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Monascus: a Reality on the Production and Application of Microbial Pigments

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Abstract *Monascus* species can produce yellow, orange, and red pigments, depending on the employed cultivation conditions. They are classified as natural pigments and can be applied for coloration of meat, fishes, cheese, beer, and pates, besides their use in inks for printer and dyes for textile, cosmetic, and pharmaceutical industries. These natural pigments also present antimicrobial activity on pathogenic microorganisms and other beneficial effects to the health as antioxidant and anticholesterol activities. Depending on the substrates, the operational conditions (temperature, pH, dissolved oxygen), and fermentation mode (state solid fermentation or submerged fermentation), the production can be directed for one specific color dye. This review has a main objective to present an approach of Monascus pigments as a reality to obtaining and application of natural pigments by microorganisms, as to highlight properties that makes this pigment as promising for worldwide industrial applications.

Keywords Microbial pigments · Biomolecules · Solid state fermentation · Submerged fermentation · Monascus

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Introduction

Color is added to food to maintain the original food appearance even after processing and during storage, to assure the color uniformity for avoiding seasonal variations in color tone, to protect the flavor and light susceptible vitamins making a light, to intensify normal color of food, and thus to maintain its quality and to increase its acceptability. This attribute is directly associated with several sensory feelings, as appearance, shape, surface, size, and brightness. It is a sensory property that can make food be accepted or rejected immediately by the consumer, even before tasting.

Food colors can be divided into four categories: natural, nature-identical, synthetic, and inorganic colors [1]. The production of synthetic coloring agents and other chemicals used as food additives is under increasing pressure due to a renewed interest in the use of natural products in food formulations and the strong interest in minimizing the use of chemical processes to produce food ingredients [2]. Since the number of permitted synthetic colorants has decreased because of undesirable toxic effects including mutagenicity and potential carcinogenicity, interest focuses on the development of food pigments from natural sources [3, 4].

Natural pigments are derived from sources such as plants, insects, and microorganisms. Actually, companies decided to color food with others food, using mainly plant extracts or pigments from plants from paprika, beetroots, berries, or tomato (red pigments); saffron or marigold (yellow pigments); annatto (orange) and leafy vegetables (green pigments) [5]. Pigments producing microorganisms and microalgae are quite common in nature. Among the molecules produced are carotenoids, melanins, flavins, quinones and more specifically monascins, violacein, phycocyanin, or indigo [6, 7]. Microorganisms have advantages of productivity and versatility over higher forms of life in the production of natural pigments and dyes. Table 1 shows some pigments produced by microorganisms. Filamentous fungi have important properties which play a significant role in the human lifestyle and in the environment, by participating in the production of food and health products, and in recycling of organic compounds in the biosphere [8].

Monascus natural fermented pigments have high economic value around the world and attracted worldwide attention as a coloring agent; they have many advantages such as easily

Table 1 Some natural pigments produced by microorganisms

Natural pigment	Microbial source
β-Carotene	*Blakeslea trispora*, *Dunadiella salina*, *Serratia marcescens*, and *Sporobolomyces ruberrimus*
Actinorhodin	*Streptomyces coelicolor*
Astaxanthin	*Chrorella pyrenoidosa*, *Haematococcus pluvialis*, and *Phaffia rhodozyma*
Canthaxanthin	*Brevibacterium* sp. and *Cantharellus cinnabarinus*
Lutein	*Chrorella pyrenoidosa* and *Spongiococcum excentricum*
Monascus	*Monascus pilosus*, *Monascus purpureus*, and *Monascus ruber*
Phycocyanin	*Cyanidium caldarium*, *Spirulina platensis*, *Synechocystis* sp.
Prodigiosin	*Serratia marcescens*
Violacein	*Chromobacterium violaceum*, *Pseudoalteromonas luteoviolacea*, and *Janthinobacterium lividum*
Zeaxanthin	*Flavobacterium* sp. and *Paracoccus zeaxanthinifaciens*

production on nonexpensive substrates, good solubility in water and ethanol, numerous bioactive metabolites, and completely safe when produced under specific conditions [9].

