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REVIEW

# *Monascus* secondary metabolites: production and biological activity

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**Abstract** The genus *Monascus*, comprising nine species, can reproduce either vegetatively with filaments and conidia or sexually by the formation of ascospores. The most well-known species of genus *Monascus*, namely, *M. purpureus*, *M. ruber* and *M. pilosus*, are often used for rice fermentation to produce red yeast rice, a special product used either for food coloring or as a food supplement with positive effects on human health. The colored appearance (red, orange or yellow) of *Monascus*-fermented substrates is produced by a mixture of oligoketide pigments that are synthesized by a combination of polyketide and fatty acid synthases. The major pigments consist of pairs of yellow (ankaflavin and monascin), orange (rubropunctatin and monascorubrin) and red (rubropunctamine and monascorubramine) compounds; however, more than 20 other colored products have recently been isolated from fermented rice or culture media. In addition to pigments, a group of monacolin substances and the mycotoxin citrinin can be produced by *Monascus*. Various non-specific biological activities (antimicrobial, antitumor, immunomodulative and others) of these pigmented compounds are, at least partly, ascribed to their reaction with amino group-containing compounds, i.e. amino acids, proteins or nucleic acids. Monacolins, in the form of β-hydroxy acids, inhibit hydroxymethylglutaryl-coenzyme A reductase, a key enzyme in cholesterol biosynthesis in animals and humans.

**Keywords** Monascus · Red yeast rice · Pigments · Monacolin K · Citrinin

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## Introduction

In Asian countries (China, Japan, Thailand, Indonesia, Taiwan, Philippines), filamentous fungi of the genus *Monascus* have been used for centuries for the production of food components, natural pigments and food supplements with positive effects on human health. The most famous product is *Monascus*-fermented rice, also known under a variety of other names, including red rice, red yeast rice, ang-kak, anka, ankak, angkhak, angquac and beni-koji [67].

A particular fungal species, under given conditions, can produce a variety of secondary metabolites (pigments, citrinin, monacolin K and others) that are usually characterized by a polyketide structure and biological activity. At the present time, research in this field is mainly focused on the isolation and characterization of new secondary metabolites [1, 10, 17, 26–28, 30, 63, 72, 82, 86, 89], as well as on the determination of their biological activities [1, 27, 35, 41, 63]. The main aim of this review is to collate available knowledge on *Monascus* secondary metabolites, with emphasis on their production and the spectra of their biological activities.

## The fungus *Monascus*

The genus *Monascus* belongs to the phylum Eumycota, subphylum Ascomycotina, class Plectomycetes, order Eurotiales, family Monascaceae and currently comprises nine species, i.e. *M. floridanus*, *M. pallens*, *M. pilosus*, *M. purpureus*, *M. ruber*, *M. sanguineus*, *M. eremophilus*, *M. lunisporas* and *M. argentinensis*, for which there are a number of synonyms [77]. Most *Monascus* species are homothallic, teleomorphic fungi; an anamorph of the genus *Monascus* is *Bazipetospora*.

Asexual reproduction of this fungus is by the germination of conidia and formation of filaments that branch and form mycelia. The most common types of conidia are aleuroconidia that develop in conidiophores and occur either as single cells or in chains. Chlamydioconidia or arthroconidia are also formed, but only rarely. Sexual reproduction begins with anastomosis of the male antheridium with the trichogyne and female ascogonium and continues with migration of the male nuclei from the antheridium to the ascogonium. Male and female nuclei do not fuse, but the ascogonium enlarges and is encompassed by sterile hyphae that form a protective shield. Ascogenous hyphae inside the ascogonium are divided into cells, and each of these cells contains male and female nuclei; this is the beginning of asci formation. The nuclei only fuse in asci, and nuclear fusion is followed by meiosis and mitosis, resulting in eight haploidic daughter nuclei. These nuclei form the basis of eight ascospores located in the asci. The asci are associated into cleistothecia of varying sizes, which are the true and frequently observed fruiting bodies. Thus, *Monascus* has an ascohymenial development of fruiting bodies during which fruiting body formation occurs at the site of ascogonium fertilization. The development of fruiting bodies occurs simultaneously with the growth of ascogennous hyphae, and asci are formed in primary cavities of the developing fruiting body. The ascus wall is transparent, and after reaching maturity, it dissolves and ascospores are released into the cleistothecium. After rupture of the cleistothecium wall, ascospores are released to the environment [11, 69, 84].

