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Comparison of *Monascus purpureus* growth, pigment production and composition on different cereal substrates with solid state fermentation

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ABSTRACT

The growth and pigment production of *Monascus purpureus* during 14 days solid state fermentation on different cereal substrates i.e. rice, corn, whole sorghum grain (WSG), dehulled sorghum grain (DSG) and sorghum bran (SB); and pigment composition of the fermented-products have been evaluated. Fungal biomass was used as a basis of its growth. Pigment content was measured by using spectrophotometer and thin-layer chromatography, and its composition was analyzed by using liquid chromatography coupled with tandem mass spectrometry. *M. purpureus* grew faster on rice substrate than did on other substrates. Production of pigments was observed at the end of logarithmic phase on all substrates tested. Similar pigment compounds were found on all substrates and the highest production of pigments was on rice, followed by DSG > WSG > Corn > SB. Twelve pigments, six of which were well-known, were detected on the *Monascus*-fermented products at different levels. Among those, Monapilol B, found in *Monascus*-fermented dioscorea, was found. On all cases, the red pigment Rubropunctamine was the major one (57–87%), except on SB substrate which produced Yellow II as the major one. Interestingly, fermented-DSG contained a large amount of Rubropunctatin compared to other fermented products. Among the non-rice substrates, DSG is the most potential substrate, on which the fungus exhibited the highest growth and pigment production. These data suggest that the fermented products are good candidates for development of natural food colorant, food supplement, functional food and or medicine with antiinflammation, anticancer and antimicrobial activities.

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1. Introduction

Monascus purpureus, an edible fungus, has been used in solid state fermentation for centuries in Asian countries. Rice is the common substrate of the *Monascus*-solid state fermentation, and the *Monascus*-fermented rice has been widely consumed by people in China, Japan and South East Asian countries (Dufossé et al., 2005; Feng et al., 2012). Corn and sorghum (whole sorghum grain (WSG), dehulled sorghum grain (DSG), and sorghum bran (SB)) are non-rice cereals as potential alternative substrates for the *Monascus*-solid state fermentation (Kraboun et al., 2013; Kongbangkerd et al., 2014; Srianta and Harijono, 2015).

During the solid state fermentation, *M. purpureus* produces various secondary metabolites, mainly pigment. The *Monascus* pigment is a mixture of red, orange and yellow compounds, which are classified into polyketide. Monascin and Ankaflavin (yellow pigments); Rubropunctatin and Monascorubrin (orange pigments); Rubropunctamine and Monascorubramine (red pigments) are six well-known *Monascus*-pigments. Monascus fungi synthesize the pigments through polyketide biosynthesis pathway, in which polyketide synthase and fatty acid synthase play essential roles (Juzlova et al., 1996; Hajjaj et al., 2000). Different carbon sources seem to affect the pigment production (Carvalho et al., 2007; Nimnoi and Lumyong, 2011).

In the application, the *Monascus* pigment has been used as

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natural food colorant, food supplement and traditional medicine. As a natural food colorant, red pigments as well as the yellow pigments have been widely used in food industries e.g. meat, edible oil, biscuit, bread, cakes and beverages (Srianta et al., 2014). Moreover, bioactivities, such as antiinflammation, anticancer, antimicrobial, antidiabetes and antiobesity, of isolated *Monascus* pigments have been reported (Feng et al., 2012). The six well-known pigments inhibit inflammation (Akihisa et al. (2005b) and Monascin, Ankaflavin, Rubropunctatin, Rubropunctamine, Monascorubramine and Monapilol A-D possess anticancer activity (Akihisa et al., 2005a, 2005b; Hsu et al., 2011; Knecht and Humpf, 2006; Su et al., 2005; Zheng et al., 2010). Antimicrobial activities of Rubropunctatin and Monascorubrin against bacteria, yeast and filamentous fungi (Martinkova et al., 1995); and of red and orange pigments against some pathogenic bacteria (Vendruscolo et al., 2014) have been found. Monascin has been reported as a potential antiobesity through reducing triglyceride accumulation (Jou et al., 2010) and possesses a therapeutic potential on diabetes (Shi et al., 2012). Analysis of pigment composition is thus important for prediction of the potential of fermented materials and their application.

Concerning the analysis of pigment composition, Miyake et al. (2005) have developed a simple and sensitive liquid chromatography-mass spectrometry (LC–MS) method for the detection of M+H ion of pigment compounds to estimate their contents, and detected 11 pigments in different *Monascus* strains grown under various culture conditions. In potato dextrose broth medium, *M. purpureus* NBRC4478 produced mainly Rubropunctatin, Rubropunctamine and Monascin, while another strain, *M. purpureus* SM50 produced a range of pigments with Rubropunctatin, Ankaflavin and Monascin as major pigments. *M. pilosus* NBRC4520 produced mainly Xanthomonascin A and Monascorubrin in the potato dextrose broth medium, but mainly Rubropunctatin in a medium containing glucose, glycerol and peptone (Miyake et al., 2008).

