalassemia; Sickle cell anemia; Antiviral;	Jeong-Cho et al. 2013
Pyranocoumarin linear type		Xanthyletin	Antibacterial;	Venugopala et al. 2013
Pyranocoumarin angular type		Alloxanthoxyletin	Antibacterial; Antiviral;	Cao et al. 2013;
Bicoumarin		Dicoumarol	Anticoagulant;	Molina and Zanusso Júnior 2014.

There are some companies specialized in the synthesis, research, and development of the coumarin production processes. The INDOFINE Chemical Company, Cayman Chemical, and SLN Pharmachem produce several coumarins and derivatives in their database for use in the pharmaceutical, agriculture, fragrances, cosmetics, and other industries. However, the most common ways of getting coumarins are through plant cell extraction or chemical synthesis. Alternative methods involving biotechnological processes are still little elucidated and their potential requires to be further explored.

Coumarins biosynthesis

Metabolic engineering and synthetic biology have enabled the construction of novel metabolic pathways that do not exist in nature or enhance existing ones (Sun et al. 2015). Microorganisms such as *Saccharomyces cerevisiae*, *Bacillus* strains, *Streptomyces* strains, *Corynebacterium glutamicum*, and *Aspergillus oryzae* are selected as a host for fermentations depending on their specific metabolic pathways to synthesize target products (Vannelli et al. 2007; Hara et al. 2014; Sun et al. 2015). Coumarin synthesis using metabolic engineering allows to better identify the reactions and enzymes involved in the process and to increase the likelihood of developing effective strategies with the goal of large-scale manufacture.

However, despite the properties and pharmaceutical importance of coumarins, little information is available regarding the microbial biosynthesis of simple coumarin molecules, which serve as a gateway to other coumarin derivatives (Lin et al. 2013b). In one route, known to function in plants (Fig. 1), coumarin is formed from the conversion of the glucose (**1**), starts from L-phenylalanine (**3**) in which phenylalanine ammonia-lyase (PAL, EC 4.3.1.24) removes the (*pro*-3S)-hydrogen and $-NH_3^+$ from L-phenylalanine to yield *trans*-cinnamic acid (CA) (**4**) (Koukol and Conn 1961; Vannelli et al. 2007). In the next step, the Cytochrome P450 enzyme cinnamate 2-hydroxylase (C2H) catalyzes hydroxylation at the 2-position of *trans*-cinnamate yielding *trans*-2-coumarate (**5**) (Gestetner and Conn 1974; Lin et al. 2013b). In the sequence, the 2-coumarate *O*-β-glucosyltransferase (2GT) transfers the glucosyl group from UDP-glucose to *trans*-2-coumarate generating *trans*-2-coumarate-β-D-glucoside (**6**). The *trans*-2-coumarate-β-D-glucoside undergoes *trans*/*cis* isomerization yielding *cis*-2-coumarate-β-D-glucoside. A β-glucosidase (GBA) has been assigned to hydrolysate *cis*-2-coumarate-β-D-glucoside specifically releasing *cis*-2-coumarate (**8**) and shows no activity towards the *trans*-isomer.

