The role of carbon and nitrogen sources in the production of bioactive compounds in Monascus fermentation products: a mini review

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Abstract. Angkak is one of the Monascus fermented products with rice as a substrate. In addition, Chinese yam, potatoes, soybean, ginseng and onions can be used as a substrate for fermentation products by Monascus sp. It was known that the fermented product by Monascus sp. produces several bioactive compounds that have antihyperlipidemic activity. These bioactive compounds are monacolin-K and pigment compounds (monascin and ankaflavin). Each of these compounds has different cholesterol inhibitory activity. The production of these bioactive compounds is strongly influenced by the nutritional composition of the fermentation media. The addition of the right carbon and nitrogen sources can accelerate the production of bioactive compounds by Monascus sp.

1. Introduction
Fermentation by Monascus sp. has been widely used throughout the Asian region. Fermented products by Monascus sp. are known as antihyperlipidemic agents [1]. Angkak, one of the Monascus sp. fermented products known in Asia, is rice fermentation by Monascus purpureus. Metabolite produced during the fermentation process of Angkak is monakolin-K which has an identical structure to Lovastatin [2]. Lovastatin is widely used as a cholesterol drug, produced from the fermentation process by Aspergillus terreus [3, 4]. Lee et al. have developed a fermentation product by Monascus that produced higher monacolin-K and monascin than angkak, Red Mold Dioscorea [1].

Monascin and ankaflavin are yellow pigment compounds produced by Monascus sp. during the fermentation process. Both compounds are known to have an antihyperlipidemic effect in wistar rats. The mechanism of antihyperlipidemia in both compounds is known through an increase in LDLR and an increase in ApoA1 protein. Many yellow pigment compounds are produced from the fermentation of Monascus sp. with the dioscorea group of substrates. Dioscorea sp. belonging to the Dioscoreaceae family contains more than 70% starch and high amylose so that it can be easily utilized by microorganisms as a substrate. In addition, Dioscora sp. also contains several amino acids such as...
aspartic acid, glutamic acid, leucine, glycine and others that can increase the growth of Monascus sp. [1, 5, 6]. Mucilage found in Dioscorea can be used as a carbon and nitrogen source that has the potential to accelerate the growth of Monascus sp. This causes an increase in the production of Monakoline-K compounds through the polyketide biosynthesis pathway.

Several factors that affect the growth of Monascus sp. Among them are sources of nutrients in the form of nitrogen sources and carbon sources, temperature, and pH. The availability of nitrogen sources determines the biomass production and the quantity of pigment compounds produced by Monascus [7]. Carvalho et al. stated that the production of Angkak supplemented with a nitrogen source can increase the intensity of the color pigment produced and can increase the production of mold biomass. It is known that the protein content in rice is 5-8% so that by adding a source of free N can increase the growth of mold. The higher the addition of N sources can reduce the production of red pigment Monascus sp [8]. Ammonium chloride is the best source of N in the production of Monascus sp pigments, then ammonium nitrate and glutamate [9]. In addition to nitrogen sources, carbon sources are one of the factors that can affect the growth of Monascus sp. Some carbon sources that can be added include glucose, fructose, maltose, lactose, and galactose. Besides being able to increase pigment compounds and biomass, the right carbon source can produce high secondary metabolites [9]. The addition of a C source of 50g/L can increase the growth rate of molds, increase the synthesis of pigment compounds, cell biomass and ethanol production [10].

Fermented product by Monascus sp. has been developed every year. Rice is not the main substrate in the fermentation by Monascus sp. Soybean, garlic, ginseng, potato, cassava to dioscorea tubers are currently widely used as substrates. The diversification and supplementation of this substrate aim to find the most appropriate media for use in fermentation by Monascus sp. to produce more secondary metabolites and lower mycotoxins. This mini-review explains the role of carbon and nitrogen sources in the production of bioactive compounds by monascus fermented products.

2. Monascus fermented product
Fermented products by Monascus sp. can be produced through two fermentation methods, solid fermentation, and liquid fermentation. In addition to Angkak, researchers are currently also developing fermented products Monascus sp. with soybean as a substrate. Soybeans are a source of protein and antioxidants. Monascus-fermented soybean (MFS) was recently developed to combine the functional properties of soybean and the metabolites produced during the Monascus fermentation process. MFS has a greater ability to scavenge free radicals and hydroxyl radicals compared to unfermented soybeans. MFS is relatively more effective in its activity as an antioxidant and has the potential to be applied in food products. Pyo and Seong reported that MFS had greater anti-hyperlipidemic activity compared to unfermented soybeans. The results showed a significant reduction in fat content through in vivo testing using experimental animals. Oral administration of MFS (200 and 400 mg/Kg body weight) significantly reduced low-density lipoprotein-cholesterol (LDL-C) levels and increased high-density lipoprotein-cholesterol (HDL-C) levels [11, 12].