The formation of conidia and biomass growth are stimulated by the presence of organic nitrogen sources in the medium, especially amino acids, although amino acids do suppress sexual reproduction. Nitrates stimulate both ascospore and conidia formation, whereas ammonium ions suppress both types of spores [12]. Red and—to some extent—blue light increase spore formation of *M. purpureus* [59, 80]. A mutual relationship between mycelium differentiation, sporogenesis and secondary metabolism is well described for filamentous fungi of the *Aspergillus* genus [9] but has not been thoroughly explored for the genus *Monascus*.

The genus *Monascus* can be characterized as aerobic, saprophytic, prototrophic, mesophilic (temperature optimum 30–35 °C), weakly xerophilous (growth up to 0.85 water activity), with respiro-fermentative metabolism. It can produce lytic enzymes enabling growth on a spectrum of different substrates, including monosaccharides, disaccharides, starch, pectin and, in the case of *M. ruber*, also cellulose and ethanol [8]. Excess glucose in the culture medium results in ethanol formation under aerobic conditions and, therefore, this fungus can be classified as Crabtree negative with limited respiration [14].

## Production of secondary metabolites

Certain species and strains of the fungus *Monascus* can produce pigments, lovastatin (monacolin K), citrinin, dimerumic acid and γ-amino butyric acid, usually in stationary growth phase.

### Pigments and citrinin: structure and biosynthesis

The main pigments produced by *Monascus* sp., especially *M. purpureus*, *M. ruber* and *M. pilosus*, are six compounds, i.e. monascin and ankaflavin (yellow), rubropunctatin and monascorubrin (orange) and rubropunctamine and monascorubramine (red). The structures of these compounds (see Fig. 1), together with a probable biosynthesis of orange and red pigments by a reaction with amino group-containing compounds, are well documented [33, 46, 53]. The pigments are both polyketides and azaphilones, i.e. they are compounds with an oxygenated bicyclic nucleus and a quaternary center

Although the structures of the major pigments were determined between 1950 and 1970 [13, 20, 22, 24, 37, 53], their biosynthetic pathways have still not been described in detail and remain relatively unknown. A probable biosynthetic pathway for the orange pigment, monascorubrin, that consists of a combination of polyketide and fatty acids was confirmed by experiments with the addition of radioactively labeled octanoic acid to the culture medium [23]. The generation of red pigments by the reaction of orange pigments and amino group-containing compounds has been verified repeatedly [33, 46, 53]; the addition of individual amino acids into the culture medium resulted in differently colored derivatives of rubropunctamine and monascorubramine [31].

In addition to the six main pigments mentioned above, minor pigments have also been isolated from different *Monascus* species cultured under various conditions (Table 1). These include xanthomonasin A [72], yellow II pigment [89], monascopyridines A and B [82], monascusones A and B [30], xanthomonasin B [1], compounds R3 and Y3 [10], monasfluor A and B [28], monafilones A, B, C [26], monarubrin and rubropunctin [52], purpureosone [17], monasnicotinates A, B, C, D [86], a new red pigment [63] and monapurpyridine A [27]. Some of these minor pigments have been found in pairs, having side chains containing six or eight carbons, the same as the main pigments. The most frequently occurring minor pigments are xanthomonasins A and B (furanoisophtalides) [1, 62]. Some of these minor pigments might be intermediates or degradation products of the main pigments, and a possible relationship between monascusones A and B and the major yellow pigment, monascin, has been proposed [30].

The production of the mycotoxin citrinin, also a polyketide compound (see Fig. 2), was described in