Monascus species can convert organic substrates into several metabolites such as pigments, alcohols, antibiotic agents, antihypertensives, enzymes, fatty acids, flavor compounds, flocculants, ketones, organic acids, antioxidants and vitamins also can be applied in the cardiovascular diseases, anti-inflammatory, digestive, gastrointestinal, and cancer prevention [10–21].

This review has a main objective to present an approach of Monascus pigments as a reality to obtaining and application of natural pigments by microorganisms, as to highlight properties that makes this pigment as promising for worldwide industrial applications.

Monascus Production

Strains Used to Monascus Production

Monascus spp., a kind of filamentous fungi, isolated from red mold rice (RMR), was classified and named by van Tieghem in 1884. *Monascus* is a genus of Ascomycetes characterized by the production of colorless to pale brown cleistothecia and aleurioconidia. Each cleistothecium is borne from a knot of hyphae on a well defined stalk, in 7-day-old cultures resembling a clenched fist on a narrow forearm. Asci break down rapidly so that, when ascospores are mature, the impression under the microscope is of a sac filled with a mass of ellipsoidal, smooth walled, and refractile spores [22].

More than twenty *Monascus* species are presented in the literature; however, only nine have been internationally recognized in the genus; these are *M. argentinensis*, *M. eremophilus*, *M. floridanus*, *M. lunisporas*, *M. pallens*, *M. pilosus*, *M. purpureus*, *M. ruber*, and *M. sanguineus* [23]. Among them, *M. pilosus* [24], *M. purpureus* [15, 25, 26], and *M. ruber* [27–30] are the most common species used in industrial applications.

Species of the genus *Monascus* (Table 2) have been used as coloring agents for many years in the manufacture of traditional foods (yoghurt, sausages, red wines, tofu, hams, meats, and other products) in East Asian countries [6, 32, 50]. Chinese traditional medicine practitioners utilize RMR to treat abdominal pain due to stagnant blood and dysentery, as well as external and internal trauma [51].

Monascus pigments are a group of fungal secondary metabolites called azaphilones, which have similar molecular structures as well as similar chemical properties. These pigments are produced mainly in the cell-bound state [52]. *Monascus* can produce yellow, orange, and red pigments (Fig. 1). The condensation of one mole of acetate with five moles of malonate in the cytosol leads to the formation of a hexaketide chromophore by the multienzyme complex polyketide synthase. Medium chain fatty acids, for example, octanoic acid, are synthesized by the fatty acid pathway and bind to the structure of the chromophore through a trans-etherification reaction, generating the orange pigment monascorubrin—$C_{23}H_{26}O_5$ or rubropunctatin—$C_{21}H_{22}O_5$ by trans-etherification of the octanoic acid. The reduction of the orange pigment monascorubrin forms the yellow pigment ankaflavin—$C_{23}H_{30}O_5$, or monascin—$C_{21}H_{26}O_5$ for rubropunctatin, whereas the amination of orange pigments gives rise to red pigments monascorubramine—$C_{23}H_{27}NO_4$ and rubropunctamine—$C_{21}H_{23}NO_4$ [8, 10, 32, 53]. Due to the affinity by amine groups, the Monascus pigments are frequently associated to proteins or to the cell wall, forming complex pigments, difficult of being extracted. Several factors such as strain selection [11], substrate [18, 50, 54], pH [55, 56], nitrogen source [15, 44, 54], light intensity

Table 2 Major species used in the *Monascus* pigments production

Process	Strain	Reference
SSF	*Monascus ruber* ATCC 96218	[8, 31]
	Monascus sp. KCCM 10093	[32]
	Monascus ruber LEB A 1-3	[33]
	Monascus purpureus	[34, 35]
	Monascus sp. TTWMB 6093	[36]
	Monascus sp. ATCC 16436	[37]
	Monascus sp. J101	[38]
	Monascus kaoliang ATCC 26264	[39]
	Monascus sp. KB20M10.2	[40]
SmF	*Monascus purpureus* IMI 210765	[2]
	Monascus ruber CCT 3802	[14, 18, 20]
	Monascus purpureus NRRL 1992	[41]
	Monascus ruber 102w	[42]
	Monascus sp. B683	[43]
	Monascus ruber	[44]
	Monascus purpureus CCM8152	[45, 46]
	Monascus ruber ATCC 96218	[47, 48]
	Monascus pallens, *Monascus ruber*	[49]

[29, 57, 58], temperature [59], broth rheology [14, 59], and oxygen [20] can influence the red pigment production by *Monascus*.