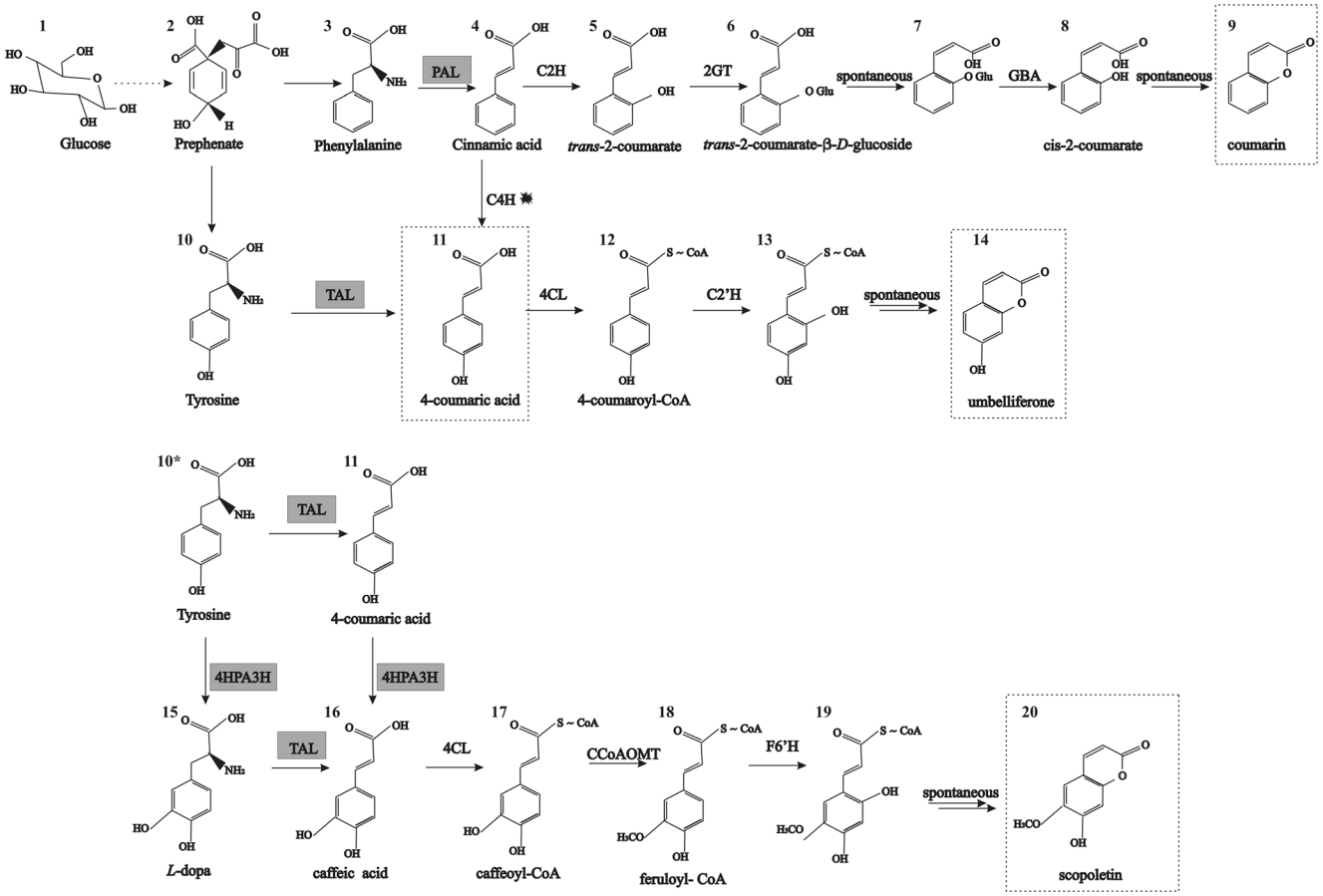


Fig. 1 The previously proposed coumarin biosynthetic pathway in plants (*1–9*) and synthetic designed pathways of the biosynthetic routes of *p*-hydroxycinnamic acid (pHCA) (*1–4*, *11*; *1–2*, *10–11*), umbelliferone (*10–14*), and scopoletin (*10**–*11*, *16–20* or *10**, *15–20*). Black-dial enzyme indicates the cytochrome 450 dependent hydroxylases. Enzymes in *gray-colored boxes* originated from microorganisms including bacteria and yeast. Enzyme PAL (*3–4*) is equivalent to originated plants and microorganisms. *PAL* phenylalanine ammonia lyase, *C2H* cinnamate 2-hydroxylase, *2GT* 2-coumarate *O*-β-glucosyltransferase, *GBA* β-glucosidase, *TAL* tyrosine ammonia lyase, *C4H* cinnamate 4-hydroxylase, *4CL* 4-coumarate:CoA ligase, *C2'H* coumaroyl-CoA 2′-hydroxylase, *4HPA3H* 4-hydroxyphenylacetate 3-hydroxylase, *CCoAOMT* caffeoyl-CoA *O*-methyltransferase, *F6'H* feruloyl-CoA 6′-hydroxylase

The last step is the spontaneous lactonization of *cis*-2-coumarate forming coumarin (**9**) (Haskins et al. 1964; Kleinhofs et al. 1967; Oba et al. 1981; Lin et al. 2013b).

Biological production of *p*-hydroxycinnamic acid (pHCA, also known as 4-coumaric acid) from glucose (**1**) can be achieved via deamination of the aromatic amino acids L-tyrosine (**10**) or L-phenylalanine (**3**) (Fig. 1). Some PAL enzymes convert L-phenylalanine (**3**) for CA (**4**) and the Cytochrome P450 enzyme cinnamate 4-hydroxylase (C4H) hydroxylates at the *para* position to produce pHCA (**11**). When tyrosine (**10**) is used as substrate, *trans*-pHCA (**11**) is produced in one step and is accomplished by phenylalanine ammonia-lyase (PAL)/tyrosine ammonia-lyase (TAL) (Vannelli et al. 2007; Sun et al. 2015). The gene from yeast *Rhodotorula glutinis*, screened for their ability to produce the PAL/TAL enzyme with high TAL activity, was cloned and expressed in *Escherichia coli* for the production of pHCA (Vannelli et al. 2007). In *E. coli* strain, containing only the PAL/TAL enzyme, either PAL activity was used to convert L-phenylalanine (**3**) to CA (**4**), or TAL activity caused deamination of L-tyrosine (**10**) to form pHCA (**11**). pHCA in *E. coli* strain, when grown on glucose (**1**), was formed in the absence of the Cytochrome P-450 and the P-450 reductase enzymes underlining its production via the TAL route without CA (**4**) intermediacy. In *S. cerevisiae*, deamination of L-phenylalanine (**3**) by PAL was followed by subsequent hydroxylation of CA (**4**) using a higher plant C4H Cytochrome P-450 and Cytochrome P-450 reductase. pHCA was also formed from direct deamination of tyrosine (**10**) to pHCA; however, the majority of pHCA (**11**) was formed via the PAL route (Vannelli et al. 2007). It is observed that *S. cerevisiae* synthesized the majority of pHCA by the same pathway of the plants, while *E. coli* synthesized pHCA through a different pathway.

Umbelliferone (4.3 mg L^{-1}) and scopoletin (27.8 mg L^{-1}) (Fig. 1) were produced via the TAL route without CA (**4**) intermediacy also in *E. coli* strains engineered to convert inexpensive phenylpropanoid acid (Lin et al. 2013b). Functional

expression of plant P450 enzymes is always hard to be achieved in bacterial systems. The route of umbelliferone production in plants is generated from L-phenylalanine (**3**). In order to circumvent the barrier of C4H, we replaced PAL and C4H enzymes for TAL enzyme, using L-tyrosine (**10**) as the entrance substrate (Lin et al. 2013b). Apart from TAL enzyme, 4-coumarate:CoA ligase (4CL) and coumaroyl-CoA 2′hydroxylase (C2′H) are required for the formation of the compound. For scopoletin (Fig. 1), the *E. coli* native 4-hydroxyphenylacetate 3-hydroxylase (4HPA3H) was used as a surrogate to cytochrome P450-dependent enzyme, 4-coumarate 3-hydroxylase (C3H) in plants, to be able to hydroxylate 4-coumarate forming caffeic acid (**16**). 4HPA3H can also hydroxylate L-tyrosine (**10**) affording L-dopa (**15**), which can be converted to caffeic acid (**16**) by TAL. The enzymes 4CL and caffeoyl-CoA *O*-methyltransferase (CCoAOMT) are necessitated to complete the pathway (Lin et al. 2013b).

Recently, the biosynthetic mechanism leading to the microbial biosynthesis of 4-hydroxycoumarin (4-HC) by *E. coli* was identified by introducing isochorismate synthase (ICS), isochorismate pyruvate lyase, salicoyl-CoA ligase, and a promiscuous bacterial quinolone synthase (PqsD). Endogenous metabolites chorismate and malonyl-CoA were converted to 4-HC via salicylate. The semisynthesis of warfarin was performed indicating 43.7 ± 2.6 mg L^{-1} warfarin with molar yield of 4.6 % (Lin et al. 2013a; Sun et al. 2015). 4HC is commonly used to treat thromboembolic diseases (Mueller 2004; Lin et al. 2013a; Molina and Zanusso Júnior 2014) and serves as an immediate precursor of these synthetic anticoagulants (Lin et al. 2013a). The most notable ones are dicoumarol, brodifacoum, difenacoum, acenocoumarol, phenprocoumon (Lin et al. 2013a; Keri et al. 2015), and warfarin, discovered in 1920 and identified in 1940 by Karl Link and Harold Campbell. It is estimated that in the United Kingdom, at least 1 % of the population and 8 % of those over 80 years are treated with warfarin and had a global market value of $300 million in 2008 (Pirmohamed 2006; Lin et al. 2013a). This fact refers to the importance of developing new areas of scale-up production of these compounds due to increased life expectancy of the population. Pharmaceutical company Nycomed, recently acquired by Takeda Pharmaceutical and Craveri, markets many drugs with vasodilatory action and anticoagulants worldwide.

