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Production and Extraction of Biopigments from Rice Malt by *Monascus purpureus*

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Biopigments are such microbial product which has greater impact over chemically synthetic pigments. Certain organism like *Monascus purpureus* has the ability to produce monascorubramine, rubropunctamine etc. which are red coloured pigments. The aim of this work was to produce biopigments from organic waste, such as banana peel, chikoo peel, papaya peel, rice malt and molasses, to bring down the production cost and to reduce the organic load from the environment. Rice malt was found to be the best suitable substrate among these organic wastes when screened for production of biopigment using *Monascus purpureus*. The fermentation was carried out in different culture conditions and the maximum pigment production was achieved at 30 °C for 10 days of incubation and at pH 6. The down-streaming was performed with rice malt as the substrate and the pigment from *Monascus purpureus* was obtained effectively using ethanol as a solvent. The presence of red pigment was confirmed by HPLC.

Key words: *Monascus purpureus*, biopigments, rice malt, ethanol extract, HPLC.

Colour and flavors act as signals which are instantly sensed and determines savor of the food[1]. The quality of food depends on its additives in the food[3]. Temperature, light, oxygen and acids affects the stability hence quality of colour and flavors gets deteriorated. Food industries utilizes chemosynthetic and plant derived flavors and colorants to replenish the genuine stock[2]. There are 29 accepted food colours, 16 are chemically prepared and their long term ingestion may cause various health related problems[4].

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Natural pigments are obtained from plants and micro organisms which are produced as secondary metabolite. Certain organic compounds which are not directly engaged in the normal growth, reproduction or development of an organisms are secondary metabolites, benefit to humankind in various aspects. *Monascus* pigments, are such a secondary metabolite produced by various species of *Monascus*. It has been used as a natural colorant for decades[5,6]. *Monascus purpureus* is a species of fungus which is purplish red in colour. It is also called as angkak rice mold, maize silage mold and corn silage mold. Angkak or red fermented rice by *Monascus* sp. has been in vogue in East Asian countries[7]. Since first century A.D. rice was traditionally used as Solid-state fermentation of *Monascus* and consumed extensively in Asia for food coloring of fish, Chinese cheese, red wine and sausages[8-14]. In

Western countries *Monascus* pigments are also utilized for the processing of meat in the industries[15].

Monascus sp. synthesizes three types of pigments, such as red, orange and yellow, with each having two components of polyketide origin with a common azaphilone skeleton [16, 7]. The orange pigment includes monascorubrin and rubropunctatin. The red pigments are the aminated orange pigments such as monascorubramine and rubropunctamine. The yellow pigment includes monascin and ankaflavin[17]. Among three, the red pigments are in the great demand, as it is used in meat products to substitute nitrites[18].

Monascus pigments have been used in food industries and have the potential for therapeutic use[19]. The antimicrobial effect of *Monascus* culture, due of monascidin A, confirmed by scientific investigations, was proved against some bacterial and fungal strains. The brute pigment obtained by growing the *Monascus* strain in surface culture had an antifungal action against some species of *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium* genus. The yellow pigment isolated from red yeast rice also inhibits bacteria of the genera of *Bacillus*, *Pseudomonas* and *Escherichia*. These bacteriostatic and antifungal effects have lead to the consideration of the preservative value of the pigments of *Monascus purpureus* besides the tinctorial properties. *M. purpureus*, cultured in a shrimp and crab shell powder (SCSP) medium, displayed protease activities and the ability of enhancing the growth of rape[20, 21]. cytotoxic activity of Monascus pigments was also observed in the ethanol extract of *M. purpureus* fermented rice[22].

Various organic wastes such as banana peel, chikoo peel, papaya peel, rice malt and molasses were screened for pigment production. Hence only rice malt had shown better yield, it was chosen as substrate for this experiment. Malt is generated as waste while cooking rice hence it can serve as the cheapest source for biopigment production. It can bring down the production cost when used for large scale production. Previous research and studies has shown that growth of *Monascus* require sucrose or dextrose as carbon source. Rice malt has rich source of sucrose[23] and easily availability makes it a better substrate. Added dextrose with different concentration, initial pH of the medium, initial moisture content, temperature and incubation time plays a greater role in the growth of *Monascus* as well as in the production of pigment.

MATERIALS AND METHODS

Culture

A culture of *Monascus purpureus* (MTCC 410) was obtained from the Microbial Type Culture Collection (MTCC, Chandigarh, India) and used for the experiment. It was maintained on potato dextrose agar medium (Hi-Media, India) and preserved at 4 °C. Sub-culturing was done once in every three weeks [24,1].

