Phytochem Rev (2013) 12:773–789
DOI 10.1007/s11101-013-9305-5

Classical and epigenetic approaches to metabolite diversification in filamentous fungi

Jacqueline Aparecida Takahashi · Ana Paula Campos Teles · Adriana de Almeida Pinto Bracarense · Dhionne Corrêia Gomes

Received: 19 December 2012 / Accepted: 3 May 2013 / Published online: 12 May 2013

Abstract Studies on fungal metabolites have produced an overwhelming expectation concerning the production of novel bioactive compounds for pharmaceutical applications. The adding of various biosynthetic precursors and the changing of nutritional components in the fermentation medium can change biosynthesis pathways, also leading to the production of novel metabolites. In addition, several growing conditions can be classically manipulated to modify fungal metabolite profiles. Recently, modern genome sequence tools have shown that not all gene clusters are regularly expressed in conventional growing conditions, thus expanding the possibilities of modulating the chemical metabolite profiles produced by filamentous fungi. This review discusses and exemplifies classical and epigenetic tools successfully applied to diversify metabolite production and to produce fungal metabolites from silent metabolic pathways.

Keywords *Aspergillus* · Culture media · Epigenetic modifiers · Fungal metabolites · OSMAC · *Penicillium*

J. A. Takahashi (✉) · A. P. C. Teles · A. de Almeida Pinto Bracarense · D. C. Gomes
Departamento de Química, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Belo Horizonte, MG 31270-901, Brazil
e-mail: jat@qui.ufmg.br

A. P. C. Teles
Escola de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

D. C. Gomes
Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Introduction

Fungi have been gaining attention regarding the production of novel bioactive metabolites used in several applications. They have been found to be prolific sources of chemicals to be used as prototypes in pharmaceutical, agrochemical, food, and cosmetic industries. This success story began in 1928 with Fleming's isolation of penicillin from *Penicillium crysogenum* (Fleming 1944). For years, this metabolite seemed to have no match in importance and potential of application. However, over time, several other interesting substances, such as cephalosporins, micophenolic acid, statins, and other compounds, not only sustained, but also broadened the search for bioactive compounds from filamentous fungi (Demain and Sanchez 2009). Fungal secondary metabolites are quite diverse structurally, and their function mostly relies on self-defense against other microorganisms, considering that natural habitats provide ecological challenges (Shwab and Keller 2008; Sun et al. 2011). In addition to their important ecological functions, fungal metabolites are also outstanding, because they

can act outside of the fungal habitat. Therefore, they are widely used as antioxidant (Zheng et al. 2010; Huang et al. 2012), antitumor (Godio and Martín 2009; Zheng et al. 2011), antiviral (Zhang et al. 2011), and antiparasitic (Gao et al. 2012a; Stadler et al. 2003) agents.

Fungi possess a mechanism for the regulation of metabolism and the control of metabolite biosynthesis so as to assure that energy and chemical precursors are only used in environments where metabolite production is advantageous (Hoffmeister and Keller 2007). Surprisingly, several other compounds can also be produced in the laboratory, although under these conditions, there is no need to produce defensive chemicals.

General remarks in fungal metabolites expression

Two major approaches have been used in the discovery and production of new bioactive fungal secondary metabolites, the major targets of which are generally increases in yields or enhancements of chemical diversity by producing novel metabolites. The first approach concerns the isolation of fungi from new biomes (tropical environments, deserts, glacial areas, etc.), or from new sources (endophytes, endolichenic, etc.) (Gunatilaka 2006), aimed at the isolation of new useful species. Although fungal libraries isolated from different environments have been widely reported in the literature (Takahashi et al. 2008), mycologists infer that many fungi have yet to be isolated and chemically studied (Hawksworth 2001).

The second approach relies on the manipulation of fermentation conditions in order to control biosynthetic pathways, given that secondary metabolite production is directly dependent on the conditions under which the fungus is grown. Nutritional manipulation, the addition of components to simulate native environments, as well as changes in temperature, pH, luminosity, and oxygen uptake have been traditionally performed without taking into account the genetic sequencing of the studied species (Bode et al. 2002).

Although in most eukaryotic cells the genes involved in a single metabolic pathway are usually scattered throughout the genome, in fungi the genes responsible for the production of secondary metabolites are instead grouped in clusters (Shwab and Keller 2008). Gene clusters are either a few or several genes, arranged alongside the chromosomes. Modern genome achievements showed that, although fungi possess the ability to produce a great number of metabolites, most clusters responsible for the regulation of biosynthetic pathways remain silent within the cultivation patterns most commonly employed in research laboratories (Asai et al. 2012b).

With the development of genetic sequencing techniques, metabolite production by fungi started to be performed under a new perspective when researchers began to understand that the production of certain secondary metabolites is related to silent genes that may not be expressed because of inappropriate culturing conditions (Wijeratne et al. 2010). New tools have been developed to fix this gap and nowadays many recent studies involving the discovery of new metabolic pathways focus not only on classical modifications in the culturing conditions, but also on genetic manipulation and epigenetic modulation.

Cultivation-dependent approaches

Considering the assumption that even small alterations in the culture medium modulate secondary metabolite biosynthesis, some strategies were developed to maximize fungal metabolite productivity, varying the composition of the culture medium, pH, temperature, aeration, and luminosity (Bode et al. 2002). This methodology can also include the addition of precursors or inhibitors of metabolite biosynthesis (Rateb and Ebel 2011). The term widely used for this approach is one strain, many compounds (OSMAC) (Bills et al. 2008; Elias et al. 2006). The success of OSMAC lies in the fact that suitable culture media and optimal growing conditions play important roles in fungal growth and mycelium formation, consequently influencing the efficiency of secondary metabolite biosynthesis. OSMAC methodology has proven to be versatile in generating intracellular signals that maximize the enzymatic synthetic potential of a fungal species (Paranagama et al. 2007), often resulting in the production of metabolites considered unusual in natural biosynthesis.

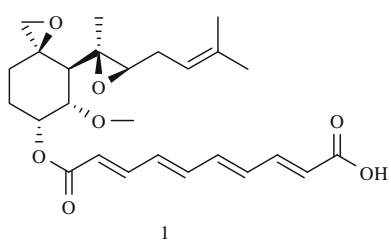
Modifications of culturing media

Fungi are heterotrophic organisms and therefore require a variety of organic nutrients to carry out all

biochemical functions. Nevertheless, carbohydrates are still their best source of energy. The influence of glucose concentrations in secondary metabolite production and in the amount of biomass has been widely reported for *Penicillium* species. *Penicillium scabrosum* produced a maximum fumagillin yield (**1**, Fig. 1) when the medium contained 1 % glucose, while 3 % glucose was necessary to attain a higher biomass formation. Therefore, when biomass growth reached a maximum level, fumagillin production was repressed (Barboráková et al. 2012). In *Penicillium janthinellum*, the number of metabolites produced also decreased in direct proportion to the increase in carbohydrate concentrations—while seven metabolites were produced upon adding 10 % sucrose, only two metabolites were produced when the sucrose concentration was raised to 40 %. The same was reported for *Penicillium duclauxii*. From the eight metabolites produced in a medium containing 10 % sucrose, only three were expressed when the sucrose concentration was raised (Zain et al. 2011).

In an attempt to obtain bioactive substances from *Penicillium verrucosum*, four culture media and different cultivation times were studied. Extracts with greater *Trypanossoma* sp. inhibition were obtained utilizing Takeuchi medium, a starch-based culture medium (Elias et al. 2006). *Penicillium citrinum*, grown in a mixture of salts (10 %) and nutrients (60 %), achieved optimum conditions for the production of several metabolites, including citrinalin A (**2**) and B (**3**, Fig. 2). A nearly tenfold increase in the yield of citrinalin B and the suppression of citranilin A was reported when the proportion of salts and nutrients varied within the medium (Pimenta et al. 2010).

Metabolic diversification of an *Aspergillus tubingensis* strain isolated from the Southern China sea was achieved by using rice as a carbohydrate source to produce eight compounds, four of which were new dimeric nafto-γ-pirones, as well as rubasperones D (**4**),



1

Fig. 1 Molecular structure of fumagillin (**1**)

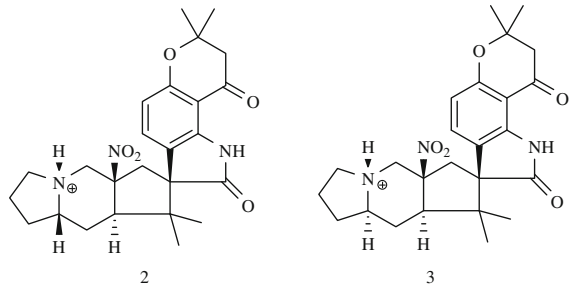


Fig. 2 Molecular structures of citrinalin A (**2**) and B (**3**)

E (**5**), F (**6**), and G (**7**) (Fig. 3). Moreover, rubasperone D proved to be active in toxicity assays against tumor cells (Huang et al. 2011a).

Corn bran, varied carbon, and nitrogen sources at different temperatures were screened for improvements in mevastatin (**8**, Fig. 4) production by *P. citrinum*. In this case, inoculum volume proved to be an important parameter, as did medium supplementation with low amounts of glucose and sodium (Mahesh et al. 2012).

Less common carbon sources, such as linoleic acid, have been used to improve lovastatin production by *Aspergillus terreus* (Barrios-González and Miranda 2010). The production of the hypoglycemic agent acarbose by *Actinoplanes utahensis* was performed using maltose, glycerol, and glutamate (Wang et al. 2012). The production of the immunosuppressant agent cyclosporin A by *Beauveria nivea* was activated by fructose (Huijun et al. 2011), while *Gibberella fujikuroi* required a mixture of glucose and corn oil on an experiment to produce gibberellic acid, a plant growth hormone (Rios-Iribe et al. 2011). These illustrate good examples of how different fungi have different carbohydrate requirements.