The antibiotic novobiocin produced by *Streptomyces niveus* (Steffensky et al. 2000) used against multidrug-resistant bacteria, especially gram-positive (Sandhu et al. 2014), already exists on the market. Bacterial DNA gyrase represents the target of these coumarins, and novobiocin inhibits this enzyme by interacting with the N-terminal 24-kDa subdomain of the *gyr*B subunit (Steffensky et al. 2000). Synthetic bioengineering represents a novel approach to create optimized microbial cell factories for efficient production of target compounds through fermentation, allowing its use in large scale.

Fungal coumarins

Basidiomycetes fungi

The fungal group basidiomycota have been researched as one of the new producer agents for the development of biotechnology. Most recently, several coumarins have been obtained from fungal synthesis using basidiomycetes. Armillarisin A is a coumarin derivative extracted from the fungus *Armillariella tabescens*. This compound is used as a choleretic to improve bile secretion and regulate the pressure of the bile duct to ease inflammation and adjust liver function (Wang et al. 2007).

Hwang et al. (2013) isolated two new coumarins (Fig. 2), 6-chloro-4-phenyl-2H-chromen-2-one (1) molecular formula $C_{15}H_9ClO_2$, with 0.5 mg when isolated, and ethyl 6-chloro-2-oxo-4-phenyl-2H-chromen-3-carboxylate, with 0.2 mg when isolated, from an EtOH extract of the mushroom *Fomitopsis officinalis*, aimed for the application in tuberculosis treatment (anti-TB).

In addition to compounds naturally occurring, one analog each with chlorine substituted at C-7 was synthesized as 7-chloro-4-phenyl-2H-chromen-2-one and ethyl 7-chloro-2-oxo-4-phenyl-2H-chromen-3-carboxylate. Based on the observations obtained from the four molecules in assays to *Mycobacterium tuberculosis* (anti-TB), the authors concluded that the anti-TB activity is higher when the ethyl ester introduces chlorine in position 6, while coumarins with a chlorine substituted at the 7-position are less active (Hwang et al. 2013). Mammalian cell cytotoxicity using Vero cells was observed by the authors at a similar concentration that causes significant inhibition against *M. tuberculosis* and *Mycobacterium bovis*. However, the authors did not make it clear that they are hitting the target similar between *M. tuberculosis* complex species and Vero cells (Hwang et al. 2013).

Molecules of coumarin are also evidenced in the fruiting body of fungi basidiomycetes. In a comparative study by Islam et al. (2015), the phytochemical and antibacterial properties of edible fungus *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Lentinula edodes* were evaluated. The authors found the presence of coumarin in extracts *G. lucidum* fruiting bodies, which showed antibacterial activity against *Bacillus cereus*.

The correlation between the content of phenolic and antioxidant activity the fungus *Macrolepiota mastoidea* extract

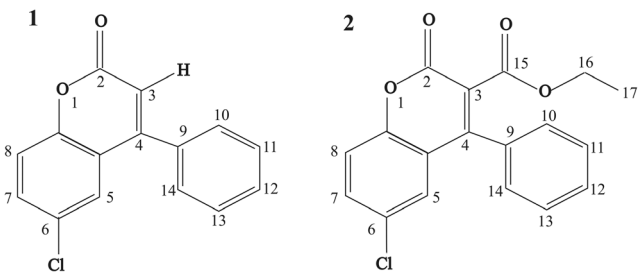


Fig. 2 Coumarin molecules synthesized by *Fomitopsis officinalis*

revealed the presence of 5.5 ± 0.151 mg g^{-1} of phenolic compounds. Through HPLC analysis, they identified coumarin compounds (3.1 min) and *p*-coumaric acid (3.2 min); a linear relationship between phenolic content and antioxidant extract assays was observed (Shirmila and Radhamany 2013).

Eleven phenolic compounds were also observed in solid cultivation of *G. lucidum* in brown rice germinated. These compounds were quantified and the authors obtained 0.02 mg mg^{-1} dry weight (Hasnat et al. 2015). A similar cultivation by Hasnat et al. (2013) using the same fungus identified 13 components, such as quercetin, ursolic acid, keampferol, terpenoil, coumarin, catechin, myricetin, ferulic acid, thymol, rutin, lupane, cyanidin, and ellagic acid, in the product composition. In this cultivation, 5.65 mg mL^{-1} of coumarin was obtained. *Agaricus bisporus*, *Agaricus bernardii*, *Agaricus arvensis*, *Agaricus porphyrocephalus*, *Agaricus silvicola*, *Coprinus comatus*, and *Lepiota cristata* also showed the presence of coumarin in fungal extracts (Mogland and Saadabi 2012).

Pleurotus euosmus grown on synthetic glucose-asparagine-mineral salt solution synthesized compounds as coumarin, linalool, and *cis*- and *trans*-linalool oxide after 35 days of cultivation. Maximum concentration of coumarin was found after 48 days, 2640 μg L^{-1}, and maximum concentration of linalool reached 900 μg L^{-1} after 35 days (Drawert et al. 1983).

The influence of the addition of coumarin or naphthaleneacetic acid (NAA) on cell growth and bioproduction of flavonoids by the fungus *Phellinus* sp. P0988 was studied by Ma et al. (2015). The authors observed that supplementation of coumarin and NAA has contributed to the formation of flavonoids with less glucose in the medium. When used in concentrations up to 0.03 g L^{-1} in the medium, coumarin peak values of 1.83 g L^{-1} flavonoids were obtained, causing a favorable effect on the survival of the cells and formation of fungal flavonoids.