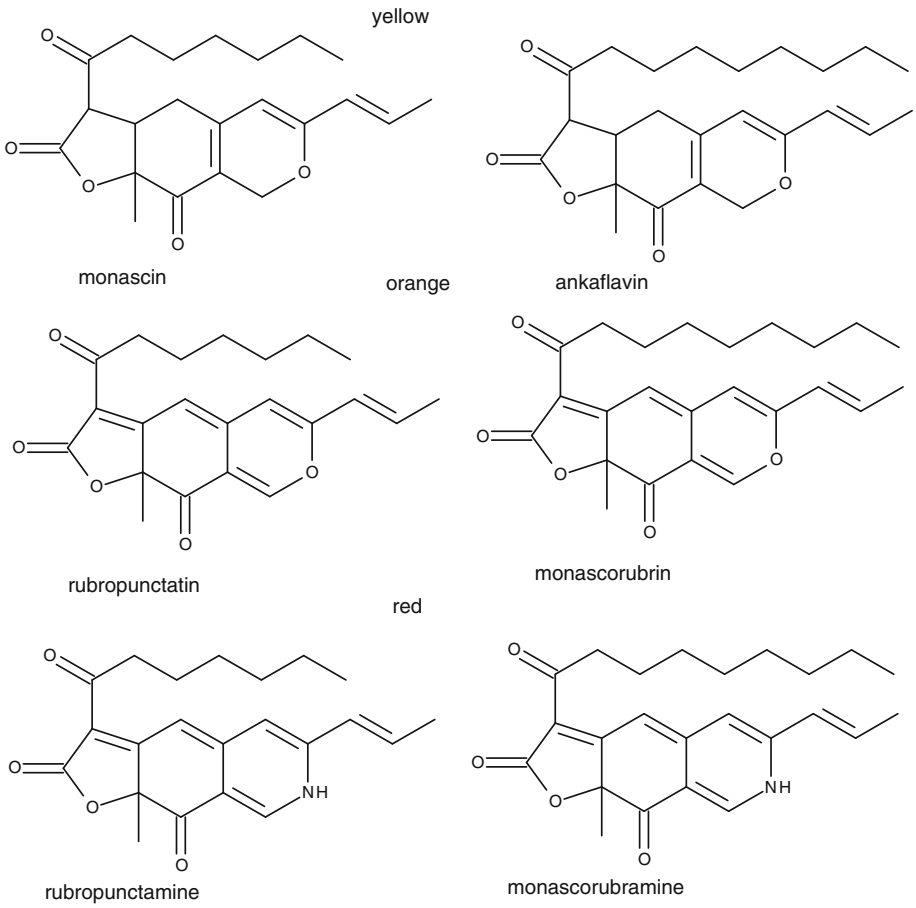



**Fig. 1** Major *Monascus* pigments

*M. purpureus* and *M. ruber* in 1995 [5]. However, it was subsequently shown [15] that the *M. ruber* used in the original study [5] was not classified properly and was in fact *M. purpureus* because the *pksCT* gene for citrinin polyketide synthase is only found in *M. purpureus* and *M. kaoliang* (*M. kaoliang* is a synonym for *M. purpureus*) and not in *M. pilosus*, *M. ruber*, *M. floridanus*, *M. sanguineus*, *M. barkeri* or *M. lunisporas.* Nevertheless, citrinin production in *M. ruber* was later demonstrated by other authors [38, 43].

Most pigments and citrinin are aromatic polyketides, and their biosynthesis consists of repeated Claisen condensations of acetyl-coenzyme A (CoA) and malonyl-CoA units, catalyzed by polyketide synthase type I [74]. In general, signal transfer from the environment to a fungal cell is mediated by a G protein signaling pathway and plays a key role in regulating the formation of fungal secondary metabolites [91]. In *Monascus,* both citrinin and pigment formation are controlled by the α-subunit of signal protein G. If the *Mga1* gene responsible for the α-subunit is inactivated in *M. ruber* M7, then both citrinin and pigment formation would be stimulated [43]. The key role of a signaling pathway of G proteins and signal transfer mediated by a cAMP-activated protein kinase A were confirmed by the addition of cAMP to the culture medium. A correlation between cAMP concentration, ranging from 0 to 10 mmol/l, and secondary metabolite (pigments, citrinin and monacolin K) formation has been found [38]. Smaller concentrations of cAMP (0.5–2 mmol/l) stimulated the production of the metabolites, but higher concentrations (10 mmol/l) inhibited their production [38]. The inhibition of secondary metabolite formation by 10 mM cAMP has also been confirmed in *M. pilosus* IFO4520 and *M. purpureus* IFO4478 [61].

The circadian regulation of the expression of genes coding for carotenoid pigments or conidia formation has been described in *Neurospora crassa* [51, 75]. In *Aspergillus nidulans* [7, 70], phytochrome FphA responding to red light (at a wavelength of approx. 600 nm) was found to influence the formation of conidia, cleistothecia and sterigmatocystine. In *M. purpureus,* pigment formation was observed to be stimulated under total darkness and was completely inhibited under full light [3, 80]. In another experiment, the synthesis of pigments and citrinin by *M. pilosus* and *M. purpureus* was stimulated by red light and repressed by blue light [59].

