

Research & Reviews: Journal of Pharmacology and Toxicological Studies

Pharmacological Products and Pigments from *Monascus* Sp by Solid State Fermentation

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Review Article

Received: 26-08-2016

Accepted: 29-08-2016

Published: 05-06-2106

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Keywords: Pigments, *Monascus* sp, Solid state fermentation

ABSTRACT

The metabolites produced by the microbes are having many beneficial usage in health care systems, textile and food industries. Recently natural pigments are encouraged above synthetic ones to overcome the hazardous effects of the later on mankind as well as the environment. Traditionally, colorants or pigments were naturally extracted from plants and animal skin. Then the era of synthetic dyes were prevalent for its availability, low cost extraction and effectiveness. But gradually it started to have dangerous results on the environment. Hence, researches on natural pigments from natural sources such as microbes are in vogue now as microbes are easily available and fast growing. Productions of antibiotics and other pharmacological substances were known from the past. Numerous microorganisms, including bacteria, fungi, yeast and mold etc. are utilized for the industrial production of various pigments, medicine, enzymes etc. by using fermentation technology. Solid state fermentation and submerged fermentation are followed for the production of the pigments from the microbes. Here the review gives a brief view about the various types of pigments that can be produced, different substrates used as a platform for the microbial growth and the factors such as pH, temperature, solvents, light, nitrogen source etc., affecting the color intensity and production.

INTRODUCTION

Pigments are secondary metabolites defined as substance that changes the color of reflected or transmitted light as the result of wavelength-selective absorption. Due to the reflection and absorption of specific wavelengths

of visible light, pigments appear as the colors that can be seen. Pigments are mostly colored, organic or inorganic solid powder, and generally are insoluble.

Normal colors are an imperative contrasting option over potentially harmful synthetic dyes. Since numerous sorts of synthetic colorants have been observed to be dangerous to human wellbeing, just restricted sorts of such dyes are allowed to be utilized in food in numerous nations, and in this way, there is a need to create alternative sources of natural food colorants.

There are various regular colorants, however just a couple of accessible in adequate amount are of industrial use since they are specifically extracted from plant flowers, fruits, leaves and roots [1]. It is consequently profitable to produce natural coloring agents from microorganisms, such as, from *Monascus* [2,3], *Streptomyces* [4], and *Serratia* [5]. Pigments produced by the fungi *Monascus* spp. have been generally used in Asia for colouring and preserving various fermented foods [6]. Moreover, their remedial properties and their moderately high strength concerning pH and temperature are intriguing elements that advance their utilization as substitutes for synthetic colorants [7]. *Monascus* sp can produce six major related pigments [8], which can be divided into three groups: two are orange (rubropunctatin and monascoubrin), two are yellow (monascin and ankaflavin) and two are red (rubropunctaminea and monascorubramine). Particularly the red pigment is of much interest as it is most suitable for the colouring of food [9].

Monascus rice products (MRPs) are now being used as health foods in the United States as well as Asian countries such as Japan, Taiwan, China, Korea, Thailand, the Philippines, and Indonesia. It has been reported that *Monascus* spp. produces profitably viable metabolites, including food colorants, cholesterol-lowering agents, and antibiotics [10].

Nowadays food industries, pharmaceutical industries and textile industries use microbial pigments. β -carotene, Arpink Red, Riboflavin lycopene and *Monascus* pigments are used as colourants in food industry. Pigments like Anthocyanin, Prodigiosin and Violacein are widely used to treat diseases in pharmaceutical industry and several microbial pigments are also used in textile industry [11].

Two main natural fermentation processes are carried for the pigment production namely, solid state fermentation and submerged fermentation, where various natural solid and liquid substrates are used.

MONASCUS SP

Monascus belongs to the mold family. Various strains of the *Monascus* sp has been found and utilized but the red-pigment produced by *Monascus purpureus* is considered as the most important because of its application in fermented foods in East Asia, mainly China and Japan. Health care systems too include production of various pharmacological substances such as antibiotics, antiatherosclerotics, antidiabetics, enzymes, etc. Some of the strains are *Monascus argentinensis*, *Monascus eremophilus*, *Monascus floridanus*, *Monascus lunispora*, *Monascus pallens*, *Monascus pilosus*, *Monascus purpureus*, *Monascus ruber*, *Monascus sanguineus*.

PIGMENTS FROM MONASCUS SP

Pigments are produced as secondary metabolites when the starch substrate is broken down by the microbes. Various studies report that microorganisms of the genus *Monascus* produce red pigments that can be used as coloring agents in food and textile industries^[12-14]. Coloring of few food items in several Asian countries is done by the pigments produced by the *Monascus* species, grown on rice grains^[15]. This fermented mass, known as ang-kak, is dried and ground to powder which is directly used as a colouring agent.

Monascus produces red biopigments which are important alternatives for the synthetic pigment such as erythrosine^[16]. These biopigments are stable in temperature and pH (in the range of 2-10)^[17]. Substrates required for the production of *Monascus* biopigments are very diverse, ranging from defined compositions to natural substrates. Extraction of the pigments can be done by various solvents to obtain it in concentrated form.

PHARMACOLOGICAL PRODUCTS FROM MONASCUS SP

Various pharmacological compounds such as enzymes, antibiotics, anti-diabetics, anti-inflammatory, inhibitory neuronal signal transmitters etc are being produced by microbes. An effective remedy for the inhibition of the HMG-CoA reductase causing osteoporosis is also found in the microbial secondary metabolites^[18].