Basidiomycetes fungi have been extensively studied due to their ability to degrade a wide range of xenobiotics by the ligninolytic system that comprises enzymes such as laccases, manganese peroxidase (MnP), and lignin peroxidase (LiP). Hadibarata and Kristanti (2012) observed the degradation of approximately 73 % of the initial benzo[*a*]pyrene (BaP) concentration within 30 days using *Polyporus* sp. S133 fungus. In their studies, the authors verified that the fungus *Polyporus* sp. S133 transformed BaP in BaP-1,6-quinone, being degraded in two ways: BaP-1,6-quinone was decarboxylated and oxidized to form coumarin, which was hydroxylated to hydroxycoumarin and finally hydroxyphenyl acetic acid by the addition of the epoxide group. BaP-1,6-quinone compound was also converted in 1-hydroxy-2-naphthoic acid. Laccase production was detected during the degradation of BaP with reduced activity of lignin peroxidase, manganese-dependent peroxidase, and 2,3-dioxygenase.

The capacity of the basidiomycete yeast *Trichosporon asahii* SBUG-Y 833 to assimilate phenylalkanes with alkyl chain lengths from 7 to 12 carbon atoms was studied by Awe et al. (2009). Among the identified metabolites, three coumarins (Fig. 3), 4-hydroxycoumarin (**1**), 4,6-dihydroxycoumarin (**2**), and 4,8-dihydroxycoumarin (**3**), were obtained. Through the kinetic experiments, the authors found that after 1 h of incubation of the yeast with phenylheptane (**A**) (0.1 % [*v*/*v*], 4.88 mM), 4-hydroxycoumarin (**1**) was detected. After 4 h, 4,6-dihydroxycoumarin (**2**) was found, and after 8 h, 4,8-dihydroxycoumarin (**3**) was identified. Due to the ability of *T. asahii* to form hydroxylated coumarins, the transformation of 7-hydroxycoumarin (**B**) and 2,4-dihydroxyphenylpropanoic acid (**C**) was investigated. Yeast cells supplemented with 7-hydroxycoumarin formed 6,7-dihydroxycoumarin (**4**) and 4,7-dihydroxycoumarin (**5**). The supplementation with 2,4-dihydroxyphenylpropanoic acid yielded 4,7-dihydroxycoumarin. According to the authors, this study describes for the first time that the yeast *T. asahii* SBUG 833 utilizes phenylalkanes as carbon and energy sources for coumarin synthesis.

Ascomycetes fungi

The ascomycetes are considered great decomposers and have great use in food and pharmaceutical industries. The fungus

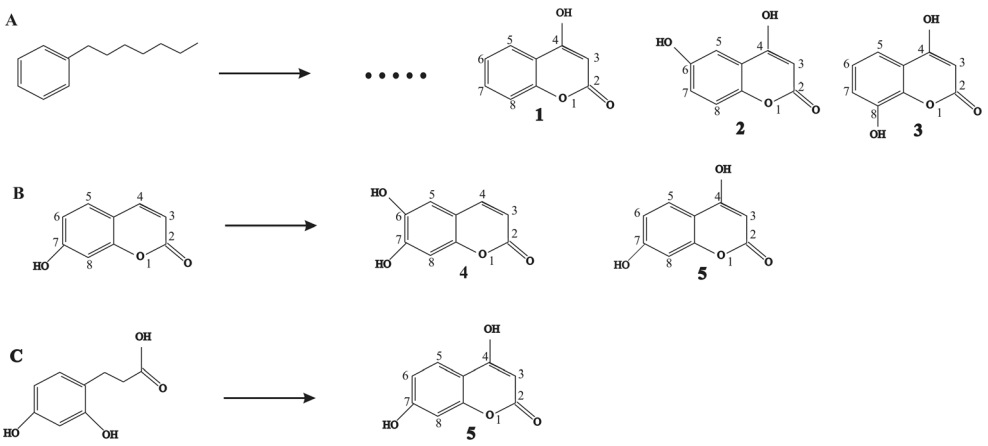


Fig. 3 Molecules derived from phenylalkanes and biotransformation from yeast *Trichosporon asahii* SBUG-Y 833

Penicillium sp. 091402, isolated from *Bruguiera sexangula* plant of mangrove, synthesized six coumarin molecules, among these (3*R**,4*S**)-6,8-dihydroxy-3,4,7-trimethylisocoumarin (**1**) and (3*R*,4*S*)-6–8-dihydroxy-3,4,5-trimethylisocoumarin (**2**). In the primary bioassay, the compound molecular formula $C_{12}H_{14}O_4$ showed moderate cytotoxic activity against tumor cell line K562 (IC50 = 18.9 μg mL^{-1}) (Han et al. 2009). K562 cells are of the erythroleukemia type, one human myelogenous leukemia line. The umbelliferone molecule (7-hydroxycoumarin) has been reported to have growth-inhibitory effects on human cancer cell lines including leukemia (Lin et al. 2013b).

7-Amino-4-methylcoumarin (AMC) was developed originally for fluorescent labeling of some peptides for the preparation of substrates of some proteases, enabling specific detection of the derivatives because it imparts excitation at the near violet region (ca. 350 nm) (Yodoshi et al. 2008). However, AMC was isolated by *Xylaria* sp. and showed strong antibacterial and antifungal activities in vitro against *Aeromonas hydrophila* (minimal inhibitory concentrations (MIC), 4 μg mL^{-1}), *Escherichia coli* (MIC, 10 μg mL^{-1}), *Salmonella enteritidis* (MIC, 8.5 μg mL^{-1}), *Salmonella typhi* (MIC, 20 μg mL^{-1}), *Salmonella typhimurium* (MIC, 15 μg mL^{-1}), *Shigella* sp. (MIC, 6.3 μg mL^{-1}), *Staphylococcus aureus* (MIC, 16 μg mL^{-1}), *Yersinia* sp. (MIC, 12.5 μg mL^{-1}), *Vibrio anguillarum* (MIC, 25 μg mL^{-1}), *Vibrio parahaemolyticus* (MIC, 12.5 μg mL^{-1}), *Candida albicans* (MIC, 15 μg mL^{-1}), *Aspergillus niger* (MIC, 25 μg mL^{-1}), and *Penicillium expansum* (MIC,40 μg mL^{-1}). The authors suggest that the extract of *Xylaria* sp. YX-28 isolated from *Ginkgo biloba* L. may be used as natural preservative in food (Liu et al. 2008). AMC also exhibited maximum antitubercular activity, with MIC of 1 mg L^{-1} against *M. tuberculosis* when produced by synthetic pathway (Tandon et al. 2011).