The objective of the research was to compare the growth of *M. purpureus* M9 and pigments production during solid state fermentation on different cereal substrates: polished rice, corn, whole sorghum grain (WSG), de-hulled sorghum grain (DSG) and sorghum bran (SB); and pigment composition of the *Monascus*-fermented products. Such a comparison study was never been reported elsewhere.

2. Materials and methods

2.1. Microorganism

M. purpureus was isolated from commercial *Monascus*-fermented rice (MFR) and identified as *M. purpureus* M9 (NCBI Accession Number: HM188425.1). *M. purpureus* culture was maintained on Potato Dextrose Agar (PDA) slant and sub-cultured monthly. *M. purpureus* starter was prepared by inoculating *M. purpureus* culture stock onto a PDA slant, incubated at 30 °C for 7 days, and then used for solid state fermentation.

2.2. Solid state fermentation

Substrates of rice, corn, WSG, DSG and SB were separately prepared. About 20 g of each substrate in a jar was added with 15 mL distilled water and sterilized at 121 °C for 20 min Solid state fermentation was carried out by inoculation of 1.5 mL of *M. purpureus* starter culture containing 5×10^5 spores/mL into the sterilized substrate. It was then incubated at 30 °C for 14 days. A sample of fermented material was taken daily, dried at 45 °C for 24 h, and analyzed for the biomass level and pigments.

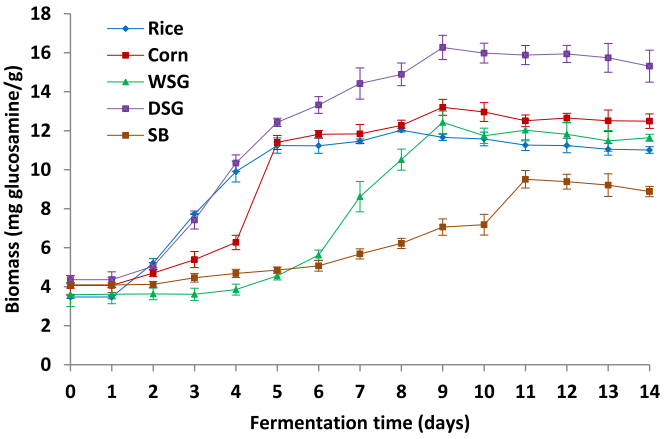

Fig. 1. Growth of *M. purpureus* on different substrates. Solid state fermentation was carried out in the same conditions (30 °C, 14 days).

2.3. Biomass estimation

The fungal biomass was estimated by determining the amount of N-acetyl glucosamine released by acid hydrolysis of chitin, present in the mycelia cell wall (Babitha et al., 2006). Chitin hydrolysis was carried out by using 10 M HCl and autoclaving at 130 °C for 2 h. The hydrolysate was neutralized to pH 7.0, mixed with acetyl acetone reagent and followed by Ehrlich reagent. The optical density was measured at 530 nm against the reagent blank. N-acetyl glucosamine (Sigma Aldrich Co. LLC) was used as a standard.

2.4. Pigment extraction and analysis

An accurately weighed fermented matter of about 0.1 g each was transferred into a tube and mixed with 2 mL of 75% ethanol. The mixture was treated in an ultrasonic bath for 60 min, followed by centrifugation at 3000 rpm for 15 min. The solid was re-extracted twice by the same procedure. The collected supernatant was mixed with 75% ethanol until 10 mL in a volumetric flask.

Pigments analysis of the extracts was carried out by using three methods i.e. spectrophotometry, thin layer chromatography (TLC) and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Spectrophotometry and TLC method was performed for monitoring pigments production during fermentation, while LC–MS/MS for analysis of pigment composition of the fermented-products.

The absorbance of pigment extracts was measured by using UV–vis spectrophotometer at 400 nm, 470 nm and 500 nm. The results were expressed as absorbance unit at the corresponding wavelength per gram (AU/g). TLC method was performed according to Nimnoi and Lumyong (2011). Ethanol extract of 3 μL was applied onto a Silica Gel 60 F254 plate (Merck, Germany) and pigments were separated with a mobile phase consisting of chloroform:methanol:water=90:25:4.

Pigment compounds were determined according to Miyake et al. (2008) with some modifications. The analysis was conducted by LC–MS/MS with an EMS mode using the 3200 Q-TRAP LC–MS/MS System (AB Sciex, Framingham, MA, USA) equipped with a Prominence UFLC (Shimadzu, Kyoto, Japan). Pigments were separated on a Mightysil RP18 column (150-mm × 2-mm i.d.) with a linear gradient of mobile phase of acetonitrile-water containing 0.1% formic acid (60:40, vol/vol) to acetonitrile-water containing 0.1% formic acid (100:0, vol/vol); flow rate of 0.2 mL/min; oven temperature of 40 °C; run time for 25 min. Pigment compounds were detected by MS/MS using electro-spray ionization in the positive ion mode (IS: 2000; CUR: 40; CAD: set to 'high'; TEM:

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