The nitrogen source is another important factor in secondary metabolite production. Microorganisms are able to use a large amount of organic and inorganic molecules, as well as complex mixtures, such as peptones and beef, yeast, corn, and soy extracts, as a source of nitrogen in generating the growth and production of secondary metabolites. Experiments that have studied the effects of the different nitrogen sources necessary to produce PP-V (a strong reddish pigment) by a strain of *Penicillium* sp. have shown that the use of ammonium was in fact more effective than nitrate, whereas the use of a yeast extract proved ineffective (Arai et al. 2012).

Mannitol and sucrose were reported to promote alkaloid biosynthesis caused by *P. citrinum*. The

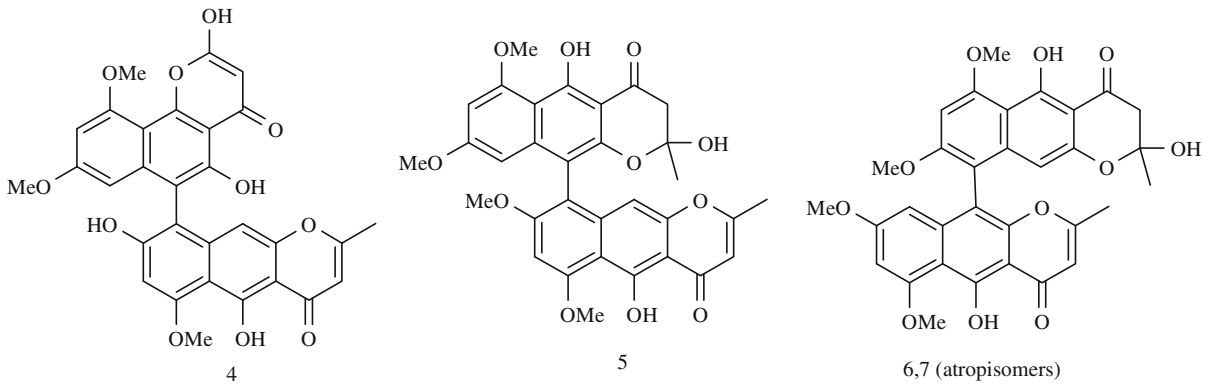


Fig. 3 Molecular structures of the dimeric nafto-γ-pirones, rubasperone D (**4**), E (**5**), F (**6**) and G (**7**)

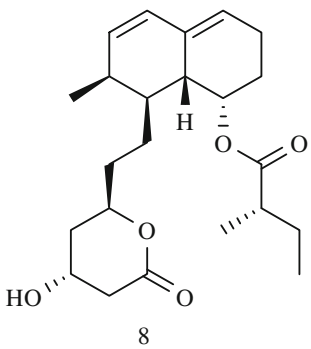


Fig. 4 Molecular structure of mevastatin (**8**)

concentration and the proportion of sucrose/mannitol influenced the type of alkaloid produced. Using urea as a nitrogen source favored flavonoid biosynthesis, while adding iron and copper inhibited flavonoid production (Kozlovsky et al. 2010).

The OSMAC approach was used to modify the metabolic profile of the fungal species *Spicaria elegans* isolated from marine sediments collected in Jiaozhou Bay, China. The fungus was cultivated for 8 days to produce six new compounds: spicochalasin A (**9**) and five new aspochalasins (**10**–**14**). Compounds **9** and **10** presented modest cytotoxic activity against HL-60 cells (Lin et al. 2009). Later, three new aspochalasins R–T (**15**–**17**) were isolated from the same fungus, *S. elegans,* grown in the same medium for a longer fermentation length (14 days) (Lin et al. 2010). Structures of compounds **10**–**17** can be found in Fig. 5. Additional compounds were isolated from the same strain of *S. elegans*, again applying the OSMAC strategy by adding D or L-tryptophan to the fermentation. Three new cytochalasins (**18**–**20**, Fig. 6) were obtained and evaluated for their cytotoxic activities against the HL-60, A-549, BEL-7402, and P388 cell lines. Compounds **18** and **19** showed cytotoxic activity against A-549 cell lines (Wang et al. 2011a).

Modifications of culturing conditions

The pH of the fermentation broth is a key feature to generate metabolic diversification, given that, along with temperature, pH also is directly involved in enzyme activation/deactivation. Together, they act by broadening or limiting the number of active enzymes in the secondary metabolite production (Sood 2011). Other physical conditions, such as the availability of light, are also mandatory in the activation of physiological biochemical functions. The manipulation of these experimental growing conditions usually results in a great differentiation among metabolic profiles.

Jain and Pundir (2011) investigated the effect of pH and temperature variation in the production of biologically active metabolites caused by a bioactive *A. terreus* species grown in potato dextrose broth at different pH levels (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0). The extracts obtained at pH 6.0 presented a massive production of metabolites that proved to be active against *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus casei*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Saccharomyces cerevisiae*. An increase in the active metabolite production could also be observed when the temperature was raised from 20 to 25 °C and a decline when the temperature was raised beyond this level (from 25 to 35 °C).

Among the physical factors able to activate the expression of silent compounds, light exposure, its intensity, and wavelength are able to affect fungal

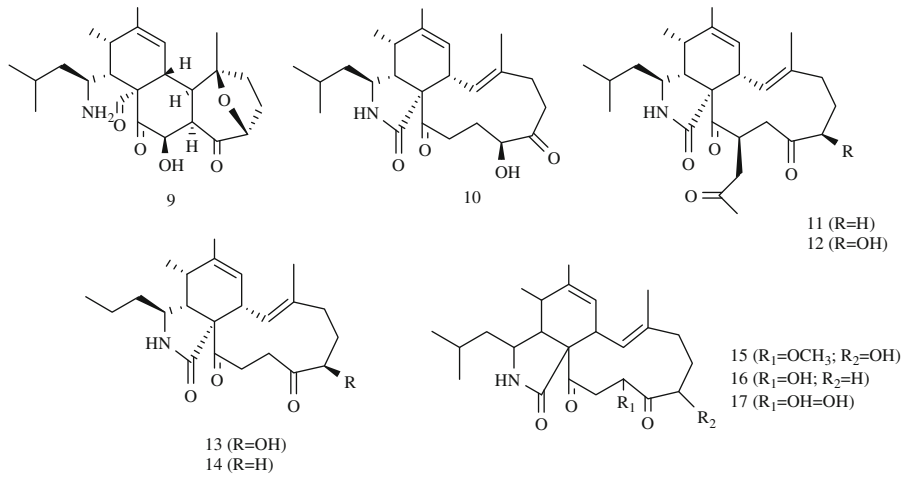


Fig. 5 Molecular structures of spicochalasin A (**9**) and Aspochalasins (M–T) (**10**–**17**)

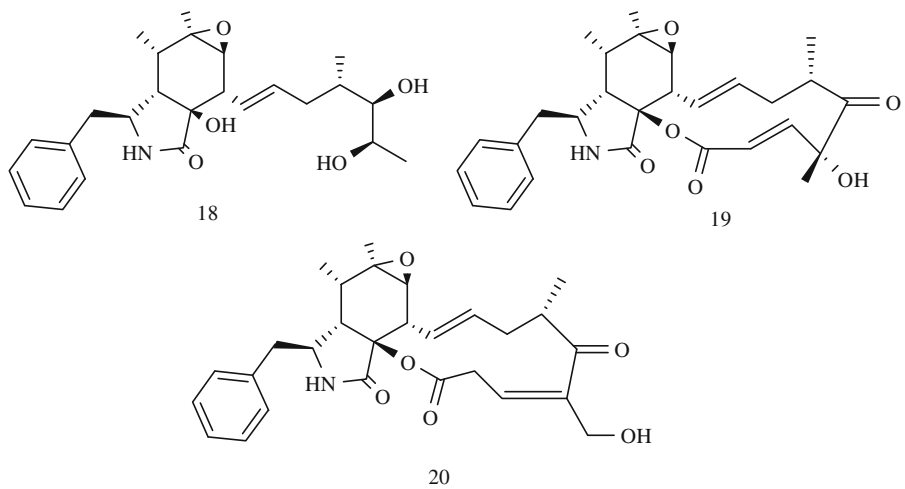


Fig. 6 Molecular structures of cytochalasins (**18**–**20**)

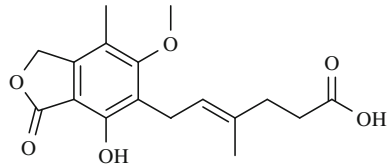
species that contain photoreceptors. It has been reported that the use of red or blue lights stimulates the formation of mycophenolic acid (**21**, Fig. 7) by *Penicillium brevicompactum.* Maximum production was reached at 900 lux, using red light, and at 600 lux, using blue light. It could also be observed that red light stimulates biomass production, while blue light inhibited it (Shu et al. 2010).

Metabolic diversification upon exposure of the fungus *Sphaeropsidales* sp. to ultraviolet light led to the isolation of a new metabolite, mutolide (**22**, Fig. 8), which presented antibacterial activity against *Bacillus subtilis* and *E. coli* (Bode et al. 2000).

Modifications in the cultivation conditions, in addition to promoting the biosynthesis of silent metabolites, can be alternatively used to delete metabolic routes of toxic metabolite biosynthesis. The production of food toxin ochratoxin (**23**, Fig. 9), by species such as *Penicillium nordicum* and *P. verrucosum*, proved to be sensitive to temperature. The suppression of these factors minimized or suppressed the production of this toxic metabolite by the fungi (Schmidt-Heydt et al. 2011).