Dioscorea batatas or Chinese yam is one of the functional foods and has high antioxidant, anti-inflammatory and hypolipidemic activity. Red Mold Dioscorea (RMD), not only contains metabolites fermented by Monascus, but the tubers also contain bioactive compounds that have biological activity. Therefore, RMD had a better hypolipidemic and antiatherosclerotic effect than unfermented Dioscorea [6]. Sweet potato (Ipomoea batatas), potato (Solanum tuberosum), cassava (Manihot esculenta), and dioscorea (Dioscorea batatas) were used as Monascus substrates to produce more monascolin K. The results showed that Monascus purpureus NTU 301, with dioscorea as a substrate, can produce monacolin K of 2,584 mg.kg–1, so it is 5.37 times greater than rice used as a substrate. So it is believed that dioscorea is a better substrate than rice for Monascus species to produce monacolin-K cholesterol-lowering compounds and anti-inflammatory compounds – monascin [1, 13]. The molecular structure of bioactive compounds (monacolin-K, GABA, monascin, and ankaflavin) produced by Monascus can be seen in Figure 1.
Figure 1. Structure of Bioactive compounds by Monascus sp. (a. Monacolin K; b. GABA; c. Monascin; d. Ankaflavin) [1].

Red ginseng fermented by Monascus purpureus KCCM 12002 has hepatoprotection, antihyperlipidemic activity, and has higher antioxidant activity when compared to unfermented red ginseng [14]. In addition to producing bioactive compounds, the pigment compounds produced by Monascus sp. have the potential to be developed. Potato pomace has been investigated to produce pigments effectively by shallow tray fermentation using Monascus purpureus [15]. Biopigments have potential use in gastronomy and food application. Monascus fermented wheat grain has been applied in bread formulation to improve new colored bread products with biological effects [16]. Waste can also be used to produce biopigments. Maltose syrup residue was also utilised as a substrate for pigment production by Monascus ruber. The production of yellow pigment occurred by low pH (2.0 – 2.5) [17]. Yellow pigments are known to have better antihyperlipidemic activity than orange and red pigments. Different types of substrate affect the pigment produced by Monascus, as in Figure 2, the fermented monascus in rice was red while the Dioscorea was orange.

Figure 2. a) Red Fermented Rice (RFR); b) Red Mold Dioscorea (RMD) by Monascus sp. [6]
3. The role of nitrogen source in *Monascus* fermented product

Nitrogen is an important nutrient needed by *Monascus* sp. for biomass growth and also the production of bioactive compounds. Various nitrogen sources have been widely used in *Monascus* fermentation. Nitrogen has an important role in the production of pigment compounds through the polyketide pathway. One source of nitrogen that is widely used in *Monascus* fermentation is peptone. Peptone produced from the hydrolysis of chicken feathers can be developed as an alternative nitrogen source in pigment production by *Monascus purpureus*. Chicken feathers are a waste that is not widely developed, through a proper hydrolysis process, the protein content of CFP (Chicken Feathers Peptone) is 67.2g/100g. The combination of CFP and glucose can produce a more dominant yellow pigment [18].

The orange pigment is the basic pigment formed by *Monascus* sp. then be derivates to yellow pigment and red pigment. The pigment can be produced by intracellular and extracellular mechanisms, intracellular orange pigment production can be accelerated at low pH (2.5 – 4.0) with the addition of ammonium as a nitrogen source [19]. The availability of nitrogen sources determines the production of biomass and the quantity of pigment compounds produced by *Monascus* [7]. The higher the addition of N sources can reduce the production of the red pigment of *Monascus* sp [8]. Ammonium chloride is the best source of N in the production of the pigment *Monascus* sp, then ammonium nitrate and glutamate [9]. The addition of nitrogen to the MFR (*Monascus* Fermented Rice) could increase the production of t red pigment, monascorubrin. Sources of nitrogen (0.5%) added include peptone, yeast extract, ammonium nitrate and monosodium glutamate. The red pigment produced by *Monascus* fermented products was stable and can be widely applied to food products. The addition of nitrogen can increase the metabolic activity of molds, the quantity of pigment compounds can be increased. Biopigments are currently in great demand along with the development of functional foods when compared to synthetic dyes [20].

*Monascus* sp. not only produces bioactive compounds but also produces a by-product in the form of a mycotoxin known as citrinin. The addition of the right nitrogen source can suppress the production of mycotoxin compounds by *Monascus*. The addition of inorganic nitrogen sources in the fermentation media can suppress the production of citrinin. The addition of NHCl and NH4NO3 can significantly suppress the intracellular and extracellular production of citrinin. The addition of inorganic nitrogen sources increases intracellular pigment. Nitrogen has an important role in the metabolism of primary and secondary metabolites of *Monascus* sp. When NHCl was used as a nitrogen source in the fermentation medium, the metabolism of amino acids, carbon metabolism including glycolysis and pentose phosphate pathway, and lipid metabolism were increased. Meanwhile, phenylalanine metabolism and tyrosine metabolism decreased. Similarly, when NHNO3 is used as a nitrogen source, nitrogen, and amino acid metabolism include the biosynthesis of phenylalanine, tyrosine, tryptophan, glycine, serine, threonine, valine, leucine, and isoleucine was increased, while the tyrosine metabolism decreased. The addition of inorganic nitrogen sources can increase amino acid metabolism which can be used as an energy source for *Monascus* growth. Some amino acids such as phenylalanine, tyrosine and tryptophan by biological reactions act as precursors for the biosynthesis of pigment compounds [21].