**Table 1** Selected minor *Monascus* polyketide pigments

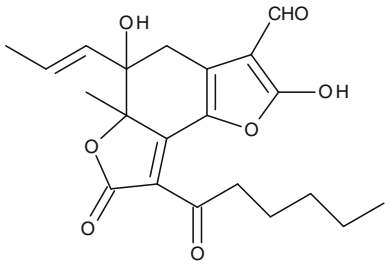
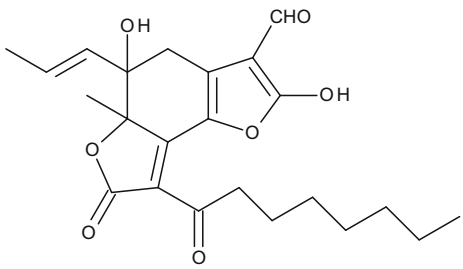
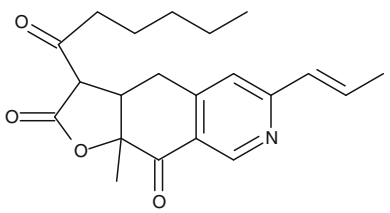
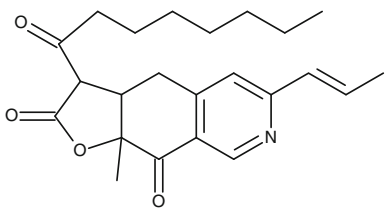
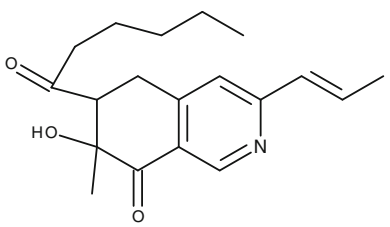
| Structure and name | Production strain and conditions | Reference |
|---|---|---|
|  xanthomonasin A (Y) | *M. pilosus* grown on rice | [1, 72] |
|  xanthomonasin B (Y) | *M. pilosus* grown on rice | [1] |
|  monascopyridine A | *M. purpureus* grown on rice | [35, 82] |
|  monascopyridine B | *M. purpureus* grown on rice | [35, 82] |
|  monascopyridine C | *M. purpureus* grown on rice | [35] |

**Table 1** continued

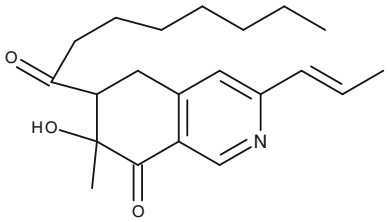
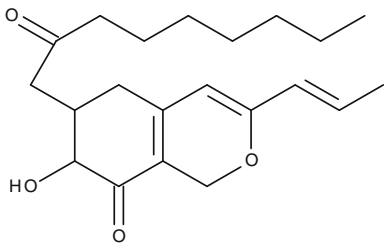
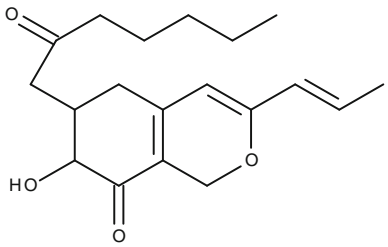
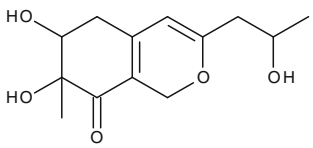
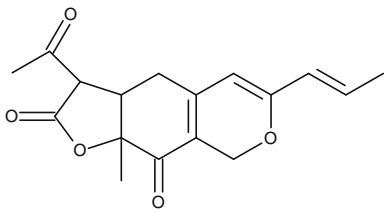
| Structure and name | Production strain and conditions | Reference |
|---|---|---|
|  monascopyridine D | *M. purpureus* grown on rice | [35] |
|  monaphilone A (Y) | Mutant of *M. purpureus* grown on rice | [26] |
|  monaphilone B (Y) | Mutant of *M. purpureus* grown on rice | [26] |
|  monascusone A (Y) | *M. kaoliang* mutant grown on rice | [30] |
|  monascusone B (Y) | *M. kaoliang* mutant grown on rice | [30] |