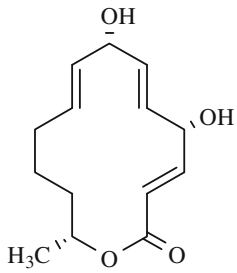
Addition of biotic and abiotic challenges

This strategy consists of providing adverse conditions to activate silent biosynthetic pathways. A marine strain of *A. terreus*, isolated from sediments of the



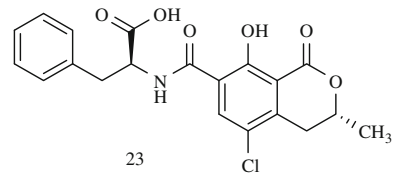
21

Fig. 7 Molecular structure of mycophenolic acid (**21**)



22

Fig. 8 Molecular structure of mutolide (**22**)



23

Fig. 9 Molecular structure of ochratoxin A (**23**)

Putian Sea, Fujian (China), when submitted to fermentation under high salinity produced twelve metabolites, including three new compounds: terremides A (**24**) and B (**25**), which are active against *P. aeruginosa* and *Enterobacter aerogenes*, respectively, and terrelactone (**26**) (Fig. 10) (Wang et al. 2011b). However, the osmotic stress caused by high concentrations of salts in the fermentation medium, in some cases, decreases the fungal growth rate (Huang et al. 2011b).

The use of sea water rather than distilled water promoted effective changes in the metabolic profile of *Aspergillus versicolor*, which had been isolated from the sea sponge, *Pestrosia* sp., resulting in the isolation of an aromatic polyketide (**27**), xanthones (**28** and **29**), and anthraquinones (**30**–**34**, Fig. 11). In vitro toxicity assays against tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15) showed cytotoxic activity for compounds **28**, **30**, and **31** (Lee et al. 2010).

The use of either sea water or sodium chloride solutions as challenging agents has been applied to many *Penicillium* species. The marine fungus *Penicillium commune*, known to produce azaphilones when cultivated in potato dextrose agar, was able to produce two new compounds, isophomenone (**35**) and 3-deacetylcitreohybridonol-3 (**36**, Fig. 12), in a peptone supplemented rice agar prepared with seawater (Gao et al. 2012b). Cultivation of *Penicillium terrestre* in a medium prepared with sea water enabled the fungus to express halogenases, which until then had remained silent, with the production of two new chlorinated sorbicillinoids, chloctanspirone A (**37**) and B (**38**), along with their precursors, terrestrol K (**39**) and L (**40**, Fig. 12) (Li et al. 2011). The production of chlorinated antimicrobial metabolite sclerotiorin by *Penicillium sclerotiorum* was 54 % higher than the control when a sodium chloride supplemented medium was employed in the fermentation (Lucas et al. 2010).

By contrast, there are examples in the literature in which an increase in sodium chloride concentrations inhibited fungal metabolic expression. From the metabolites produced by *P. janthinellum* in a sodium chloride-free medium, five were not produced when salt concentrations in the medium were >5 %, while some metabolites were only produced in a medium containing a narrow range of NaCl (between 1 and 3 %). The same effect was shown by *P. duclauxii*, which produced six metabolites in a sodium chloride-free medium; however, none of these were produced when the salt concentration in the medium was higher than 3 % (Zain et al. 2011).

Calcium bromide showed an inability to induce the production of either halogenated pyranopyranones or bromomethyilchlamydosporols A (**41**) and B (**42**) (Fig. 13) by a *Fusarium tricinctum* strain isolated from *Sargassum ringgoldium,* an edible brown marine algae (Nenkep et al. 2010).

By contrast, cadmium inhibited the biosynthesis of metabolites caused by *P. janthinellum*. From the seven metabolites produced in a cadmium nitrate-free culture medium, only two were produced when cadmium nitrate was added. *P. duclauxii*, however, produced only three metabolites in a medium containing cadmium, while nine metabolites were produced in a cadmium-free medium (Zain et al. 2011).

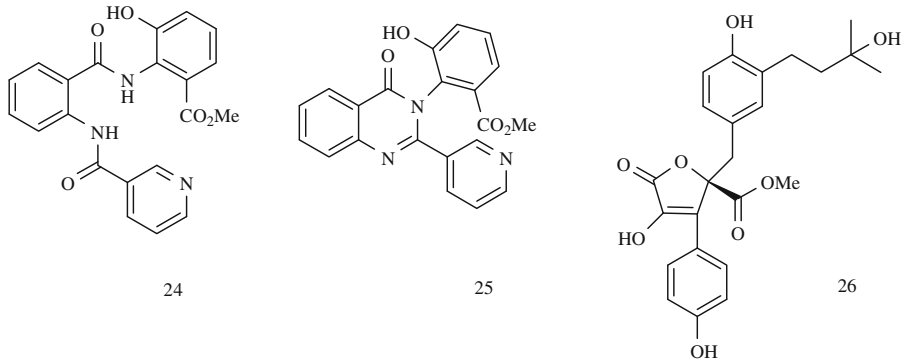


Fig. 10 Molecular structures of terremides A (**24**), B (**25**) and terrelactone (**26**)

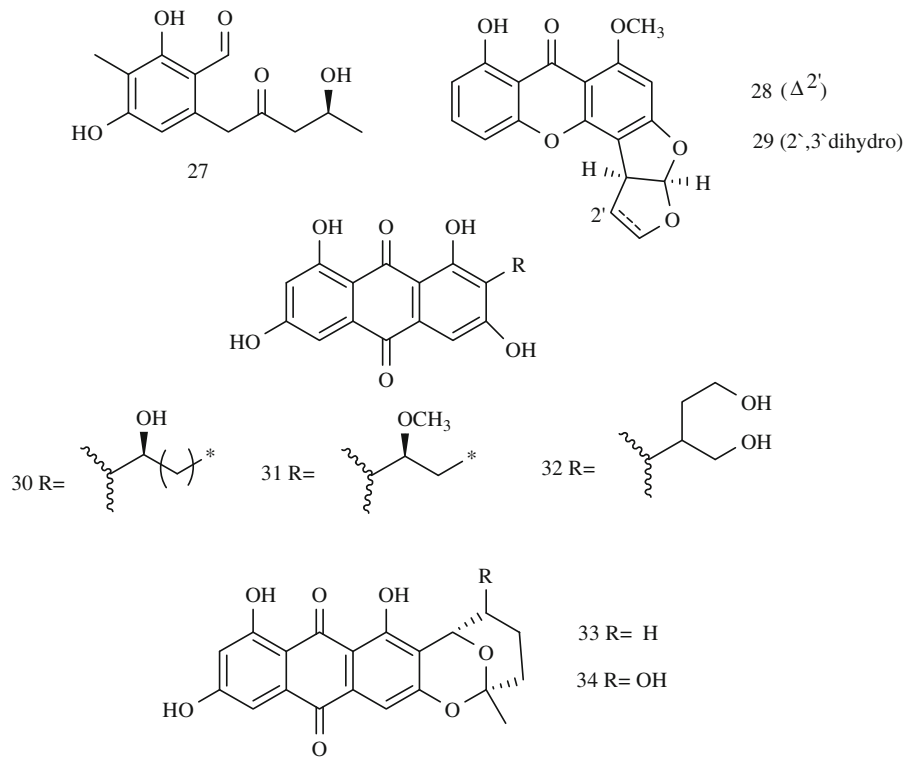


Fig. 11 Molecular structures of aromatic polyketide (**27**), two xanthones (**28** and **29**), and five anthraquinones (**30**–**34**)

In one interesting approach, the metabolic profiles of four species of *Penicillium* sp., isolated as parasites of the plant *Allium* sp. when cultivated in traditional media supplemented with *Allium* sp., enhanced secondary metabolites production (Overy et al. 2006).

Another quite effective strategy for chemical diversification is co-culturing, also called mixed culturing. In this approach, a challenging environment is provided by adding another microorganism to the fermentation broth. This is a very efficient methodology to bring about silent metabolic biosynthesis that is not expressed in environments that lack ecological competitiveness (Teles and Takahashi 2013). Antibiotic production is especially targeted by co-culturing, as can be seen in the introduction of a bacterium into the culturing medium, in turn stimulating antibiotic production by a fungal species (Cueto et al. 2001; Zuck et al. 2011; Hailei et al. 2011).

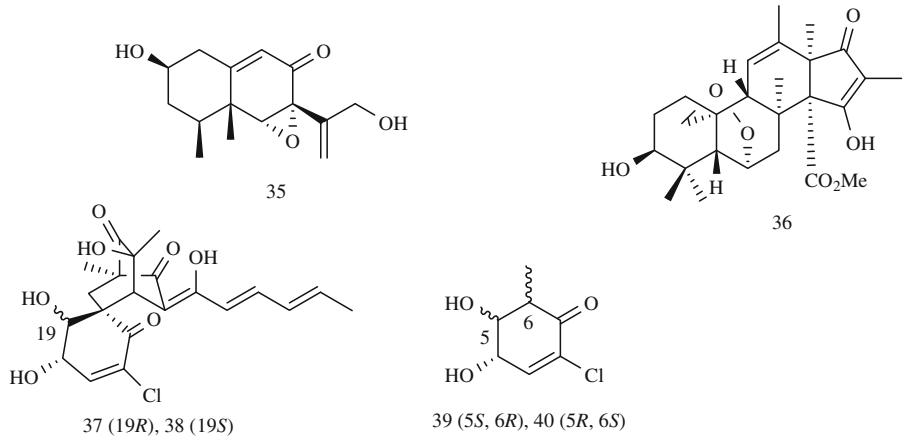


Fig. 12 Molecular structures of isophomenone (**35**), 3-deacetylcitreohybridonol (**36**), chloctanspirone A (19*R*) (**37**), B (19*S*) (**38**), terrestrol K (5*S*, 6*R*) (**39**) and L (5*R*, 6*S*) (**40**)

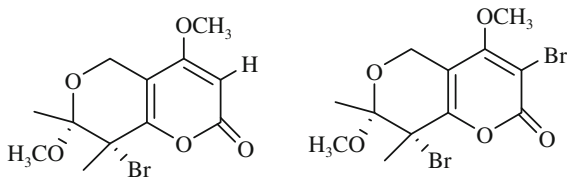


Fig. 13 Molecular structures of bromomethyilchlamydosporols A (**41**) and B (**42**)

Metabolites expression modulated by genetic manipulation

The development of advanced genetic tools has allowed for the mapping of genetic fragments responsible for important biochemical mechanisms, such as the production of polyketide synthase (PKS) enzymes and non-ribosomal peptides (NRPS). This achievement allowed for the reshaping of the biosynthetic potential of fungal species. Literature has shown that <30 % of 31 NRPS, 15 PKS, and 9 hybrid PKS–NRPS of *Aspergillus niger* biosynthetic gene clusters have been mapped under laboratory conditions to date (Henrikson et al. 2009).