Solid-state fermentation of cooked non-glutinous rice with *Monascus* species is done to produce a traditional Chinese medicine called Angkak (known as red mold rice, red yeast rice, Chinese red rice). The secondary metabolite produced by *Monascus sp*, monacolin K/lovastatin, has proven to be effective in lowering blood lipid levels^[19]. Monacolin K is an important bioactive compound isolated from *Monascus* which is identical to the potent cholesterol-lowering, antiatherosclerotic drug lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. Few species of *Monascus* produces citrinin, a mycotoxin, harmful to the hepatic and renal systems. Monacolin K and citrinin are polyketide fungal metabolites.

MRPs are used as health foods with a concern for safety and recent studies reported that MRPs contain beneficial substances like flavonoids, polyunsaturated fats, phytosterols, pyrrolic compounds etc. with a wide variety of biological activities and pharmacological potentials. Their effects in lowering blood sugar and triacylglycerol while increasing HDL-C are higher than that of monacolin K. Apart from lowering cholesterol, MRP may also be used for the treatment of metabolic syndrome^[10].

SOLID STATE FERMENTATION AND THE NATURAL SUBSTRATES

Solid state fermentation (SSF) has developed as an effective option for liquid, culture-based fermentation technology. The substrates used in SSF provide with the basic nutrients for the growth of microorganisms and serve as an anchor for the cells^[20]. Recent studies reported that SSF provides a more sufficient environment for fungi, resulting in high pigment production in a relatively low-cost process when agro-industrial wastes were used as

substrates. Agro-industrial wastes such as rice bran, wheat bran, coconut oil cake, sesame oil cake, palm kernel cake, groundnut oil cake, cassava powder, spent brewing grain, and jackfruit seed powder have been screened to select the best substrate for pigment production [20]. Corn cob substrate, i.e the central core of the maize was also used to grow *Monascus purpureus* [21]. Moreover okara (soya bean extract), sweet potato, sugarcane bagasse, rice, wheat, corn, soy, soy bran, cassava, cassava starch, cassava flour, cassava bagasse, potato has been used as carbon source substrates [22].

The cultivation of *Monascus* in solid-state fermentation (SSF) over steamed rice is excellent. Apart from rice there are other natural substrates that show same or even higher amount of carbohydrates and proteins, and are considered as good sources of C and N for the fungal growth. There are many cheap by-products and residues of food processing that might be used as substrates for *Monascus* fermentation. Such substrates have shown favorable results in the production of other metabolites in SSF [23]. It has been reported that some other raw materials such as cassava starch used as substrate for *Monascus* [24-26], prickly pear juice [27], and dairy milk [28]. Nutrient supplement such as vitamins and organic nitrogen are essential for the growth of microbes. The supplements that are introduced include sugars (glucose), micronutrients and organic nitrogen sources (amino acids, peptones) or inorganic nitrogen (ammonium nitrates) [29].

Pigment production by the *Monascus purpureus* on rice under solid state culture have been treated with various nutrient (nitrogen) sources such as peptone, NH₄Cl, MnSO₄.H₂O, dextrose, malt extract, was well described in a recent work where production showed effective results [30].

EXTRACTION

Pigments can be extracted when full microbial growth can be observed in the solid state culture. The spores of the microbes on the substrates were removed or killed by the autoclaving process and finally the substrates with the colored pigments were taken for the extraction. Various organic and inorganic solvents such as ethanol, methanol, dimethyl sulfoxide (dmsO), hexane, ethyl ether, ethyl alcohol, methyl alcohol and acetonitrile and water (deionized) can be used for the extraction process [31].

EFFECTS OF VARIOUS FACTORS

As reported in a study, many aspects affected the red rice production by *Monascus purpureus* and it was seen that rice polished for 3 minutes gave highest pigment with the concentration of 5.40 units (OD 500) whereas neither addition of nitrogen sources nor any metals stimulate the pigment production with temperature maintained at 30-35 degree celcius and 74% relative humidity [32].

Few studies suggested that pH environment created by ammonium salts were not responsible but ammonium salts itself affected the production of *Monascus* pigments and the growth of *Monascus anka*. It was seen that the inhibition effect of ammonium ion on few enzymes for *Monascus* red pigments synthesis is stronger than the *Monascus* yellow pigments synthesis [33].

The red pigment production by *Monascus purpureus* was being carried out with pH maintained at 6.0 with 0.1M HCl or NaOH and autoclaved for 20 min at 121°C and the rice was cooled based solid medium, inoculated with 10% *Monascus* seed and incubated at 30°C, 70% relative humidity for 14 days in a humidity chamber for solid-state fermentation which gave better results^[34].

Therefore external factors shows effects on the growth of the microbes and also its pigment production which can be favorable or maybe not.

CONCLUSION

The research on production of pigments from microbes can effectively overcome the usage of synthetic dyes or colorants and its hazardous effects. Moreover agro-industrial wastes and residues can be an alternative option for *Monascus* pigment production such as the use of corn cob, soya extracts, sugarcane bagasse etc. as substrate that are cost effective and environmental friendly. The factors like pH, temperature, solvents, nutrients, light etc. can be maintained to provide the favorable conditions for growth of microbes and the pigment production. Microbial extracts are not only used as food colorant, flavoring agent and preservative but also widely applied in therapeutic aspects. By encouraging these researches and the application of such natural colorants from plants and microbes in various food and textile industries can bring about a new dawn to a healthy and friendly environment.

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