The fungus *Pestalotiopsis* sp., isolated from Chinese mangrove sheets *Rhizophora mucronata*, in studies by Xu et al. (2009), synthesized five new coumarin pestalasins (1–5) and the known compound 3-hydroxymethyl-6,8-dimethoxycoumarin (6) (Fig. 4) when grown on solid fermentation using rice as the culture medium. It was possible to identify compounds 6,8-dimethoxy-3-(20-hydroxy-propyl)-coumarin (**1**), 6,8-dimethoxy-3-(10,20-dihydroxypropyl)-coumarin (**2**), 6,8-dimethoxy-3-(20, 30-dihydroxy-propyl)-coumarin (**3**), 8-hydroxy-6-methoxy-3-(20,30-dihydroxy-propyl)-coumarin (**4**), and 6-hydroxy-8-methoxy-3-propyl-coumarin (**5**) (Xu et al. 2009).

Monankarins A–F containing skeleton conjugated pyran-coumarins were isolated and their structures determined by fungus *Monascus anka* pigments (Hossain et al. 1996). The yellow pigments, monankarins A–F, were tested for inhibitory activity against monoamine oxidase (MAO). The monankarin C compound exhibited the most potent inhibitory activity (IC_{50} 10.7 μM), and when specific inhibition towards MAO-A and MAO-B of monankarin C was examined in mice liver and brain preparations, monankarin C showed higher specificity towards MAO-B than MAO-A in mice brain preparations.

Aspergilli belong to the class of imperfect filamentous fungi, and many are producers of beneficial secondary metabolites, such as antibiotics (penicillin and cephalosporin), antifungals (griseofulvin), anti-tumor drugs (terrequinone A), and cholesterol-lowering drugs (lovastatin). However, many *Aspergillus* species synthesize aflatoxins with high toxicity, making impossible their application (Leontopoulos et al. 2003; Yu 2012; Bbosa et al. 2013a; Baranyi et al. 2015; Wang et al. 2016). Aflatoxins are a group of difuranocoumarin derivatives, which consist of a double-furan ring and a coumarin moiety, where the furofuran moiety is essential for toxic and carcinogenic activity (Wang et al. 2016). The aflatoxins generally have a deleterious effect on the central nervous system, cardiovascular and respiratory systems, as well as the gastrointestinal tract. They can be teratogenic, immunosuppressive, and present hepatocarcinogenic and hepatotoxic properties (Szumski et al. 2014; Baranyi et al. 2015).

Among the aflatoxins, four are considered important (B_1, B_2, G_1, and G_2). Aflatoxins B group (B_1, B_1) contains a cyclopentane ring and the group G has a lactone ring. They

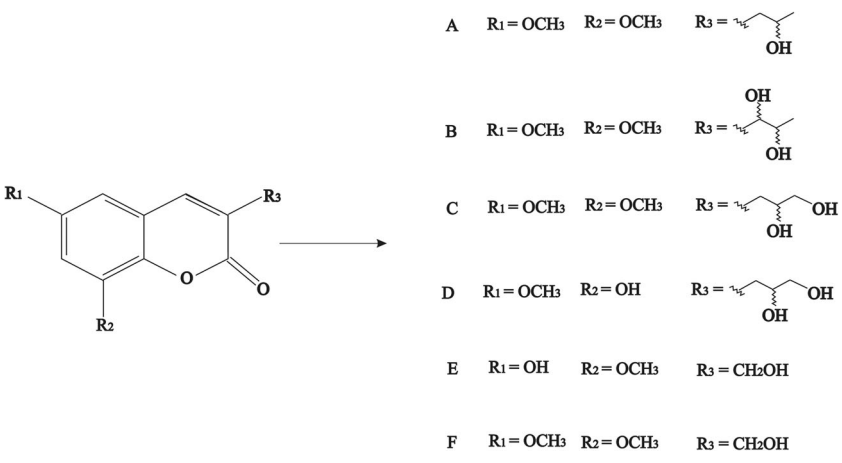


Fig. 4 Coumarin molecules synthesized by *Pestalotiopsis* sp.

may emit green or blue fluorescence (B = blue, G = green) under the irradiation of ultraviolet light, and subscript numbers 1 and 2 indicate major and minor compounds, respectively. Aflatoxins M_1 and M_2 are hydroxylated products of aflatoxin B_1 and B_2 (Fig. 5) (Bbosa et al. 2013b; Szumski et al. 2014; Wang et al. 2016).

Aflatoxins are produced by leastwise 20 species of the genus *Aspergillus*: *A. astellatus* (=*Emericella astellata*), *A. arachidicola*, *A. bombycis*, *A. flavus*, *A. minisclerotigenes*, *A. mottae*, *A. nomius*, *A. novoparasiticus*, *A. ochraceoroseus*, *A. olivicola* (=*Emericella olivicola*), *A. parasiticus*, *A. parvisclerotigenus*, *A. pseudocaelatus*, *A. pseudonomius*, *A. pseudotamarii*, *A. rambellii*, *A. sergii*, *A. togoensis*, *A. transmontanensis*, and *A. venezuelensis* (=*Emericella venezuelensis*), and aflatoxins B_1 and B_2 are the most synthesized (Bbosa et al. 2013b; Baranyi et al. 2015).

Aflatoxin B_1 (AFB1) is the most toxic and the most potent carcinogen in humans and animals. In animal models, AFB1 is metabolized by cytochrome P450 (CYP450) dependent monooxygenase in liver. The epoxide form of aflatoxin binds to guanine residues in DNA, forms guanyl-N7 adducts, and induces mutations and the mechanism for initiating carcinoma formation. The organ most severely affected by AFB1is the liver, and the primary lesions include hemorrhagic necrosis, fatty acid infiltration, and bile duct proliferation (Eaton and Gallagher 1994; Hwan Do and Choi 2007; Yu 2012; Bbosa et al. 2013b).