Citrinin production can also be suppressed by the addition of a nitrogen source and the right combination of pH. Monosodium glutamate ((NH4)SO4 and NaNO3) can be used as nitrogen sources in suppressing citrinin production. The source of nitrogen that can suppress citrinin production but still produce the optimum pigment compound is (NH4)SO4 using *Monascus anka* [22]. Other bioactive compounds produced by *Monascus* are GABA and monacolin-K. GABA (γ-amino butyric acid) has antihypertensive activity, while monacolin-K has an important role in inhibiting endogenous cholesterol synthesis. Ammonium sulfate does not play a role in the synthesis of GABA. MSG, yeast extract, peptone and sodium nitrate are known to increase GABA production. However, the addition of MSG of 1000 mg/kg can suppress the production of monacolin-K [23].

Based on the description above, nitrogen is one of the determining factors that can be used to accelerate the production of bioactive compounds from *Monascus*. The use of nitrogen sources is
based on the main substrate and other external factors, each also has an important role in the biosynthesis of bioactive compounds by Monascus sp.

4. The role of carbon source in Monascus fermented product

Carbon is the main component needed by molds for growth. The main role of carbon was to produce cell biomass other than as an accelerator for the production of bioactive compounds through the secondary metabolism pathway. Several carbon sources are added to the Monascus fermentation substrate, such as methanol, ethanol, n-propanol, isopropanol, butyl alcohol, isoamyl alcohol and 1-octanol was added to the submerged fermentation of Monascus purpureus. The carbon source from the alcohol group that produces the highest biomass as well as the yellow pigment is ethanol [24]. The addition of exogenous cofactors can increase the pigment production of Monascus. Yellow pigment with free citrinin was produced under electrolytic fermentation because these conditions affected the nioI gene [25].

Maltose syrup has the potential to be developed as a substrate in pigment production by Monascus ruber CCT 3802. pH below 4.0 with maltose syrup as a substrate can produce greater biopigments when compared to neutral pH [17]. Several species of M. purpureus, M. ruber, M. pilosus were able to produce bioactive compounds that act as antimicrobials. The addition of 1% shrimp and crab shell wastes as a carbon source can increase the production of antimicrobial compounds [26]. Some wastes such as rice straw hydrolyzate can also be used as a substrate for pigment production by Monascus purpureus through the submerged fermentation method. Lignocellulose in rice straw will be hydrolyzed and used as a carbon source by M. purpureus [27]. The utilization of waste as a fermentation medium to produce biopigment compounds continues to grow. One of the wastes that can be used is bakery production waste which has not been used optimally. Bakery waste hydrolyzate with a glucose concentration of 5g/L produces the highest pigment through solid-state fermentation by M. purpureus. Bakery waste is a good source of carbon and nitrogen so that it can be used as a medium for mold growth [28].

The addition of glycerol of 160 g/L to the synthetic growth medium of M. pilosus could increase pigment production 3.24 times higher than the control. In addition to pigment production, the resulting biomass was also 17.86 times greater. The role of glycerol in the growth of M. pilosus was to accelerate glycolysis and carbon metabolism so that the biomass and pigment produced increased [29]. One of the most interesting pigments due to their biological activity is monascin and ankaflavin. These two yellow pigment compounds have higher antihyperlipidemic activity than monacolin K [30]. Glycerol and soluble starch are known to affect the production of intracellular pigment by Monascus purpureus. The use of a combination of carbon sources between soluble starch and glycerol can support the synthesis of amino acids through primary metabolism and polyketide metabolism which is correlated with the resulting gene expression. Therefore, monascin and ankaflavin increased significantly with the addition of glycerol as a carbon source [31].

Carbon sources affected the production of monacolin K, an identical compound to lovastatin which has antihyperlipidemic activity. Several substrates such as rice, millet, broken corn, wheat, and barley were used as growth media for M. ruber. Millet produced higher monacolin K compared to other substrates using the solid-state fermentation (SSF) method. This is due to the smaller particle size so M. ruber spectrum is wider to access the substrate [32]. Carbon sources have an important role in the production of monacolin K, each carbon source has a different effect. Glucose is a simple carbon source that can be accessed directly by Monascus so that it can accelerate primary and secondary metabolism. The amount of glucose addition also needs to be considered, the addition of excess glucose can inhibit the synthesis of monacolin-K. Simple carbon sources are highly recommended as an accelerator for the production of bioactive compounds by Monacolin K [33].

5. Conclusions

Carbon and nitrogen have an important role in the biosynthesis of bioactive compounds by Monascus sp., including biopigments, monacolin K, GABA, and others. The selection of the right carbon and
nitrogen sources must be considered. Both organic and inorganic sources of carbon and nitrogen can be utilized. Simple carbon and nitrogen sources are highly desirable because they can be easily accessed to accelerate the primary and secondary metabolism of Monascus sp., so the production of bioactive compounds can be increased.

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