Mapping genetic fragments of fungal species has been helpful in the investigation of biosynthetic routes. In this context, it has been shown that the acetylation of histone H4 in gene clusters responsible for aflatoxin biosynthesis in *Aspergillus* species are related to toxin expressions; therefore, a decrease in this acetylation inhibits aflatoxin production (Roze et al. 2011). The availability of genomic sequence databases contributed to identifying the genes responsible for the biosynthesis of major bioactive metabolites (Arnaud et al. 2012).

Some interesting studies on *Botytis cinerea* genetic profiles have also been reported in the literature. *B. cinerea* is a sever phytopathogen that kills host-cells by producing two types of phyto-toxins: a sesquiterpene, called botrydial, and the polyketide botcinic acid. Recent research in this field enabled the identification of two PKS, encoding genes related to botcinic acid biosynthesis, demonstrating that botrydial plays a role of redundant virulence (Dalmais et al. 2011). *B. cinerea* mutants have been screened for mapping the proteins responsible for colonization efficiency in different host plants and tissues, as well for toxin production (Temme et al. 2012).

Metabolite diversification can be accomplished by the genetic mapping of a fungal metabolic expression, followed by gene inactivation and subsequent examination of the metabolic profile produced by the mutant, as compared to the wild type. Using this approach, six NRPS from an *Aspergillus nidulans* strain were randomly selected and deleted. The selective perturbation of each gene was verified by polymerase chain reaction (PCR) and fungal metabolic expression was analyzed to confirm an exotic metabolic profile from the mutants in relation to the control species (Chiang et al. 2008).

A study analyzed the role of COMPASS, an enzyme present in the Set1 complex, responsible for

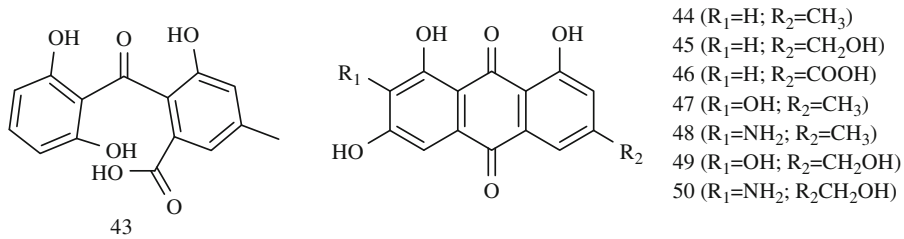


Fig. 14 Molecular structures of monodictyphenone (**43**), emodin (**44**) and emodin derivatives (**45–50**)

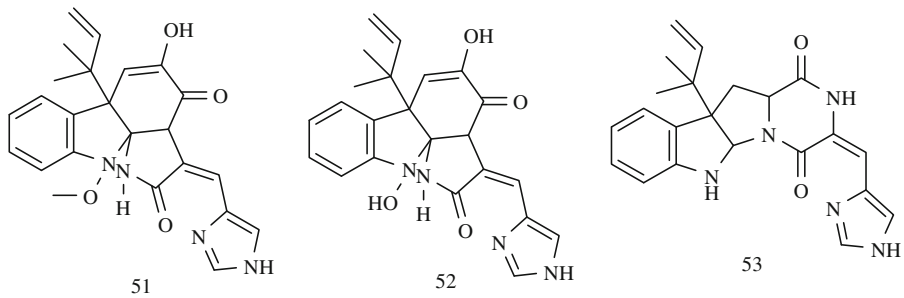


Fig. 15 Molecular structures of meleagrin (**51**), glandicolin B (**52**) and roquefortine C (**53**)

catalyzing the methylation of lysine 4 on histone H3 in *A. nidulans*. It was determined that the loss of function of a critical COMPASS component enabled the expression of clusters related to secondary metabolite production. One of the clusters, called MDP, was related to the production of monodictyphenone (**43**), emodin (**44**), and emodin derivatives (**45–50**, Fig. 14) (Chiang et al. 2010).

The inhibition of a specific gene through the use of interference RNA (RNAi or RNAi plasmid) can be carried out, considering that the knowledge of genes involved in metabolite biosynthesis allows for a broad control of their production. In *Penicillium chrysogenum,* gene suppression was responsible for the production of a methyltransferase capable of converting meleagrin (**51**) into glandicolin B (**52**), in turn inhibiting the production of **51** and enhancing the production of **52**. The suppression of two other genes located within the same cluster was able to inhibit meleagrin and roquefortine C (**53**) (Fig. 15) production, showing that this cluster is responsible for the biosynthesis of both metabolites (García-Estrada et al. 2011).

In another strain of *P. chrysogenum*, the silencing of the gene responsible for oxalate production resulted in an increased production of adipoyl-6-aminopenicillic acid (ad-6-APA), a cephalosporin precursor (**54**, Fig. 16). The gene was identified as an oxalate producer after its cloning on *S. cerevisiae*. Depletion of this gene in *P. chrysogenum* resulted in a strain with a higher production of ad-6-APA (**54**) than the control strain (Gombert et al. 2011). The depletion of genes responsible for adipic acid oxidation (**55**) (Fig. 16) has also increased the production of ad-6-APA (**54**), since adipic acid oxidation blocks the biosynthesis from proceeding (Veiga et al. 2012).

In a related experiment, a similar effect was observed for *Penicillim aethiopicum*. The inhibition of a gene responsible for griseofulvin (**56**) production resulted in the accumulation of the precursor dechlorogriseofulvin (**57**). Comparing their structures shows that the inhibited gene was responsible for the production of a chlorinase enzyme. Follow-up studies demonstrated that the two clusters responsible for the biosynthesis of this chlorinated metabolite were also responsible for producing another metabolite with a more complex structure, viridicatumtoxin (**58**) (Chooi et al. 2010). For structures of **56–58**, see Fig. 17.

In *Penicillium expansum*, the inhibition of the gene responsible for the biosynthesis of the toxin patulin (**59**, Fig. 18) was carried out in an attempt to reduce the toxicity of this microorganism associated with the

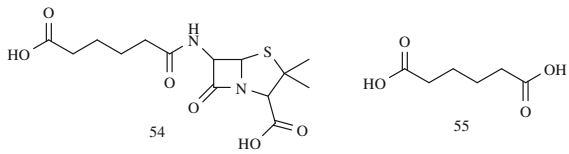


Fig. 16 Molecular structures of adipoyl-6-aminopenicillinic acid (**54**) and adipic acid (**55**)

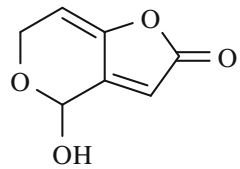


Fig. 18 Molecular structure of patulin (**59**)

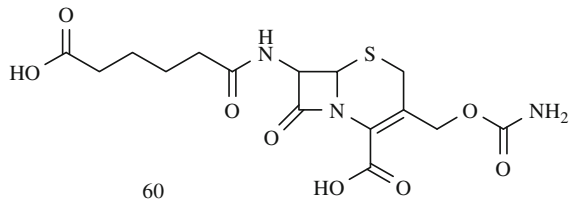


Fig. 19 Molecular structure of ad7-ACCCA (**60**)

intake of patulin as a contaminant of foods like orange juice. The gene disruption technique was able to generate two mutants with a low production of patulin (Sanzani et al. 2012).

In addition to the modulation of metabolite production, genetic engineering has also made it possible to transfer genes between fungi in an attempt to produce new molecules. A strain of *P. chrysogenum* was able to produce adipoyl-7-amino-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid (ad7-ACCCA) (**60**, Fig. 19) after cloning and expressing both an *Acremonium* gene that expresses the enzymes expandase and hydrolase as well as a *Streptomyces* gene expressing carbamoyltranferases in a *P. chrysogenum* strain (Harris et al. 2009). In the same way, a gene producer of CoA ligase induced by phenylacetic acid (PAA), present in *P. chrysogenum*, was able to be cloned and expressed in *E. coli*. Studies have shown that the new strain was able to produce PAA-CoA, resulting in an efficient gene expression (Yu et al. 2011).

Metabolite expression modulated by epigenetic modifiers

The study of the mechanisms that lead to the suppression of biosynthetic transcription, as well as the development of methodologies to induce the expression of these genes, represents an important role in the search for new secondary metabolites. The regulation of enzymes that control metabolite production can be obtained by changing epigenetic mechanisms, such as that which involves DNA methylation, which is related to gene silencing, and histone modifications. In the later, acetylation and phosphorylation are the most studied mechanisms, determining the conformation of chromatin (Fisch et al. 2009). This can be obtained using epigenetic modifiers.

Histone deacetylases (HDAC) and methyltransferase inhibitors of DNA are the most commonly used tools to track biosynthetic silent pathways (Asai et al. 2012b), as they are capable of activating silent gene clusters. The addition of epigenetic agents has been applied to change the transcription rate of some genes, thus inducing the expression of those responsible for the production of novel secondary metabolites (Henrikson et al. 2009). In some cases, this approach increases the yield of secondary metabolites already produced by the fungal species under classical conditions (Williams et al. 2008).