**Table 1** continued

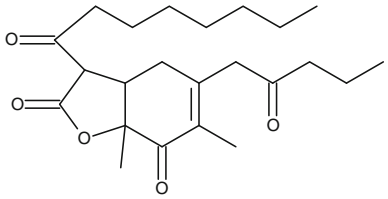
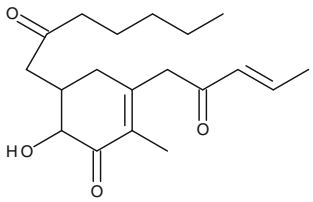
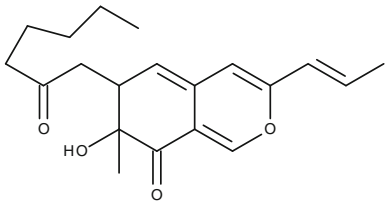
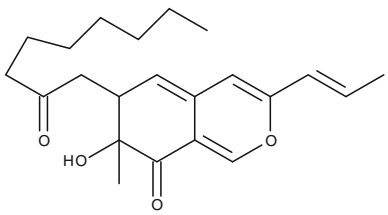
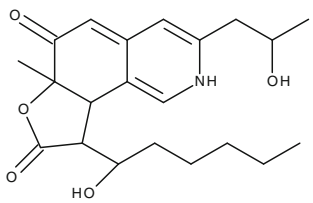
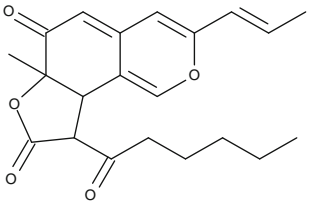
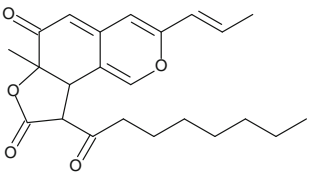
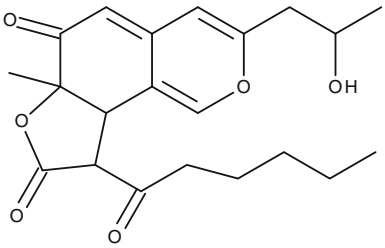
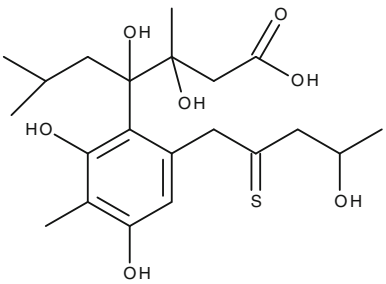
| Structure and name | Production strain and conditions | Reference |
|---|---|---|
|  purpureusone (Y) | *M. purpureus* mutant grown on rice | [17] |
|  monashexenone (Y) | Mutant of *M.purpureus* grown on rice | [26] |
|  monarubrin (Y, BF) | *M. ruber* culture medium | [52] |
|  rubropunctin (Y, BF) | *M. ruber* culture medium | [52] |
|  new red pigment | *M. purpureus* culture medium | [59] |

**Table 1** continued

| Structure and name | Production strain and conditions | Reference |
|---|---|---|
|  monasfluor A (BF) | *Monascus sp.* grown on rice | [28] |
|  monasfluor B (BF) | *Monascus sp.* grown on rice | [28] |
|  compound R3 (R) | *M. purpureus* culture medium | [10] |
|  compound Y3 (Y) | *M. purpureus* culture medium | [10] |

BF, R, Y: color of the compounds, i.e. blue fluorescent, red and yellow, respectively

## Lovastatin (monacolin K)—structure and biosynthesis

Mevastatin was the first of a group of statins isolated in the 1970s from *Penicillium citrinum* medium. Structural analogs of mevastatin, i.e. monacolins J, K and L (Fig. 3), were subsequently described as secondary metabolites of *Monascus ruber* and *Aspergillus terreus* [19]. A gene cluster for lovastatin synthesis was characterized in *M. pilosus*, and this cluster was partly homologous with that of *A. terreus* [16]. The biosynthesis of lovastatin proceeds in a similar way in *A. terreus* and *M. ruber* and requires the action of two polyketide synthases, i.e. lovastatin nonaketide synthase and lovastatin diketide synthase, with the former synthesizing dihydromonacolin L, monacolin L and monacolin J in this order and the latter providing the methylbutyryl-side chain [54].

Lovastatin is a typical secondary metabolite that is produced in the stationary growth phase, and its production is subject to glucose repression. *M. pilosus* was induced to produce lovastatin (725 mg/l) in liquid medium using a mixed substrate of maltose:glycerol (1:7) and peptone as the nitrogen source [60]. With solid substrate cultivation (SSC), an increase in lovastatin formation was induced by adding glycerol, soya meal, acetic acid or $NaNO_3$ to the

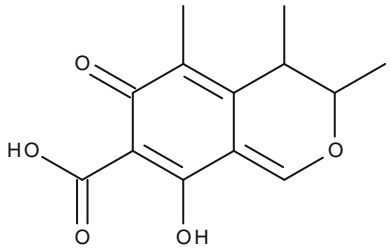



**Fig. 2** Structure of the mycotoxin, citrinin

main substrate, rice [88]. Nevertheless, *A. terreus* strains are usually used in industrial lovastatin production process and the lovastation titer can reach 7–8 g/l [76].