Unlike aflatoxins, some *Aspergillus* species synthesized coumarin derivatives with therapeutic properties. Bocks (1967) cites that the *o*-coumaric acid when incubated with *A. niger* was converted in approximately 72 % into 4-hydroxycoumarin. A different strain, *A. niger* ATCC 11394, reduced coumarin to dihydrocoumarin (3,4-dihydrochromen-2-one) and then either to 6-hydroxy-3,4-dihydrochromen-2-one or to melilotic acid, 3-(2-hydroxyphenyl)propanal and 2-(3-hydroxypropyl)phenol (Parshikov et al. 2015). According to the authors, dihydrocoumarin is widely used as a flavor and fragrance, and transformed by *A. niger* ATCC 11394 to 2-(3-hydroxypropyl)phenol, 6-hydroxy-3,4-dihydrochromen-2-one, 4-hydroxycoumarin, and three minor metabolites (Parshikov et al. 2015). 4-Hydroxycoumarin and derivatives have been effectively used as anticoagulants for the treatment of thrombophlebitis, pulmonary embolism, and certain cardiac conditions. They exhibit cytotoxic and spasmolytic activities, and also activity against HIV virus, and analgesic, anti-arthritis, anti-inflammatory, antipyretic, antibacterial, antiviral, and anti-cancer properties. They are also useful key intermediates for many industrial products such as dyes and liquid crystals (Jung and Park 2009; Stanchev et al. 2009).

Fungal transformation of coumarin molecules

Biotransformation processes have become an economically competitive technology for the modification of chemical compounds having complex structures (Lv et al. 2013). Filamentous fungi often are tolerant to these molecules when performing metabolic detoxification of these compounds (Myung et al. 2012). Basidiomycete fungus *Phanerochaete chrysosporium* grown in submerged stationary medium showed several metabolites in studies by Matsuzaki and Wariischi (2004) when using different substrates. Among the substrates tested (10), 18 metabolites were obtained. It was observed that there was a conversion of cinnamic acid in *p*-coumarin acid and coumarin compound in 8-hydroxycoumarin, 5-hydroxycoumarin, 6-hydroxycoumarin, and 7-hydroxycoumarin. A cytotoxic effect against cancer cell lines was reported in 7-hydroxycoumarin (Mao et al. 2009; Symeonidis et al. 2009) and 6-hydroxycoumarin, this against leukemia (HL-60) cancer cell line (Rehman et al. 2014). Antioxidant, antibacterial, and antihyperglycemic properties were also related (Symeonidis et al. 2009).

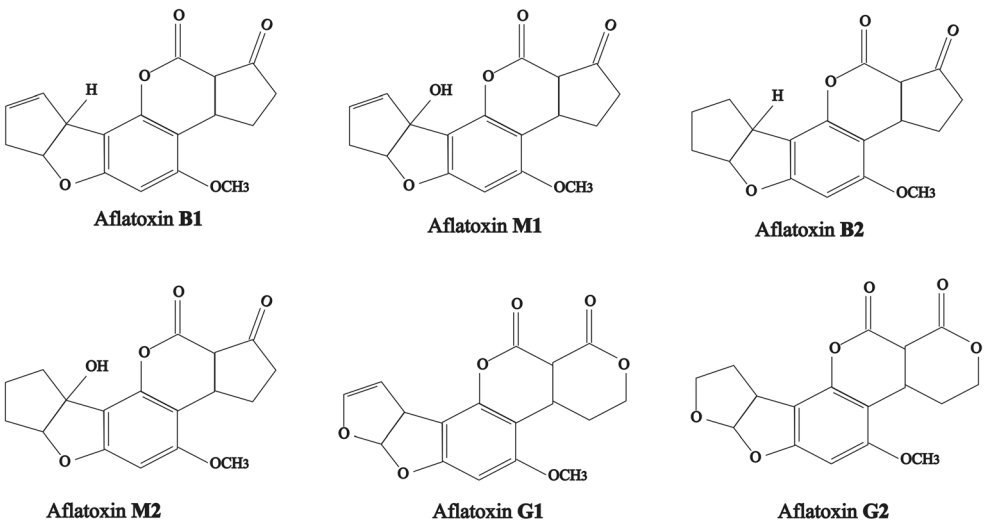


Fig. 5 Chemical structures of aflatoxins B_1, M_1, B_2, M_2, G_1, and G_2

Penicillium janthinellum showed good ability to transform imperatorin (a linear furanocoumarin) in the studies by Lv et al. (2013). Ten products of imperatorin transformed were isolated, identified, and assessed for anti-osteoporosis activity using MC3T3-E1cells. The enzymatic hydrolysis reactions, reduction at α,β-unsaturated lactone ring, hydroxylation, methylation, dehydration, and glycosylation were observed in microbial transformation processes. Some metabolites (6, 7-furano-8-(5a-hydroxyl-methyl-prenyloxy) hydrocoumaric acid and 6,7-furano-8-(2a-hydroxy-3a-en-prenyloxy) hydrocoumaric acid) demonstrated potent bioactivity and increase the growth cell of MC3T3-E1. These metabolites were cited by the authors as candidates for development of new anti-osteoporosis drugs and functional foods.

Furanocoumarins have attracted considerable interest in biotransformation studies due to their anti-inflammatory activity, analgesic, antispasmodic, and anti-cancer properties (Shi et al. 2013). In microbial biotransformation studies with isoimperatorin, Yang et al. (2013) performed a screening HPLC-DAD analysis of 20 filamentous fungi and their processing ability isoimperatorin (**1**) to assess their anti-osteoporosis activity. The selected microorganisms (*Aspergillus niger* AS 3.795, *Cunninghamella blakesleana* AS 3.970, *C. elegans* AS 3.2028, *Rhizopus oryzae* AS 3.2380, and *Penicillium janthinellum* AS 3.510) showed the ability to transform the substrate into new metabolites with more polarity. *Cunninghamella blakesleana* AS 3.970 metabolizes the substrate completely, suggesting greater potential strain for a preparative biotransformation in getting products processed from isoimperatorin. By incubating (**1**) with *C. blakesleana* AS 3.970, the authors have identified new structures: 14-hydroxyl-isoimperatorin (**2**), 11-carbonyl-14-hydroxyl isoimperatorin (**3**), 11-carbonyl- 14-hydroxyl-12,13-dihydrogen-iso-imperatorin (**4**), 14-hydroxyl-12,13-dihydrogenisoimperatorin (**5**), isoimperatorin-14-*O*-β-D-mannoside (**6**), and isoimperatorin-14-*O*-β-D-glucoside (**7**) (Fig. 6). In vitro activity of compounds (**1**–**7**) on MC3T3-E1 cells in 1, 10, and 100 μM concentrations was evaluated. Products (**2**–**7**) exhibited an increase in MC3T3-E1 cell growth effects significantly at three different test concentrations, and (**2**) and (**7**) compounds are more bioactive than (**1**). Through the obtained results, the authors suggest that glycosylation and hydroxylation at C-14 significantly increase the anti-osteoporosis activity (Yang et al. 2013).