Among the HDAC, suberoylanilide hydroxamic acid (SAHA) (**61**), presented in Fig. 20, stands out.

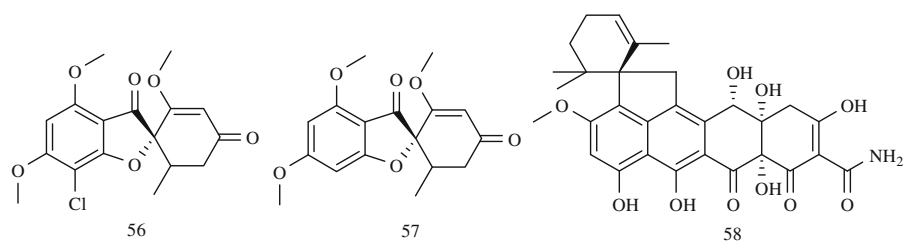


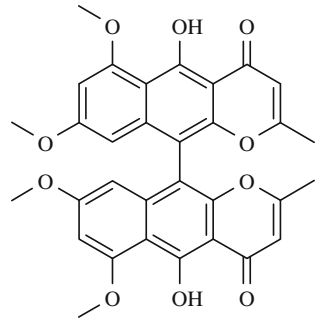
Fig. 17 Molecular structures of griseofulvin (**56**), dechlorogriseofulvin (**57**) and viridicatumtoxin (**58**)

The usefulness of this class of substances relies on the fact that, among the chemical changes that a DNA histone may undergo, its acetylation is generally associated with gene silencing. Histone acetyltransferases are responsible for this step, while HDAC follow the reverse pathway, activating inactive clusters. SAHA is able to interact with the catalytic site of histone-deacetylases. The most commonly used methyl transferase inhibitor, 5-azacytidine (5-AZA) (**62**, Fig. 20), interacts with methyltransferase, in turn resulting in DNA hypomethylation, which subsequently leads to chromatin restructuring (Fisch et al. 2009).

Aspergillus niger has been targeted by an epigenetic methodology in which this species was cultivated over a 2-week-period in a vermiculite based semi-solid medium treated with SAHA, leading to the isolation of nigerone (**63**, Fig. 21), exemplifying the importance of epigenetic modulation to produce unknown natural products (Henrikson et al. 2009). Working with the same fungal species, a successful combination of SAHA (**61**) and 5-AZA (**62**) was used to obtain secondary metabolites related to other silent genes (Fisch et al. 2009).

A class 2 histone deacetylase hdaA was used to regulate the production of secondary metabolites by *Aspergillus fumigatus*. The suppression of the hdaA gene generated an increase in the production of secondary metabolites and a reduction in the production of gliotoxin (**64**, Fig. 22), a toxin produced by this fungus. On the other hand, overexpression of the hdaA gene promoted an increase in gliotoxin production (Lee et al. 2009).

The production of new metabolites by *Penicillium citreonigrum* has been induced by AZA. The addition of this epigenetic agent to a culture medium containing rice, oatmeal, cornmeal, nutrient broth, and vermiculite induced the production of metabolites **65–72** (Fig. 23) (Wang et al. 2010).

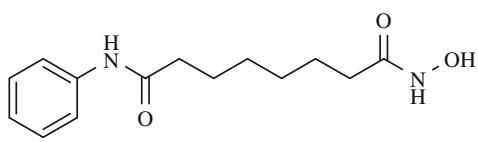


63

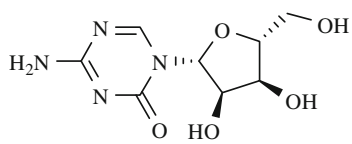
Fig. 21 Molecular structure of nigerone (**63**)

The effect of epigenetic modulators SAHA and AZA on the production of volatile organic compounds (VOCs) by the endophytic fungus *Hypoxylon* sp. has been reported in the literature. Analysis performed by gas chromatography coupled to mass spectrometry (GC/MS) of 8-day-old *Hypoxylon* sp. cultures revealed significant variations in the VOCs profiles, with the production of several new compounds when using epigenetic modulators as compared to the *Hypoxylon* sp. wild-type VOCs profile (Ul-Hassan et al. 2012).

Reports of other interesting secondary metabolites successfully obtained using epigenetic agents, such as new tryptophan derivatives (**73–75**) (Fig. 24) from *Torrubiella luteorostrata* (Asai et al. 2011), a new aromatic polyketide glycoside, and indigotide B (**76**), from *Cordyceps indigotica* (Asai et al. 2012a) have also been described in the literature. The concomitant addition of two epigenetic modifying agents, SBHA and RG-108, to the culture medium of *Isaria tenuipes,* induced obtaining tenuipyrone (**77**), a novel polyketide (Fig. 24) (Asai et al. 2012b). The use of epigenetic modulators succeeded in increasing the production of metabolites with antimalarial and anti-MRSA activity from the fungus *Leucostoma persoonii,* endophyte from *Rhizophora mangle*. The major metabolites

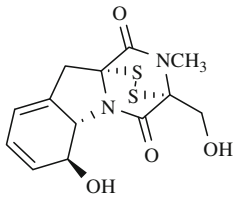


61



62

Fig. 20 Molecular structures of SAHA (**61**) and 5-AZA (**62**)



64

Fig. 22 Molecular structure of gliotoxin (**64**)

cytosporones B (**78**), C (**79**), and E (**80**) (Fig. 25) had their yields considerably enhanced in the HDAC-inhibited fermentation (360, 580, and 890 %, respectively). Moreover, cytosporone R (**81**, Fig. 25), a new compound of this class, was also isolated from HDAC-inhibited cultures (Beau et al. 2012).

Final remarks and conclusions

Medium modification, changes in culture parameters and epigenetic modifiers provide a modulation of secondary metabolite production resulting from different gene expressions. Although they are usually studied separately, these factors attempt to achieve the same goal: change the chromatin condensation level in such a way that new genes will be expressed and new compounds will be produced.

Other approaches, such as the introduction of biotic stress, are also promising. For instance, interaction between *A. nidulans* and bacterium *Streptomyces rapamycinicus* resulted in the production of orselinic acid (**82**) and derivatives (**83–85**), also influencing the production of sterigmatocystin (**86**), terrequinone A (**87**), and penicillin (**88**) (Fig. 26). This stress factor was able to promote an increase in lysines 9 and 14 acetylation on histone H3, catalyzed by the SAGA/ADA complex. This complex, containing the proteins GcnE HAT and Adab, is required to induce these metabolites production (Nutzmanna et al. 2011).

Some antibiotics, in addition to playing a role as a stress factor, can induce gene resistance expressions

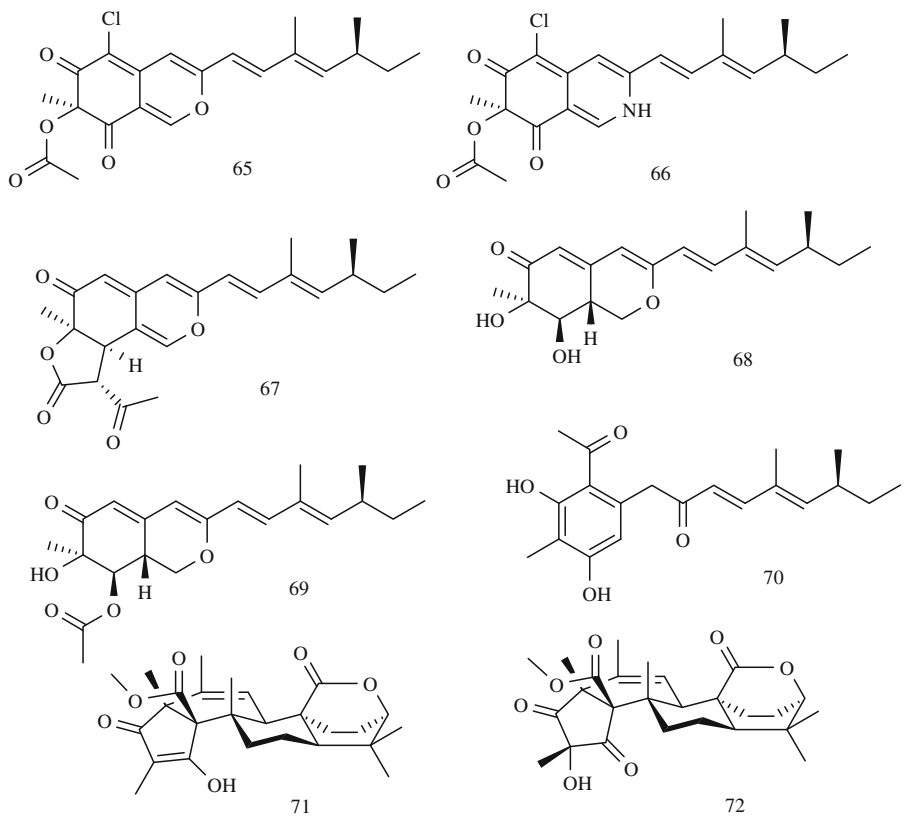


Fig. 23 Molecular structures of sclerotiorin (**65**), sclerotioriamine (**66**), ochrephilone (**67**), dechloroisochromophilone III (**68**), dechloroisochromophilone IV (**69**), 6-[(3E, 5E)-5, 7-dimethyl-2-methylenenone-3,5-dienyl]-2,4-dihydroxy-3-methylbenzaldehyde (**70**), atlantinone A (**71**) and antlatinone B (**72**)