Monascus Production Process

Several review manuscripts have compared the solid state fermentation (SSF) and submerged fermentation (SmF); however, some important questions should be answered before the choice of the conduction form of the fermentative process: The culture medium is synthetic or complex? Which substrate will be used? Which will be the application of the biomolecule? The biomolecule of interest needs extraction and purification? The extraction drags undesirable substances? How will be the treatment of the residues generated during the process?

Monascus sp. is traditionally cultivated on solid media, rice grains or bread, though such SSF does not enable the environmental parameters to be controlled and SmF in natural or synthetic media have been developed [8]. Agroindustrial residues have carbohydrates, proteins, lipids, and vitamins, being considered excellent substrates to the development of

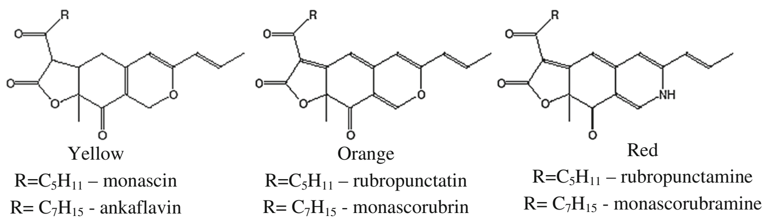

Fig. 1 Major chemical structures of the major Monascus pigments compounds

microorganisms and, consequently, to the achievement of biomolecules of industrial interest by SSF. Rice bran [60], apple pomace [61, 62], cassava [10], wheat, and many others have been used for biomolecules production from biotechnology techniques. In general, substrates for solid state fermentation (SSF) are composite and heterogeneous products from agriculture or by-products of agro-industry.

SSF consists in a fermentative process of low water content, characterized by the absence of dripping. Filamentous fungi have extreme capacities of adaptation, colonize solid substrates with facility by the hyphae penetration on the solid support, and grow in low water content, besides producing concentrated products. The temperature, pH, oxygen concentration, and homogenization of the culture medium are parameters difficult to control when compared to SmF. Generally, the SSF is advantageous when there is no need to separate from the solid matrix the produced biomolecules; in the case of pigments production, the ideal is that the pigments are used together with the solid matrix. The production of pigments from *Monascus* species are generally performed by SSF.

On the other side, the SmF is carried out with an excess of water, where the culture media are liquid, facilitating the mixture and the control of operational parameters as temperature, pH, dissolved oxygen, heat transfer, among others. The biochemical potential, ease of adaptation, and the versatility of fungi applications, as the case of *Monascus*, make possible its cultivation in submerged medium with different substrates, synthetic, or complexes.

The *Monascus* pigment produced by SSF is an unpurified product because of low productivity, high labor cost, and the control problems in the solid state fermentation; the method is not suitable for large-scale industrial production [63]. Therefore, the utilization of submerged fermentation for the production of *Monascus* pigments has been studied to overcome the problems of space, scale-up, and process control of solid culture. In the same way, the use of submerged culture can benefit the production of many secondary metabolites and decrease production costs by reducing the labor involved in solid-state fermentation [27, 64].

The production of Monascus pigments by SmF has been investigated using glucose as main substrate. Red pigments have been obtained from growth in submerged culture of glucose [27], corn syrup [65], wheat flour [2], shrimp flour and crab shell [66], glycerol [18, 28], pear juice [35], cassava starch [67], grape waste [68], and ethanol [69].

Monascus produces three kinds of described polyketides: citrinin, red pigments, and monacolin K. Studies about the relation between the pigments production and citrinin by species of *Monascus* are contradictory; however, many efforts have been carried out to minimize the production of citrinin in *Monascus* cultivation. The form of conduction, the addition of nitrogen compounds as amino acids to the cultivation medium, concentration of dissolved oxygen, pH, and genetic alteration and manipulation have been applied to minimize the production of this toxic compound in cultivation of *Monascus* species.