## Culture conditions versus secondary metabolite formation

### Solid substrate cultivation

The most famous *Monascus* product, red yeast rice, can be used as a food coloring (red koji) or as a food supplement, and its cultivation differs according to the intended utilization [67]. A common problem with all red yeast rice cultures is the possible formation of the mycotoxin citrinin, but this can be overcome by selecting a citrinin non-producing strain or by genetic modification of the producing strain [29]. The amount of citrinin in red yeast rice can be also decreased by its extraction with 45 % (v/v) ethanol containing 1.5 % (w/v) phosphate. In one study where this method was applied 92 % of the original citrinin was removed and 80 % of the original monacolin K was preserved [40].

A traditional red yeast rice product for coloring foods such as prepared fish, cheeses, soya products, vinegar, Peking duck or sausages consists of washing, soaking, draining and steaming of a convenient non-glutinous rice variety, followed by inoculation with the fungus, a 7-day culture in a heated room and drying at 45 or 60 °C. During the cultivation, rice kernels are mixed or shaken and moistened if necessary. A critical parameter for red koji cultivation is the water activity ($a_w$) of the substrate. High $a_w$ results in elevated activity of the fungal glucoamylase, which causes a rapid release of glucose from rice starch and ethanol fermentation of the substrate instead of pigment formation. In contrast, low $a_w$ results in poor fungal

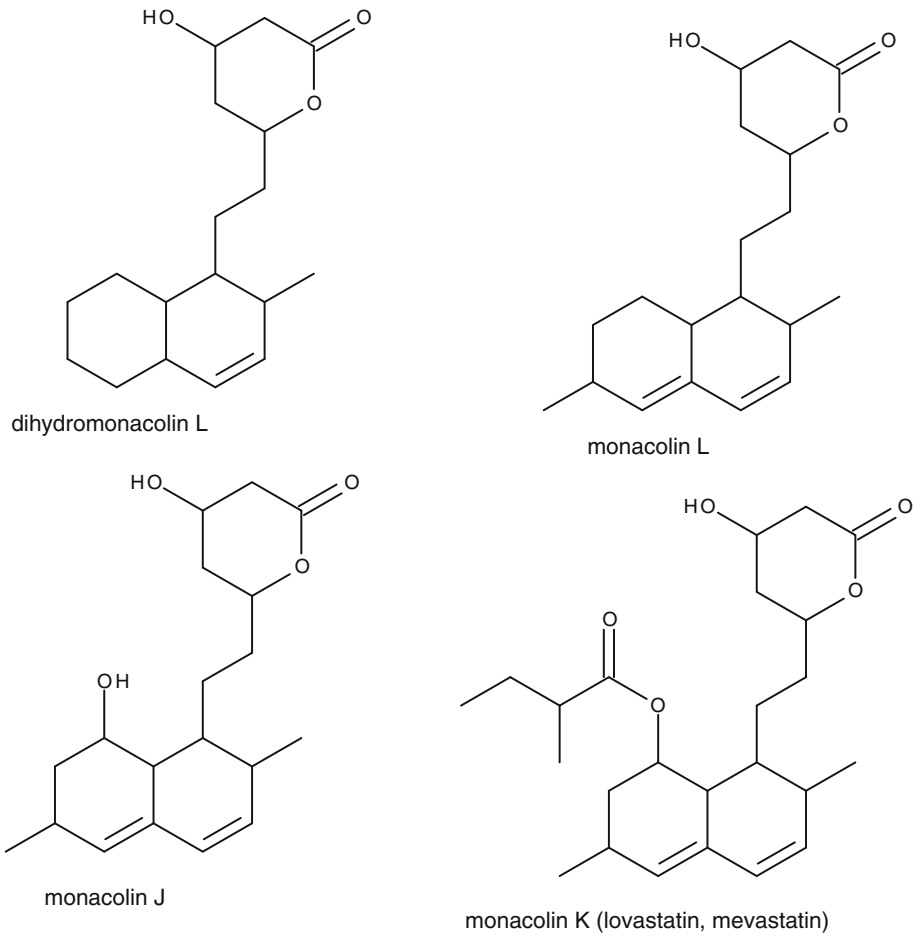



**Fig. 3** Monacolins produced by *Monascus*

growth. It is also important to ensure sufficient oxygen access to the kernels and an outlet for $CO_2$ and metabolic heat by periodic mixing [33, 50]. Nowadays, different types of bioreactors, such as cupboards with trays, rolling drums or fluid beds are used for the production of red yeast rice [18].