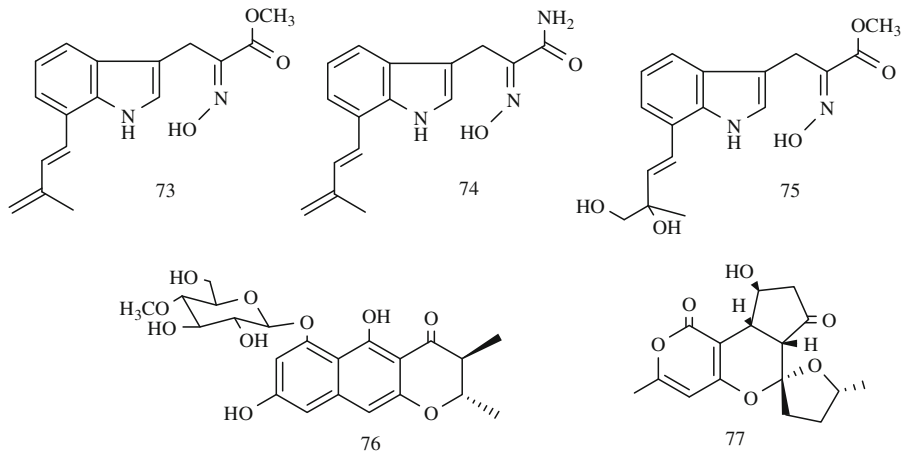


Fig. 24 Molecular structure of luteoride A (**73**), B (**74**), C (**75**), indigotide B (**76**) and tenuipyrone (**77**)

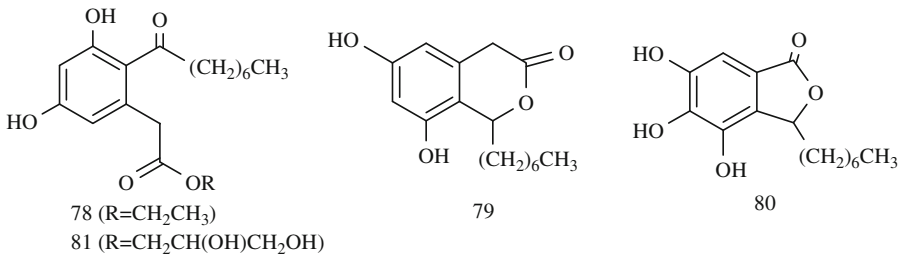


Fig. 25 Molecular structures of cytosporones B (**78**), C (**79**), E (**80**) and cytosporone R (**81**)

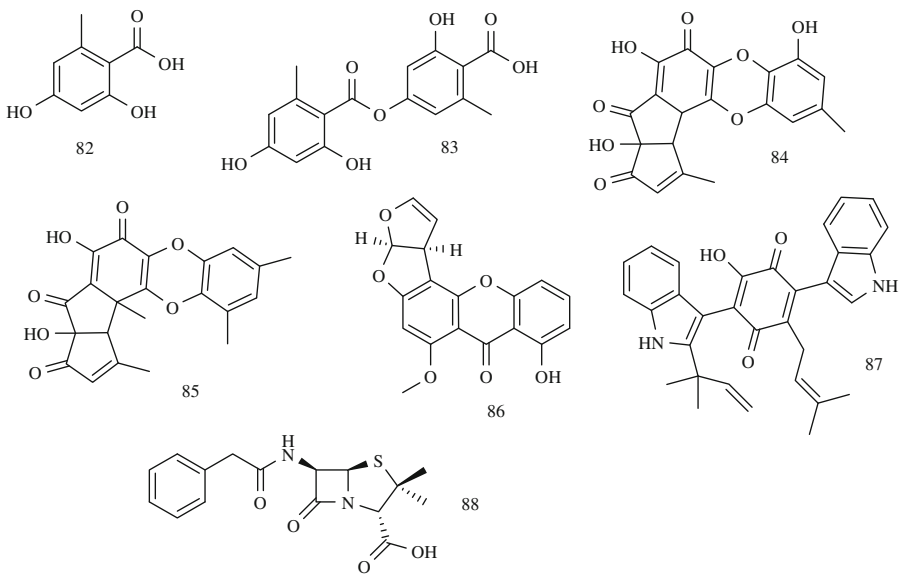


Fig. 26 Molecular structures of orselinic acid (**82**) and its derivatives (**83**–**85**), sterigmatocystin (**86**), terrequinone A (**87**) and penicillin (**88**)

that lead to the expression of other biosynthetic routes (Chai et al. 2012). In this manner, *Penicillium purpurogenum*, when treated with high concentrations of the antibiotic gentamicin, acquired resistance. The resistant mutant strain was capable of producing four new metabolites: janthinone (**89**), fructigenine (**90**),

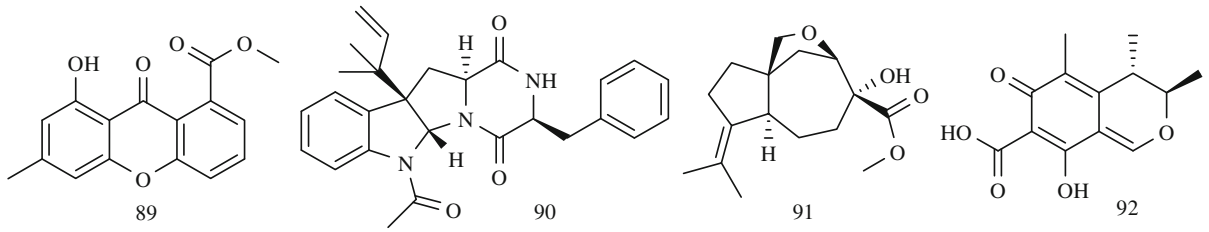


Fig. 27 Molecular structures of janthinone (**89**), fructigenine A (**90**), aspterric acid methyl ester (**91**) and citrinin (**92**)

aspterric acid methyl ester (**91**), and citrinin (**92**) (Fig. 27). Hanlon (2006) added a xenobiotic agent, cyclosporine, to the culture medium when cultivating *Aspergillus* sp. so as to induce the biosynthesis of three colored compounds.

Since there are various factors that can be changed to activate the production of silent metabolites, or even to increase their yields, the use of statistical techniques have gained importance in the simultaneous assessment of several culturing alterations. For example, antitumor metabolite production by a marine species of *Penicillium* was achieved by conducting an initial screening of seven factors (glucose, sucrose, K_2HPO_4, $FeSO_4$, pH, temperature, and stirring), using the Plackett-Burman design. The factors that proved to be significant were analyzed by response surface methodology to successfully determine the exact amounts of culture medium ingredients, as well as fermentation conditions (Guo et al. 2012).

Another broad study guided by statistical tools was carried out with *Penicillium steckii* aimed at the production of antifungal agents, in which several parameters were evaluated: the concentration of potato extracts; different carbon sources, such as dextrose and sucrose; combinations of carbon sources; effects of the addition of glycerol; glycerol concentrations, different sources of amino acids; amino acid concentrations; the addition of potassium, ammonium, and sodium salts; as well as the addition of heavy metals, hormones, and their concentrations. As the result, an optimum culture medium to promote fungal growth and the production of antifungal agents was found to contain a mixed carbohydrate source made up of potato extracts, sucrose, dextrose, and glycerol, in addition to lysine at pH 5 for 6 days. The other variables proved to be statistically irrelevant (Sabat and Gupta 2010).

The use of statistical planning associated with the use of OSMAC and epigenetic approaches has been greatly speeding up the process of bringing about new fungal secondary metabolites. The genetic mapping of gene clusters from both known and new fungi is expected to direct future studies toward microorganisms containing promising silent biosynthetic routes. It is possible that the combined use of OSMAC, epigenetic, and statistical tools to modulate and monitor secondary fungal metabolism will increase in such a way as to rationally broaden the use of fungi as small factories that produce numerous novel compounds with pharmacological potentials in the near future.

Successful examples with industrial applications cover from antibiotics (Jonge et al. 2011) and other drugs, such as cyclosporin (Tanseer and Anjum 2011) and lovastatin (Jia et al. 2009), to agricultural products (Agriquest) and broaden the activity of metabolites, such as terrein (Yin et al. 2012) and kojic acid (Terrabayashi et al. 2010). There is a great expectation that the development of the "omics" sciences in recent years will also bring about new perspectives on the production of new metabolites by fungi. These "omics" sciences, including genomics, transcriptomics, metabolomics, and proteomics, have been demonstrating that it is relatively easy to monitor, quantify, and produce secondary metabolites. Metabolomics have been greatly aiding in the identification and quantification of metabolites in different organisms, providing complete compositional representations of individual samples that can be compared with other individuals (Gomase et al. 2008). Nuclear Magnetic Resonance and Liquid Chromatography, coupled with Mass Spectroscopy techniques, are also potent tools, allowing for simultaneous analyses and the identification of small molecules found directly in biofluids (Smolinska et al. 2012).