Citrinin (IUPAC (3R,4S)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3 H-2-benzopyran-7-carboxylic acid) is a mycotoxin produced as a secondary metabolite by several fungal species, including *Monascus* and *Penicillium.* Generally, citrinin is present in a trace concentration of 0.1 to 500 mg kg^{-1} of RMR, compared with other *Monascus* metabolites [70]. According to the results of Lee et al. [43], citrinin did not reveal any nephrotoxicity and hepatotoxicity effects in animals of 1 to 200 ppm of citrinin in the RMR.

Studies indicate that high pigment production can be achieved without citrinin synthesis by incorporating histidine in the growth media [8, 71, 72]. Wang et al. (2005) surveyed type cultures of 23 species of *Monascus* for their ability to synthesize citrinin and found that all, including *M. ruber*, produced citrinin. Hajjaj et al. [49] studied the amino acids as sole nitrogen sources to examine their effects on the production of water-soluble red pigments and citrinin by *Monascus ruber* ATCC 96218. Histidine was found to be the most valuable amino acid as it resulted in the highest production of red pigments and almost completely eliminated the formation of mycotoxin.

Biochemical and genetic studies can be employed to prevent or minimize the amount of citrinin produced. The first step was demonstrated by Hajjaj et al. [48] (Fig. 2) and affirms that the citrinin biosynthesis is originated from a tetracetide rather than a pentacide, as found in studies with *Aspergillus terreus* and *Penicillium citrinum*.

Tools as molecular biology by the study and discovery of genes responsible for the synthesis of citrinin have been an advance to minimize the production of this secondary metabolite. Shimizu et al. [73] studied the polyketide synthase gene responsible for citrinin biosynthesis in *Monascus purpureus*. A polyketide synthase gene *pksCT* involved in citrinin biosynthesis has been successfully cloned from *M. purpureus* which provides us a basis to modify the industrial strain *M. purpureus* SM001 through this potential method. Jia e al. [74] eliminated the citrinin production of a industrial strain of *Monascus purpureus* SM001 by genetic manipulation where a binary vector system was constructed and successfully disrupted the polyketide synthase gene *pksCT* in *M. purpureus* SM001 through the *Agrobacterium tumefaciens* mediated transformation.

Aqueous two-phase extraction system (ATPES) has been studied as an alternative process for citrinin recovery, cost reduction, and decreasing the number of purification steps [75]. Pimental et al. [76] studied the parameters for citrinin extraction from *Penicillium citrinum* fermentation broth using aqueous two-phase systems in discontinuous and continuous modes with 98.44 % of citrinin recovery.

Monascus Properties

Pigment

Since some pigments of *Monascus* spp. are unstable at high temperatures, light, presence of oxygen, metal ions, and pH changes, they can be applied to coloring pork, poultry, fish,

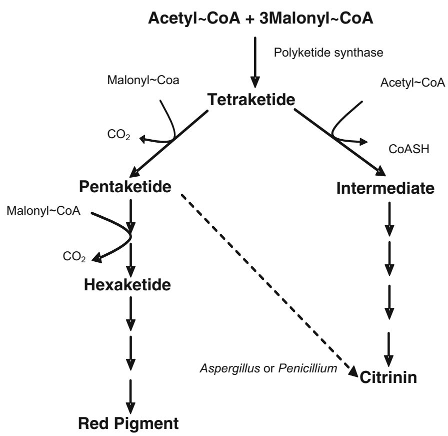

Fig. 2 Synthesis of citrinin and red pigment in *Monascus ruber*. The toxin pathway in *Aspergillus* and *Penicillium* is indicated by the *dashed arrow* (Hajjaj et al. [48])

and also in tofu to enhance the flavor and preservation of these products [77]. They appeared to be viable substitutes for nitrite salts and nitrate traditionally added to enhance flavor and color in meat products. Food applications, such as coloring processed meat, ham, sausage, fish paste, beer, and ketchup are described in the literature. Sensory evaluations of meat products (Toulousan sausage, dry sausage, Strasbourgan sausage, and liver pâté) colored by Monascus pigments showed differences between food colored whit conventional additives. Food containing natural pigments was preferred because of better flavor and texture [78].

