Production of red pigments by Monascus purpureus in solid-state culture

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Abstract

In this study various nutritional and environmental parameters such as, initial moisture content, pH, inoculum size, air rate, sample size and nutrient supplement that influence pigment production were evaluated in solid-state cultures. optimum initial moisture content and pH were determined to be 50% and 6.0, respectively. The supplement of the substrate with different carbon, nitrogen, and mineral source reveals a more inhibitive effect as the substrate concentration increase. optimum aeration rate was determined to be 2vvm in flask culture. The maximum amount of red pigment, 3500 OD/g dried fermented rice, was obtained in optimum conditions which is obtained in solid flask culture.

Introduction

The fungi *Monascus* species are known as the sources of various secondary metabolites. Pigments synthesized by the fungi *Monascus* spp. have been traditionally used in Asia for coloring and securing a number of fermented foods such as alchoholic beverage, red soybean curd, meat, and vegetables [1].

Production of *Monascus* pigments can be obtained in both solid-state and submerged culture. Extensive submerged culture studies of red pigment synthesis by M. purpureus strains have revealed that the yield is affected markedly by many factors including medium composition, pH and agitation. In addition, the pigments produced in submerged culture are mainly retained intracellularly, causing inhibition of further production. For these reasons, when compared between submerged and solid culture, the red pigment yield is superior in the latter.

In order to maximize and sustain productivity of *Monascus* pigment optimization and control of culture conditions are of essential importance. Therefore, in this study various nutritional and environmental parameters such as, inoculum size, pH, initial moisture content, air rate, sample size and nutrient supplement that influence pigment production were evaluated in solid-state cultures.

Materials and Method

Microorganism and medium

The strain used in this study was *Monascus purpureus* (ATCC 16362). The stock culture was maintained on YM agar slant [2] containing: glucose, 20 g; malt extract, 3 g; peptone, 5 g; yeast extract, 3 g; agar, 1.5 g; and distilled water, 1 L. Long rice (from Thailand) was used as substrate for the solid-state culture

Cultivation

Agar plates containing YM medium were inoculated by *M. purpureus* from colonies on 5-day old agar plate and then incubated at 30¡É for 5days. Ten ml of sterile distilled water was added to each agar plate, which was scraped with the flamed slide glass gently. This mixed

suspension of mycelia and spores was filtered through syringe containing cotton to remove mycelia and debris. A 10% volume of spore suspension was inoculated into rice and incubated in growth chamber within which 90% of relative humidity was maintained at 30¡É for 7 days.

Assays

Red pigment estimation was performed using a HP 8890 UV/VIS spectrophotometer at 500 nm. The culture samples stored at 70 C were freeze-dried. The dried samples were then ground into powder with a mortar and pestle. Twenty milliliters of 95% ethanol was added to 0.1g of powdered ample in a 100ml flask, and the pigment was extracted at 25 C for 24 hours with occasional shakings in a dark room. As an indirect measurement of cell growth, glucosamine was determined according to the method proposed by Aidoo et al. [3].

Result and Discussion

Maximum production of biomass and pigment were obtained at 50% of IMC. The moisture content rose from the initial value of approximately 50% to a final value of about 77%. To a certain degree the physicochemical parameter moisture refer mainly to the primary metabolism. An initial pH of 6.0 gave the highest production of red pigment and biomass. This is close to the natural pH of the rice samples soaked in distilled water. The supplement of the substrate with different carbon and nitrogen source reveals a more inhibitive effect as the substrate concentration increase. Mineral sources also cause inhibition to production of red pigment. optimum aeration rate and inoculum size were determined to be 2vvm and 1x104 spore cell/g dried rice, respectively.

Table 2. Optimum conditions obtained in submerged and solid-state culture for producing *Monascus* red pigments

Parameters		Optimum conditions in solid-state culture
Initial moisture content		50%
pH		6.0
Mineral supplement	Cu^{2+}	Negative effect
	Fe^{2+}	Negative effect
	Mn^{2+}	Negative effect
	Zn^{2+}	Negative effect
Other nutrient supplement	Carbon source	Negative effect
	Nitrogen source	Negative effect
Optimum inoculum size		$1x10^4$ spore/ g dried rice
Aeration		2vvm

Reference

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