In the production of red koji, the culture time is shorter, and the growth of fungal biomass together with the synthesis of hydrolytic enzymes are emphasized. Red koji is usually mixed with normal white koji that is obtained with the culture of *Aspergillus oryzae* on rice fermented to produce sake (which can be further distilled to obtain Kaoliang brandy) or for tofu fermentation into sufu (soya mold fermented cheese) [67].

Red yeast rice food supplements are based on statin compounds, such as monacolin K. The formation of monacolin K by *Monascus* can be stimulated by a suboptimal culture temperature and the addition of either 0.3 % ethanol [81] or $NaNO_3$ [79].

In addition to rice, *Monascus* has been grown on jackfruit (*Artocarpus heterophyllus*) seeds [2], adlay (seeds of *Coix lacryma-jobi*) [68], corn, breadfruit and tubers of *Discorea batatas* [39]. Some of these substrates probably positively affect consumers' health, and after *Monascus* cultivation they could be used as food supplements.

### Submerged liquid cultivation

The main advantage of submerged liquid cultivation (SLC) is the use of a defined culture medium, which permits secondary metabolite formation to be controlled. Both pigments and monacolin K are subject to strict glucose repression [60], which results in ethanol production if the glucose concentration in a medium exceeds 30 g/l [14]. The nitrogen source and the pH of the medium are critical factors affecting pigment production. Organic nitrogen sources, such as yeast extract or peptone, stimulate biomass growth and conidia formation but suppress pigment production. If they are used, free amino acids in the culture medium, at neutral pH, can react with the orange pigments monascorubrin and rubropunctatin to form red-colored complexes of monascorubramine and rubropunctamine, respectively [49]. A similar effect was found when monosodium glutamate was used as the sole nitrogen source [45]. However, in another study, the use of other individual amino acids as sole nitrogen sources for growing *Monascus* sp. cells resulted in worse pigment production in comparison with monosodium glutamate [47]. Nitrates limit growth but stimulate spore and pigment formation. They cause an increase in pH, which stimulates the reaction between orange pigments and amino group-containing compounds, but the reaction is limited by the supply of these amino compounds, the lack of which results in a gain in yellow and orange pigments. Ammonium ions decrease the pH of the culture medium and spore production is inhibited, although pigment production is stimulated. A low pH prevents the nucleophilic addition of amino groups to the oxygen atoms of orange pigments and consequently red pigment formation is limited [78]. The use of ammonium nitrate as the nitrogen source has been found to result in the formation of mainly cell-bound orange pigments by *Monascus sp*. [48].

Ethanol, as a single substrate or co-substrate, can stimulate both pigment and monacolin K formation [32, 81]. Since the production of secondary metabolites usually takes place in the stationary growth phase, it may be possible to split fungal growth and secondary metabolite formation into two distinct phases. Indeed, a two-stage cultivation in which two substrates, maltose and ethanol, were used successively [32] or two-stage cultivation conducted at different pH (5.5 and 8.5) [65] resulted in increased pigment production. Moreover, in the latter study, high pH in the second stage caused the inhibition of citrinin formation and the release of red pigments into the culture medium.

## Biological activity of secondary metabolites

The biological activity of the most famous *Monascus* product, i.e. red yeast rice, has been known for centuries [33, 57, 67].

### Biological activity of *Monascus* pigments

The major *Monascus* pigments structurally belong to a large group of fungal pigments known as azaphilones, which usually exhibit biological activity manifested by the inhibition of different enzyme activities, leading to antimicrobial, anti-human immunodeficiency virus, antitumor, antioxidant, anti-inflammatory or other characteristic activities. This non-selective effect of azaphilones is caused by their reaction with amino group-containing compounds, i.e. amino acids, proteins or nucleic acids. This reaction, in which the oxygen atom in the pyrane ring is exchanged for nitrogen, results in the creation of vinylogous γ-pyridones [33, 57, 66]. In total, there are more than 170 known azaphilones, formed by 23 fungal genera, and most of these exhibit biological activity [66].

The antimicrobial effect of rubropunctatin and monascorubrin on various microorganisms was first mentioned by Wong and Bau [64] and Nozaki et al. [83] and confirmed by Martínková et al. [55, 56]. "New red pigment" [63] also suppresses the growth of Gram-positive bacteria.