Antimicrobial Activity

Today, there is a significant consumer demand for foods that are minimally processed and free from synthetic chemical preservatives with the perception of being "natural" [79, 80]. Toxicological problems associated with the use of certain synthetic food antimicrobials have generated interest in the food industry for use of naturally occurring compounds [81]. The mechanism of antimicrobial effect of pigments produced by *Monascus* sp. is unclear but is estimated to occur through various ways such as reaction with enzymes from germinated spores and vegetative cells, restricting the use of iron and interface with cell membrane permeability, reducing transport of nutrients and metabolites. Therefore, specific modes of action of pigments such as produced by *Monascus* species with antimicrobial properties on the metabolic activities of microorganisms still need to be clearly defined.

The antimicrobial activity of Monascus pigments has been the focus of some researchers. Vendruscolo et al. [27] studied the antimicrobial activity of Monascus pigments extracts produced by *Monascus ruber* CCT 3802 from submerged fermentation against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis*. The orange pigment extract presented antimicrobial activity against *S. aureus* ATCC 25923 and red pigments extracts against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. *S. enteritidis* ATCC 13076 was not inhibited for orange and red pigments extracts. Martínková et al. [82] studied the antimicrobial activity of the pigment extracts from *Monascus purpureus*. The antimicrobial activity was influenced by the culture medium composition and growth conditions: when amino acids, peptides, or proteins were available during cultivation, as in the case of solid-state cultivation on rice, wheat, or pearl barley or submerged cultivation with an organic nitrogen source, the bioactive compounds were converted into inactive complexes and, to a lesser extent, into purple pigments which retained some biological activity. Wong and Koehler [83] studied the culturing, purification, and isolation of the antibiotic produced by *Monascus purpureus* N11S against *Bacillus subtilis*. Solid medium, without shaking, favored antibiotic production with maximum activity after about 15 to 17 days of incubation, and media containing 10 to 15 wt% glucose were most suitable for antibiotic production. Xu [84] studied the antimicrobial activity of pigments produced by *Monascus* M3428 from corn flour as substrate against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, yeast, and *Mycete*. Monascus pigments had strong inhibition against *Bacillus subtilis*, had weak inhibition function against *Escherichia coli* and yeast, but had no inhibition function against *Staphylococcus aureus*. Kim et al. [85] studied the antimicrobial activity of amino acids derivatives pigments produced by *Monascus* sp. KCCM 10093. Against *Escherichia coli*, the hydrophobic L-tyrosine and L-phenylalanine derivatives exhibited high antimicrobial activities with Minimal Inhibitory Concentration values of 8 and 16 $\mu g\ mL^{-1}$, respectively, and the hydrophilic L-glutamine and L-asparagine derivatives exhibited low activities.

Thermal Stability

Color degradation is common for natural pigments and is therefore a major concern in coloring foods, frequently compensated by the proper dosage of the pigment [11]. However, replacing synthetic dyes with natural colorants (carotenoids, anthocyanins, chlorophylls, monascins, and other) offers a great challenge, due to the greater stability of synthetic dyes with respect to temperature, pH, and emerging technologies such as high pressure, among other factors [6, 11].

According to Fabre [78], sauces and pâtés colored with red *Monascus* pigments show a residual color of 92 to 98 % after 3 months at 4 °C, with good sensorial acceptance. However, these pigments are unstable towards light (only 20 % residual color after 50 days) and heat (45 % residual color after 2 h at 100 °C). These pigments are more stable under basic or neutral pH (Fabre, 1993). Carvalho et al. [11] studied the stability of red pigment produced by *Monascus*. The pigments obtained were assayed for their stability towards pH and temperature, and it was found that these pigments are unstable at low pH values and high temperatures, so that they should be used in applications in processes with temperatures inferior to 60 °C and pHs near neutrality. Recently, Vendruscolo et al. [64] determined the thermal stability of orange and red pigments produced by submerged fermentation of *Monascus ruber* CCT 3802. The red pigments presented higher thermal stability under alkali pH values and the orange pigments under acid pH values. The activation energies of the red and orange pigments were 11.49 and 12.65 kcal mol^{-1}, respectively. These results are of extreme importance for knowledge of the thermal properties of natural compounds to be applied in food formulations.