In vivo methods for identifying isoimperatorin metabolites in combination with in vitro microbial biotransformation studies were conducted in Shi et al. (2013). Among the 15 fungi tested preliminarily (*Absidia glauca* AS 3.3385, *Aspergillus niger* AS 3.1858, *Aspergillus niger* AS 3.795, *Alternaria alternata* AS 3.4748, *Beauveria bassiana* AS 3.4273, *Cunninghamella blakesleana* AS 3.970, *Cunninghamella elegans* AS 3.1207, *Fusarium avenaceum* AS 3.4594, *Mucor subtilissimus* AS 3.2454, *Mucor alternate* AS 3.3450, *Rhizopus arrhizus* AS 3.2896, *Rhizopus stolonifer*

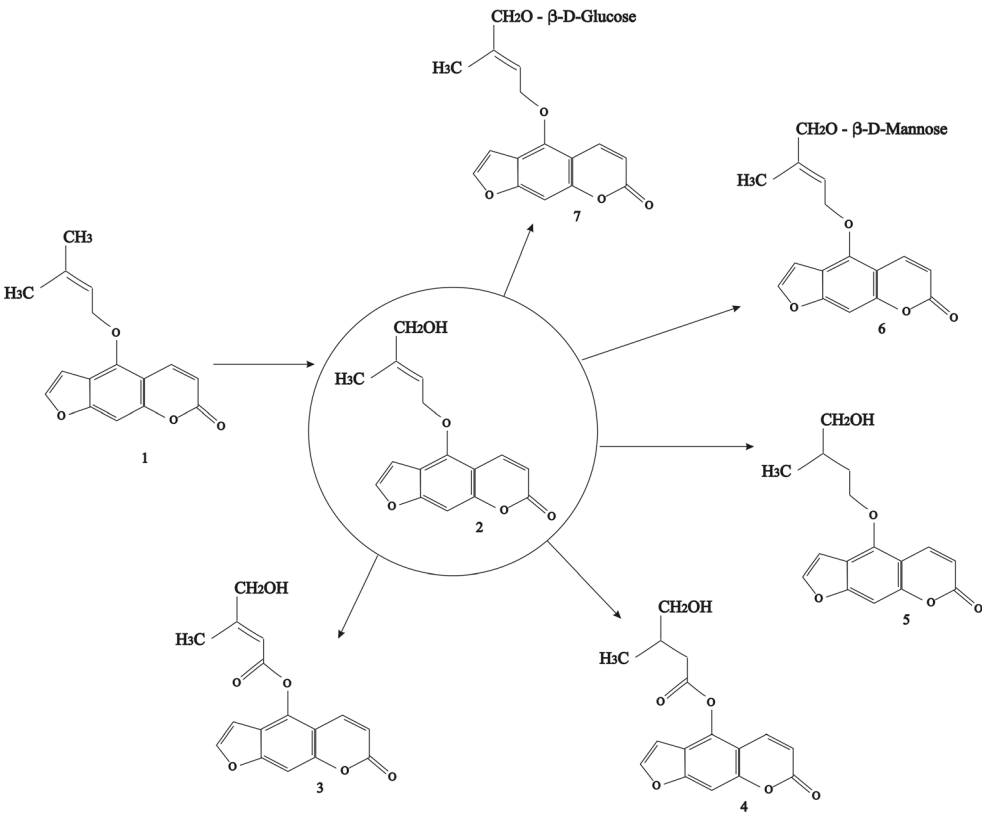


Fig. 6 Transformations of isoimperatorin products by *C. blakesleana* AS 3.970

AS 3.2050, *Penicillium melinii* AS 3.4474, *Sporotrichum* sp. AS 3.2882, *Syncephalastrum racemosum* AS 3.264), the fungus *Cunninghamella blakesleana* AS 3.970 was selected as the most potent strain in the biotransformation of isoimperatorin. The side chain of isoimperatorin was found to be the main place that suffered metabolic oxidation in vivo and in vitro. Thirteen products were detected by microbial transformation, and 11 products also exist in in vivo biological samples. According to the authors, this indicates a good correlation between isoimperatorin metabolites from in vivo samples and those from microbial transformations.

In submerged culture conditions, Myung et al. (2012) evaluated the conversion of 6′,7′-epoxybergamottin (**1**) for six filamentous fungi strains (*Botrytis cinerea*, *Geotrichum citri*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *Penicillium ulaiense*, and *Phomopsis citri* (teleomorph *Diaporthe citri*)) obtaining the products 6′,7′-dihydroxybergamottin (**2**), bergaptol (**3**), and an opened lactone ring metabolite 6,7-furano-5-(6′,7′-dihydroxy geranyloxy)-2-hydroxy-hydrocoumaric acid (**4**). The authors observed that after 5 days of fungal growth, compound 4 was the major metabolite **1**, with limited production of compounds **2** and **3** by four fungi. *Penicillium ulaiense* was the most effective in metabolism of **1** for **4** by converting ~60 %, followed by *Penicillium digitatum* (~41 %), *Botrytis cinerea* (~32 %), and *Geotrichum citri* (~31 %). *Lasiodiplodia theobromae* metabolizes **1** in **2**, followed by **4** and **3**. Only 10 % of **1** was converted to **2**–**4** by *Phomopsis citri.* Metabolites **2**–**4** were not detected in fungal cultures without exogenous addition of **1** by the authors. This suggests the need for an inducing agent in the metabolism and synthesis of coumarin compounds.