Acknowledgments The authors acknowledge support (scholarships and grants) from the following Brazilian Research

Agencies: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Agriquest: http://www.agriquest.info/. Consulted on 27 Apr 2013

Arai T, Umemura S, Ota T, Ogihara J, Kato J, Kasumi T (2012) Effects of inorganic nitrogen sources on the production of PP-V [(10Z)-12-carboxyl-monascorubramine] and the expression of the nitrate assimilation gene cluster by *Penicillium* sp. AZ. Biosci Biotechnol Biochem 76:120–124

Arnaud MB, Cerqueira GC, Inglis DO, Skrzypek MS, Bindley J, Chibucos MC, Crabtree J, Howarth C, Orvis J, Shah P, Wymore F, Binkley G, Miyasato SR, Simison M, Sherlock G, Wortman R (2012) The *Aspergillus* genome database (AspGD): recent developments in comprehensive multi-species curation, comparative genomics and community resources. Nucleic Acid Res 40:653–659

Asai T, Yamamoto T, Oshima Y (2011) Histone deacetylase inhibitor induced the production of three novel prenylated tryptophan analogs in the entomopathogenic fungus, *Torrubiella luteorostrata*. Tetrahedron Lett 52:7042–7045

Asai T, Yamamoto T, Chung YM, Chang FR, Wu YC, Yamashita K, Oshima Y (2012a) Aromatic polyketide glycosides from an entomopathogenic fungus, *Cordyceps indigotica*. Tetrahedron Lett 53:277–280

Asai T, Chung YM, Sakurai H, Ozeki T, Chang FR, Yamashita K, Oshima Y (2012b) Tenuipyrone, a novel skeletal polyketide from the entomopathogenic fungus, *Isaria tenuipes*, cultivated in the presence of epigenetic modifiers. Org Lett 14:513–515

Barboráková Z, Labuda R, Häubl G, Tančinová D (2012) Effect of glucose concentration and growth conditions on the fungal biomass, pH of media and production os fumagillin by a non-pathogenic strain *Penicillium scabrosum*. J Microbiol Biotechnol Food Sci 1:466–477

Barrios-González J, Miranda RU (2010) Biotechnological production and applications of statins. Appl Microbiol Biotechnol 85:869–883

Beau J, Mahid N, Burda WN, Harrington L, Shaw LN, Mutka T, Kyle DE, Barisic B, Olphen A, Baker BJ (2012) Epigenetic tailoring for the production of anti-infective cytosporones from the marine fungus *Leucostoma persoonii*. Marine Drugs 10:762–774

Bills GF, Platas G, Fillola A, Jiménez MR, Collado J, Vicente F, Martín J, González A, Bur-Zimmermann J, Tormo JR, Peláez F (2008) Enhancement of antibiotic and secondary metabolite detection from filamentous fungi by growth on nutritional arrays. J Appl Microbiol 104:1644–1658

Bode HB, Walker M, Zeeck A (2000) Secondary metabolites by chemical screening, structure and biosynthesis of mutolide, a novel macrolide from a UV mutant of the fungus F-249707. Eur J Org Chem 8:1451–1456

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627

Chai Y, Cui C, Li C, Wu C, Tian C, Hua W (2012) Activation of the dormant secondary metabolite production by introducing gentamicin-resintance in a marine-derived *Penicillium purpurogenum* G59. Marine Drugs 10:559–582

Chiang Y, Szewczyk E, Nayak T, Davidson AD, Sanchez JF, Lo H, Ho W, Simityan H, Kuo E, Praseuth A, Watanabe K, Oakley BR, Wang CCC (2008) Molecular genetic mining of the *Aspergillus* secondary metabolome: discovery of the emericellamide biosynthetic pathway. Chem Biol 15: 527–532

Chiang YM, Szewczyk E, Davidson AD, Entwistle R, Keller N, Wang CCC, Oakley BR (2010) Genetic characterization of the monodictyphenone gene cluster in *Aspergillus nidulans*. Environ Microb 76:2067–2074

Chooi Y, Cacho R, Tang Y (2010) Identification of the viridicatumtoxin and griseofulvin gene clusters from *Penicillium aethiopicum*. Chem Biol 17:483–494

Cueto PRJ, Kauffman C, Fenical W, Lobkovsky E, Clardy J (2001) Pestalone, a new antibiotic produced by a marine fungus in response to bacterial challenge. J Nat Prod 64: 1444–1446

Dalmais B, Schumacher J, Moraga J, Pecheur PL, Tudzynski PB, Collado IG, Viaud M (2011) The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. Mol Plant Pathol 12:564–579

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5–16

Elias BC, Said S, Albuquerque S, Pupo MT (2006) The influence of culture conditions on the biosyn-thesis of secondary metabolites by *Penicillium verrucosum* Dierck. Microbiol Res 161:273–280

Fisch KM, Gillaspy AF, Gipson M, Henrikson JC, Hoover AR, Jackson L, Najar FZ, Wägele H, Cichewicz RH (2009) Chemical induction of silent pathway transcription in *Aspergillus niger*. J Ind Microbiol Biotechnol 36: 1199–1213

Fleming A (1944) The discovery of penicillin. Brit Med Bull 2:4–5

Gao J, Radwan MM, León F, Wang X, Jacob MR, Tekwani BL, Khan SI, Lupien S, Hill RA, Dugan FM, Cutler HG, Cutler SJ (2012a) Antimicrobial and antiprotozoal activities of secondary metabolites from the fungus *Eurotium repens*. Med Chem Res 21:3080–3086

Gao S, Shang Z, Li X, Li C, Cui C, Wang B (2012b) Secondary metabolites produced by solid fermentation of the marine-derived fungus *Penicillium commune* QSD-17. Biosci Biotechnol Biochem 76:358–360

García-Estrada C, Ullán RV, Albillos SM, Fernández-Bodega MA, Durek P, Döhren H, Martín JF (2011) A single cluster of coregulated genes encodes the biosynthesis of the mycotoxins roquefortine C and meleagrin in *Penicillium chrysogenum*. Chem Biol 18:1499–1512

Godio RP, Martín JF (2009) Modified oxidosqualene cyclases in the formation of bioactive seconda-ry metabolites: biosynthesis of the antitumor clavaric acid. Fungal Genet Biol 46:232–242

Gomase VS, Changbhale SS, Patil SA, Kale KV (2008) Metabolomics. Curr Drug Metab 9:89–98

Gombert AK, Veiga T, Puig-Martinez M, Lamboo F, Nijland JG, Driessen AJM, Pronk JT, Daran JM (2011) Function

characterization of the oxaloacetase encoding gene and elimination of oxalate formation in the β-lactam producer *Penicillium chrysogenum*. Fungal Genet Biol 48:831–839

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity and implications of their occurrence. J Nat Prod 69:509–526

Guo L, Zhu W, Liu C (2012) Fermentation conditions optimization of anti-tumor active metabolites from marine *Penicillium* sp. HGQ6. Adv Mater Res 340:344–350

Hailei W, Zhifang R, Ping L, Yanchang G, Guosheng L, Jianming Y (2011) Improvement of the production of a red pigment in *Penicillium* sp. HSD07B synthesized during coculture with *Candida tropicalis*. Bioresour Technol 102: 6082–6087

Hanlon A (2006) Chemical modulation of secondary metabolite production in *Aspergillus* sp. MS thesis, Massachusetts Institute of Technology

Harris DM, Westerlaken I, Schipper D, Krogt ZA, Gombert AK, Sutherland J, Raamsdonk LM, Berg MA, Bovenberg RAL, Pronk JT, Daran J (2009) Engineering of *Penicillium chrysogenum* for fermentative production of a novel carbamoylated cephem antibiotic precursor. Metab Eng 11: 125–137

Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res 105: 1422–1432

Henrikson JC, Hoover AR, Joyner PM, Cichewicz RH (2009) A chemical epigenetics approach for engineering the in situ biosynthesis of a cryptic natural product from *Aspergillus niger*. Org Biomol Chem 7:435–438

Hoffmeister D, Keller NP (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep 24:393–416

Huang HB, Xiao ZE, Feng XJ, Huang CH, Zhu X, Ju JH, Li MF, Lin YC, Liu L, She ZG (2011a) Cytotoxic naphtho-γ-pyrones from the mangrove endophytic fungus *Aspergillus tubingensis* (GX1-5E). Helv Chim Acta 94:1732–1740

Huang J, Lu C, Qian X, Huang Y, Zheng Z, Shen Y (2011b) Effect of salinity on the growth, biological activity and secondary metabolites of some marine fungi. Acta Oceanol Sin 30:118–123

Huang X, Zhu Y, Guan X, Tian K, Guo J, Wang H, Fu G (2012) A novel antioxidant isobenzofuranone derivative from fungus *Cephalosporium* sp. AL031. Molecules 17:4219–4224

Huijun D, Junyun J, Tongshun Y, Junjie Z (2011) Optimization of cyclosporin A production by *Beauveria nivea* in continuous fed-batch fermentation. Arch Biol Sci 63:907–914

Jain P, Pundir RK (2011) Effect of fermentation medium, pH and temperature varations on antibacterial soil fungal metabolite production. J Agric Technol 7:247–269

Jia Z, Zhang X, Cao X (2009) Effects of carbon sources on fungal morphology and lovastatin biosynthesis by submerged cultivation of *Aspergillus terreus*. Asia Pac J Chem Eng 4:672–677

Jonge LP, Bujis NAA, Pierick A, Deshmukh A, Zhao Z, Kiel JAKW, Heijnen JJ, Gulik WM (2011) Scale-down of penicillin production in *Penicillium chrysogenum*. Biotechnol J 6:944–958

Kozlovsky AG, Zhelifonova VP, Antipova TV, Zelenkova NF (2010) The influence of medium composition on alkaloid biosynthesis by *P. citrinum*. Appl Biochem Microbiol 46:525–529

Lee I, Oh JH, Shwab EK, Dagenais TRT, Andes D, Keller NP (2009) HdaA, a class 2 histone deacetylase of *Aspergillus fumigatus*, affects germination and secondary metabolite production. Fungal Genet Biol 46:782–790

Lee YM, Li H, Hong J, Cho HY, Bae KS, Kim MA, Kim DK, Jung JH (2010) Bioactive metabolites from the sponge-derived fungs *Aspergillus versicolor*. Arch Pharm Res 33:231–235

Li D, Chen L, Zhu T, Kurtán T, Mándi A, Zhao Z, Li J, Gu Q (2011) Chloctanspirones A and B, novel chlorinated polyketides with an unprecedented skeleton, from marine sediment derived fungus *Penicillium terrestre*. Tetrahedron 67:7913–7918