Both orange (rubropunctatin and monascorubrin) and yellow (ankaflavin and monascin) pigments have significant

toxic and teratogenic effects on chicken embryos, but they also have immunomodulative effects on mouse T-cells. In contrast, red pigments, or their complexes with amino group containing compounds, and extracts from red yeast rice have neither of these effects [55, 56]. No negative effects of red rice on human health have been described [6, 36, 90]. Nevertheless, cytotoxic effects of pure rubropunctamine, monascorubramine and monascopyridines A and B on immortalized human kidney cells have been reported [35]. Antitumor activity has been reported for yellow, orange and red pigments together with monascumic acids isolated from *M. pilosus* red yeast rice [1], monapurpyridine A [27] and orange pigments [92].

The yellow pigments ankaflavin and monascin were recently found to exhibit similar anticholesterolemic effects as monacolin K [41].

## Biological activity of citrinin

The discovery of citrinin production by *Monascus* [5] resulted in the cessation of efforts to use *Monascus* pigments as food colorants in Europe and the USA. In Asian countries, where the coloring of foods with red yeast rice is traditional, this discovery led to a thorough monitoring of citrinin levels in red yeast rice and the establishment of maximum tolerance limits for citrinin in food. This limit varies in different countries and is 50 μg/kg in South Korea [34] and 200 μg/kg in Japan [21]; the EU recommends 100 μg/kg.

Citrinin is hepatotoxic and nephrotoxic in various animals and humans and is also a probable cause of endemic Balkan nephropathy. In the Ames test for mutagenicity, citrinin exhibited no mutagenic effect, but the pre-cultivation of citrinin with hepatocytes resulted in the formation of a mutagenic citrinin-derived product [71]. Citrinin also possesses antimicrobial activity against Gram-positive and Gram-negative bacteria [58, 85].

## Biological activity of lovastatin (monacolin K) and red rice

In 1987, lovastatin became the first drug isolated from a group of statins with anticholesterolemic effects that was approved by the U.S. Food and Drug Administration (FDA). In general, statins are competitive inhibitors of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis, due to a structural analogy between the β-hydroxy acids of statins and an HMG-CoA intermediate. The affinity of a statin towards HMG-CoA reductase is several fold higher than that of the HMG-CoA intermediate. The hypocholesterolemic effect of statins, i.e. a decrease in total blood cholesterol concentration, is significant several days after the beginning of treatment [54].

In addition to their confirmed anticholesterolemic effect, statins have other positive influences on human health, such as anti-inflammatory activity, improvement in the state of the blood vessels, decreased risk of thrombosis and accelerated healing of fractures. Recent research [76] has also shown a decreased risk of Alzheimer's disease and cancerostatic effects. Negative effects on human health (especially muscle myopathy and kidney disease) are not frequent and are usually reversible [54].

Nowadays, approved statin drugs and food supplements containing varying amounts of different statins are available based on a powder or an extract from red yeast rice [67], either as a sole component or in a mixture with other ingredients. The use of red yeast rice as a food supplement has a demonstrable effect on human health, frequently comparable with that of higher doses of statin drugs [6, 25, 50]. The healing effect of red rice is ascribed to the synergistic action of various components of red yeast rice, i.e. different statins, gamma-aminobutyric acid (GABA), pigments and β-sitosterol. The treatment of cardiovascular diseases with red rice was described in the sixteenth century in the Chinese Pharmacopoeia published (Shi-Zhen Li (1518-1593) [50]). In the USA, some physicians recommend the use of red yeast rice to patients with statin intolerance [4]. A review summarizing the effect of red yeast rice on Alzheimer's disease has recently been published [42], as has a study showing a reduction in an abdominal aortal aneurysm [87]. The main problem associated with use of red yeast rice is a lack of control during and after the culture process because Good Manufacturing Practice is not required in the production of food supplements. This can result in possible citrinin contamination and also varying concentrations of different statins [44]. As a result, in 2007 the FDA warned consumers not to use red yeast rice because it might contain varying amounts of lovastatin, which could damage human health if taken without medical supervision.

*Monascus ruber* is frequently isolated from silage, where it can produce lovastatin and similar compounds that can act as inhibitors of ergosterol synthesis in ruminal cellulolytic fungi such as *Neocallimastix*. As a result of this inhibition, the growth and metabolic activity of these fungi is restricted, leading to poor fiber digestion by cattle [73].

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