Aguirre-Pranzoni et al. (2011) studied the behavior of six *Aspergillus* fungus species in the coumarin biotransformation. The fungi (*Aspergillus niger* ATCC 11394, *A. niger* ATCC 16404, *A. terreus* INM 031783, *A. flavus* UBA 294, *A. ochraceus* RC 33, *Aspergillus fumigatus* RC 78) were grown in submerged culture and at resting. The biotransformation of isolated metabolites was analyzed by GC-MS, H NMR, and C NMR. The authors observed (Fig. 7) that the fungi *A. niger*, *A. flavus*, and *A. ochraceus* were more active when compared with the other tested fungi. Resting cells of fungus *A. ochraceus* gradually consumed the coumarin compound (**1**) until the fifth day without performing biotransformation. Already in submerged culture, there was a reduction of the C3–C4 double bond, obtaining dihydrocoumarin (**2**) in 24 h. The *A. flavus* metabolism showed a difference. The resting cells were more efficient than submerged culture, converting the coumarin compound in 92 %. The main product of biotransformation on resting cells was identified as 5-hydroxycoumarin (**3**). In experiments with growing cells of *A. niger* ATCC 11394, it was verified that coumarin has been completely converted into dihydrocoumarin (**2**) within 48 h of biotransformation. In 7 days, different metabolites were

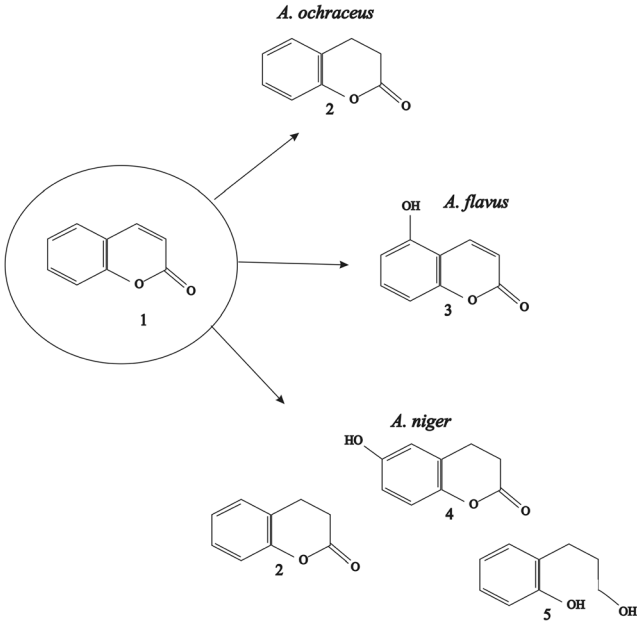


Fig. 7 Coumarin (**1**) biotransformation by *Aspergillus* species

isolated: 6-hydroxy-3,4-dihydrochromen-2-one (**4**) and 2-(3-hydroxypropyl)-phenol (**5**).

The dihydrocoumarin production was obtained from the yeast *Saccharomyces cerevisiae* found in studies by Häser et al. (2006). Experiments using isolated coumarin and tonka bean meal as a source of coumarin showed melilotic acid as an intermediary product. It was observed that a concentration of substrate (coumarin) to 0.5 g L^{-1} was completely processed in the product. Its use in higher concentrations (1.0, 2.0, and 5.0 g L^{-1}) was toxic to yeast. By using the coumarin in tonka bean meal (25 g L^{-1} equivalent to 0.5 g L^{-1} free coumarin), the authors observed that after 6 h, the coumarin maximum level reached (0.55 g L^{-1}), with subsequent reduction of coumarin and an increase in melilotic acid. After distillation of melilotic acid with citric acid, dihydrocoumarin compound was obtained with purity >90 % (HPLC-DAD), obtained by high performance using tonka bean meal as substrate (1.0 g L^{-1} product was obtained from 25 g L^{-1} tonka bean meal), within 147 h. According to the authors, the compound dihydrocoumarin can be used as a natural flavoring due to the criteria procedure of a natural feedstock.

Conclusions

Coumarins have potential application in different areas, and new findings related to these molecules are highlighted, especially as therapeutic agents. Stable and scalable sources of natural coumarins are required to meet its production in the pharmaceutical, cosmetics, fragrances, and food industry. Obtaining these compounds through fungal production could meet this demand. However, we need more research efforts in

the field of production and characterization of coumarin and to identify the mechanisms of synthesis of these molecules from non-pathogenic fungal species.

An initial view is the comparison of production and characterization of natural coumarins produced by fungi with the those extracted from plants. Improving microbial synthesis is required for a competitive amount with plant coumarins. Several studies have attempted to increase the yields of extractive molecules from plants since the concentration of the compound obtained is related to seasonal reasons and the type of extraction performed. Drying temperature analysis and extraction techniques involving biological processes combined with physical and chemical processes have become increasingly utilized to reduce the use of organic solvents in extractive processes.

Another investigation area is the comparison of fungal coumarins with chemically synthesized ones, widely used due to the possibility of obtaining complex molecules more efficiently than the plant extract which is difficult to remove and purify. Along with chemical synthesis, unnecessary derivatives are obtained, requiring an appropriate treatment. The obtaining of simple and complex coumarins can be carried out by fungal processes, which use lower temperatures, pH, and pressure than those used in chemical processes. In special cases, chemical synthesis cannot reproduce molecules with a high degree of purity required for use in pharmaceuticals; that does make us look again to obtain coumarins through natural and biological processes.

Although the coumarin production detected compounds of the secondary fungal metabolism, there are few studies of applications and molecules identified during processing limited to the laboratory scale. Scale-up studies using reactors and process controllers to reduce production and deployment costs are keys to making the process more competitive.

Little is known about the mechanism of regulation and synthesis of this compound within the microorganisms as well as the biotransformation process performed by the fungus. It is known that there is a need to use substrate inducers capable of stimulating the desired reactions for the production of these molecules. An initial screening of fungi and culture media makes it possible to identify which classes of inductors or compounds are most effective in stimulating the microorganisms to express and expand their catabolic potential. By determining this mechanism, it is possible to produce specific molecules containing coumarin skeleton.

Some research groups have succeeded in the production of coumarin produced by fungi, and these molecules have been shown to be effective in therapeutic application and food. However, this is still a relatively unexplored field when compared to plant extraction and chemical synthesis of coumarin, requiring major engineering efforts for future applications.

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Compliance with ethical standards

Conflict of interest Tania Maria Costa declares that she has no conflict of interest.

Lorena Benathar Ballod Tavares declares that she has no conflict of interest.

Débora de Oliveira declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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