Lin Z, Zhu T, Wei H, Zhang G, Wang H, Gu Q (2009) Spicochalasin A and new aspochalasins from the marine-derived fungus *Spicaria elegans*. Eur J Org Chem 18: 3045–3051

Lin Z, Zhu T, Chen L, Gu Q (2010) Three new aspochalasin derivatives from the marine-derived fungus *Spicaria elegans*. Chin Chem Lett 21:824–826

Lucas EMF, Machado Y, Ferreira AA, Dolabella LMP, Takahashi JA (2010) Improved production of pharmacologically active sclerotiorin by *P. sclerotiorum*. Trop J Pharm Res 9:365–371

Mahesh N, Balakumar S, Indumathi P, Ayyadurai A, Vivek R (2012) Production and optimization of mevastatin using *Penicillium citrinum* NCIM 768. J Microbial Biochem Technol 4:1–4

Nenkep V, Yun K, Zhang D, Choi HD, Kang JS, Son BW (2010) Induced production of bromomethylchlamydosporols A and B from the marine-derived fungus *Fusarium tricinctum*. J Nat Prod 73:2061–2063

Nutzmanna HW, Dominguez YR, Scherlache K, Schroeckh V, Hom F, Gacek A, Schumann J, Hertweck C, Strauss J, Brakhage AA (2011) Bacteria-induced natural product formation in the fungus *A. nidulans* requires Saga/Ada-mediated histone acetylation. Proc Natl Acad Sci 108:14282–14287

Overy DP, Smedsgaard J, Frisvad JC, Phipps RK, Thrane U (2006) Host-derived media used as a predictor for low abundant, in plant metabolite production from necrotrophic fungi. J Appl Microbiol 101(6):1292–1300

Paranagama PA, Wijeratne EMK, Gunatilaka AAL (2007) Uncovering biosynthetic potential of plant-associated fungi: effect of culture conditions on metabolite production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*. J Nat Prod 70:1939–1945

Pimenta EF, Vita-Marques AM, Tininis A, Seleghim MHR, Sette LD, Veloso K, Ferreira AG, Williams DE, Patrick BO, Dalisay DS, Andersen RJ, Berlinck RGS (2010) Use of experimental design for the optimization of the production of new secondary metabolites by two *Penicillium* species. J Nat Prod 73(11):1821–1832

Rateb ME, Ebel R (2011) Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28:290–344

Rios-Iribe EY, Flores-Cotera LB, Chávira MMG, González-Alatorre G, Escamilla-Silva EM (2011) Inductive effect produced by a mixture of carbon source in the production of gibberellic acid by *Gibberella fujikuroi*. World J Microbiol Biotechnol 27:1499–1505

Roze LV, Koptina AV, Laivenieks M, Beaudry RM, Jones DA, Kanarsky AV, Linz JE (2011) Willow volatiles influence growth, development, and secondary metabolism in *Aspergillus parasiticus*. Appl Microb Biotechnol 92:359–370

Sabat J, Gupta N (2010) Nutritional factors affecting the antifungal activity of *Penicillium steckii* of mangrove origin. Afr J Microb Res 4:126–135

Sanzani SM, Reverberi M, Punelli M, Ippolito A, Fanelli C (2012) Study on the role of patulin on pathogenicity and virulence of *Penicillium expansum*. Int J Food Microbiol 153:323–331

Schmidt-Heydt M, Graf E, Batzler J, Geisen R (2011) The application of transcriptomics to understand the ecological reason of ochratoxin a biosynthesis by *Penicillium nordicum* on sodium chloride rich dry cured foods. Trends Food Sci Technol 22:S39–S48

Shu C, Peng J, Tsai C (2010) Effects of light intensity and light wavelength on the production of mycophenolic acid by *Penicillium brevicompactum* in batch cultures. Enzym Microbial Technol 46:466–471

Shwab EK, Keller NP (2008) Regulation of secondary metabolite production in filamentous ascomycetes. Mycol Res 112:225–230

Smolinska A, Blanchet L, Buydens LMC, Wijmenga SS (2012) NMR and pattern recognition methods in metabolomics: from data acquisition to biomarker discovery: a review. Anal Chim Acta 750:82–97

Sood M (2011) Cultural physiology: effect of culture mediums and pH on the growth, sporulation and secondary metabolites production of *Aspergillus umbrosus*. J Ecobiotechnol 3:8–11

Stadler M, Tichy H, Katsiou E, Hellwig V (2003) Chemotaxonomy of *Pochonia* and other conidial fungi with *Verticillium*-like anamorphs. Mycol Prog 2:95–122

Sun Z, Zhang M, Zhang J, Feng J (2011) Antifungal and cytotoxic activities of the secondary metabolites from endophytic fungus *Massrison* sp. Phytomedicine 18:859–862

Takahashi JA, Castro MCM, Souza GG, Lucas EMF, Bracarense AAP, Abreu LM, Marriel IE, Oliveira MS, Floreano MB, Oliveira TS (2008) Isolation and screening of fungal species isolated from Brazilian cerrado soil for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pyogenes* and *Listeria monocytogenes*. J Myc Médicale 18:198–204

Tanseer S, Anjum T (2011) Modification of C and N sources for enhnced production of cyclosporin 'A' by *Aspergillus terreus*. Braz J Microbiol 42:1374–1383

Teles APC, Takahashi JA (2013) Paecilomide, a new acetylcholinesterase inhibitor from *Paecilomyces lilacinus*. Microbiol Res 168:204–210

Temme N, Oeser B, Massaroli M, Heller J, Simon A, Collado IG, Viaud M, Tudzynski P (2012) BcAtf1, a global reguladtor, controls various differentiation processes and phytotoxin production in *Botrytis cinerea*. Mol Plant Pathol 13:704–718

Terrabayashi Y, Sano M, Yamane N, Marui J, Tamano K, Sagara J, Dohmoto M, Oda K, Ohshima E, Tachibana K, Higa Y, Ohashi S, Koike H, Machida M (2010) Identification and characterization of genes responsible for biosynthesis of kojic acid, an industrially importat compund from *Aspergillus oryzae*. Fungal Genet Biol 47:953–996

Ul-Hassan SR, Strobel GA, Booth E, Knighton B, Floerchinger C, Sear J (2012) Modulation of volatile organic compound formation in the mycodiesel-producing endophyte *Hypoxylon* sp. CI-4. Microbiology 158:465–473

Veiga T, Gombert AK, Landes N, Verhoeven MD, Kiel JAKW, Krikken AM, Nijland JG, Touw H, Luttik MAH, Toorn JC, Driessen AJM, Bovenberg RAL, Berg MA, Klei IJ, Pronk JT, Daran J (2012) Metabolic engineering of β-oxidation in *Penicillium chrysogenum* for improved semi-synthetic cephalosporin biosynthesis. Metab Eng 14:437–448

Wang X, Filho JGS, Hoover AR, King JB, Ellis TK, Powell DR, Cichewicz RH (2010) Chemical epigenetics alters the secondary metabolite composition of guttate excreted by an atlantic-florest-soil-derived *Penicillium citreonigrum*. J Nat Prod 73:942–948

Wang FZ, Wei HJ, Zhu TJ, Li DH, Lin ZJ, Gu Q (2011a) Three new cytochalasins from the marine-derived fungus *Spicaria elegans* KLA03 by supplementing the cultures with l- and D-tryptophan. Chem Biodivers 8:887–894

Wang Y, Zheng J, Liu P, Wang W, Zhu W (2011b) Three new compounds from *Aspergillus terreus* PT06-2 grown in a high salt medium. Marine Drugs 9:1368–1378

Wang Y, Liu L, Wang Y, Xue Y, Zheng Y, Shen Y (2012) *Actinoplanes utahensis* ZJB-08196 fed-batch fermentation at elevated osmolality for enhancing acarbose production. Bioresour Technol 103:337–342

Wijeratne EMK, Bashyal BP, Gunatilaka MK, Arnold AE, Gunatilaka AAL (2010) Maximizing chemical diversity of fungal metabolites: biogenetically related heptaketides of the endolichenic fungus *Corynespora* sp. J Nat Prod 73: 1156–1159

Williams RB, Henrikson JC, Hoover AR, Lee AE, Cichewicz RH (2008) Epigenetic remodeling of the fungal secondary metabolome. Org Biomol Chem 6:1895–1897

Yin Y, Gao Q, Zhang F, Li Z (2012) Medium optimization for the high yield production of single (+)-terrein by *Aspergillus terrenus* strain PF26 derived from marine sponge *Phakellia fusca*. Process Biochem 47(5):887–891

Yu Z, Liu J, Wang F, Dai M, Zhao B, He J, Zhang H (2011) Cloning characterization of a novel CoA-ligase gene from *Penicillium chrysogenum*. Folia Microbiol 56:246–252

Zain ME, El-Sheikh HH, Soliman HG, Khalil AM (2011) Effect of certain chemical compounds on secondary metabolites of *Penicillium janthinellum* and *P. duclauxii*. J Saudi Chem Soc 15:239–249

Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, Gu Q (2011) Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegiceras corniculatum*. Phytochemistry 72:1436–1442

Zheng W, Dai Y, Sun J, Zhao Y, Miao K, Pan S, Zhang M, Wei J (2010) Metabonomic analysis on production of antioxidant secondary metabolites by two geographically isolated strains of *Inonotus obliquus* in submerged cultures. Mycosystema 29:897–910

Zheng W, Zhao Y, Zheng X, Liu Y, Pan S, Dai Y, Liu F (2011) Production of antioxidant and antitumor metabolites by submerged cultures of *Inonotus obliquus* cocultured with *Phellinus punctatus*. Appl Microbiol Biotechnol 89: 157–167

Zuck KM, Shipley S, Newman D (2011) Induced production of *n*-formyl alkaloids from *Aspergillus fumigatus* by coculture with *Streptomyces peucetius*. J Nat Prod 74: 1653–1657