Antioxidant Activity

In addition to their cholesterol-lowering effect, *Monascus* sp. fermented products also have antioxidant activities [12, 13, 86]. Monascus products with antioxidant properties might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage [13].

Tseng et al. [12] studied the antioxidant properties of a Monascus product obtained by inoculating the fungus *Monascus* into cooked adlay. They observed that Monascus adlay products exhibited higher antioxidant activity, reducing power, scavenging and chelating abilities, and higher in total phenol content that uninoculated adlay products. Rice products inoculated with *Monascus* fungus also exhibited antioxidant activity according to the study of Yang et al. [13]. The antioxidant activity of *Monascus*-fermented soybean was verified by Pyo and Lee [86]. They suggest that *Monascus*-fermented soybean may be used as natural and multifunctional dietary food additives or supplements due to its effective activities on free radical scavenging and angiotensin I-converting enzyme.

A nutraceutical with greater anti-atherosclerotic value can be created by the levels of the antioxidant capacity of *Monascus* sp. fermented products. In order to increase the nutraceutical value of *Monascus* sp. fermented products, Kuo et al. [87] showed that modification of culture medium by addition of ginger juice significantly enhanced the antioxidant activities of *Monascus pilosus* fermented products.

Health Benefits

Recent studies confirm that *Monascus*-fermented products are not due to any adverse health effect. Choe et al. [88] studied 47 amine derivatives of *Monascus* pigment produced via a series of fermentation and chemical derivation on anti-obesity activities using cellular and mice tests. Among the derivatives, 16 derivatives showed an inhibitory activity against adipogenic differentiation in 3T3-L1 cells. Reductions in the weight gain and epididymal weight of mice are known to affect the amounts of lipids in serum, e.g., triglyceride, total cholesterol, and HDL and LDL cholesterol.

Monascus-fermented rice functions in invigorating spleen and digestion, promoting blood circulation and resolving blood stasis. γ-Aminobutyric acid existing in *Monascus*-fermented rice works well in preventing hypertension as a hypotensive agent. Also, γ-aminobutyric acid that is a natural inhibitory neurotransmitter in the central nervous system and essential for brain metabolism and function in vertebrates improves visual cortical function in senescent monkeys and signaled the nitrogen uptake in plants [89].

Statin drugs, which are widely known as cholesterol-lowering medications, are among the most widely prescribed drugs in the world with annual sales of tens of billions of dollars. Commercially lovastatin is produced by a variety of filamentous fungi including *Penicillium* species, *Monascus ruber*, and *Aspergillus terreus* as a secondary metabolite. Production of lovastatin by fermentation decreases the production cost compared to costs of chemical synthesis. In recent years, lovastatin has also been reported as a potential therapeutic agent for the treatment of various types of tumors and also play a tremendous role in the regulation of the inflammatory and immune response, coagulation process, bone turnover, neovascularization, vascular tone, and arterial pressure [90]. Lovastatin is the first reported in *Monascus ruber* and by competitively inhibiting HMG Co-A reductase. Monacolin M, a specific inhibitor of cholesterol biosynthesis structurally related to monacolin K (mevinolin), was isolated from cultures of a strain of *Monascus ruber*.

Final Remarks

From the pointed out in this review, it is possible to verify the potentiality of this natural pigment that beyond its dyeing power presents several properties important to the industry. It is worth to mention that Monascus pigments have been the research target for application in food industry, but the pharmaceutical, textile, and cosmetic industries also can make use of these promising biomolecules. The *Monascus* is a versatile fungus, since it has facility of adaptation to adverse conditions, being cultivated both in solid and submerged fermentation, characteristic that helps the reduction of costs of lab and industrial processes of production and downstream, depending on the destination, application, and purity necessary. The employment of molecular biology and genetic alterations of *Monascus* species as well the study of biotechnological processes will contribute to the advance of this promising research area.

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