Inoculum preparation

Inoculum was prepared in potato dextrose broth (Hi-Media, Mumbai, India). It was incubated for 6 to 8 days, at 30 °C. The sterilized substrate was directly inoculated from the broth culture[26].

Substrate

Rice malt was chosen as a substrate. It was collected from canteen (VIT University Vellore, Tamilnadu, India) and nearby hotels. Experiments were conducted in 500 ml Erlenmeyer flasks containing 200 ml of substrate. To get red pigment pH of substrate was maintained at 6.0 using pH meter. Substrate was autoclaved at 121 °C for 15min and cooled to room temperature. It was inoculated with *M. purpureus* culture from the broth culture and incubated at 30 °C. These conditions were maintained throughout the experiment[24].

Pigment extraction

From the fermented liquid substrate, the cells were removed using Watmann's filter paper #1. Cells were dried at 55 °C in a hot air oven till it gets fully dried and then powdered. Pigment was extracted using 99% ethyl alcohol as a solvent and separated using separating funnel. The solvent fraction was collected and concentrated by keeping in rotary shaker at 200 rpm over night.

Purification

To check the presence of the pigment the concentrated extract was analyzed on C18 column (Techsphere 5ODS 4_m, 250 mm×4.6 mm) using an analytical HPLC equipped with a 2487 dual absorbance detector, set at 500 nm. The flow rate was 0.5mLmin^{-1} using the mobile phase of acetonitrile mixed with water (80:20, v/v). The red

pigment obtained from preparative TLC was further purified by semi-preparative HPLC (Waters, USA) on a C18 column (100mm×19mm, XBridge Prep, Waters, USA) by using a separation gradient of water mixed with methanol (80:20 to 0:100, v/v) over 30 min. The flow rate was 0.8mL per min[24].

RESULTS AND DISCUSSION

Selection of culture

Monascus purpureus (Fig. 1) was selected as a culture because of its ability to produce red pigment and other pigments at wide range of pH[4].

Selection of substrate

Organic wastes such as banana peel, chikoo peel, papaya peel, pineapple peel, rice malt and molasses were screened to select the best substrate for the production of red pigment. Highest pigment production was achieved with rice malt (fig. 2). Others either yielded negligible or showed poor yields. Hence, rice malt was selected as a substrate for subsequent studies. Cheap, easy availability and high sugar content of rice malt made it a suitable substrate for the pigment production.

Effect of incubation time on pigment production

Maximum pigment production was obtained after 10 days of incubation (Fig. 2 and 3). From the results it was evident that up to 4 days of incubation, the maximum growth of the fungus was observed. After 3 days the pigment production took place which was increased till 10 days of incubation. A report was also given by Gunjan Mukherjee where the pigment production was observed from 3 days of incubation[24].

Effect of temperature on pigment production

Temperature is an important factor which has greater impact in pigment production as well as in growth of the fungus. It was observed that maximum production of red pigment was obtained at 30 °C[26]. As the temperature increased the concentration of red pigment was found to decrease[7].

Effect of initial pH of the medium

The pH of the medium is a major factor in pigment production. It was observed that at lower pH, there was no red pigment produced. The pH, between 6 to 8 has shown the appearance of red pigments[27]. Maximum red pigments were observed at pH 6. For the growth of fungus pH 4 was found as the optimal pH and at pH of 2 and 2.5, there was no fungal growth.

Extraction of red pigment

Ethyl alcohol was found as a suitable solvent for the extraction of red pigment (Fig. 4 and 5). It was concentrated and used for HPLC analysis.

HPLC analysis

The HPLC was done at 500nm. The chromatogram showed a sharp peak at retention time 2 minute fig. 6 which indicates the presence of red pigment which was similar to the study of Gunjan Mukharjee[24].

Fig. 1.

Fig. 2.

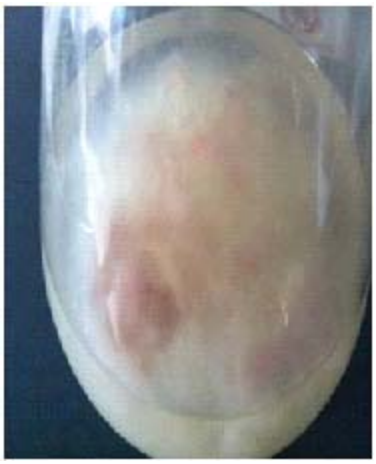

Fig. 3.

Fig. 4.

Fig. 5.

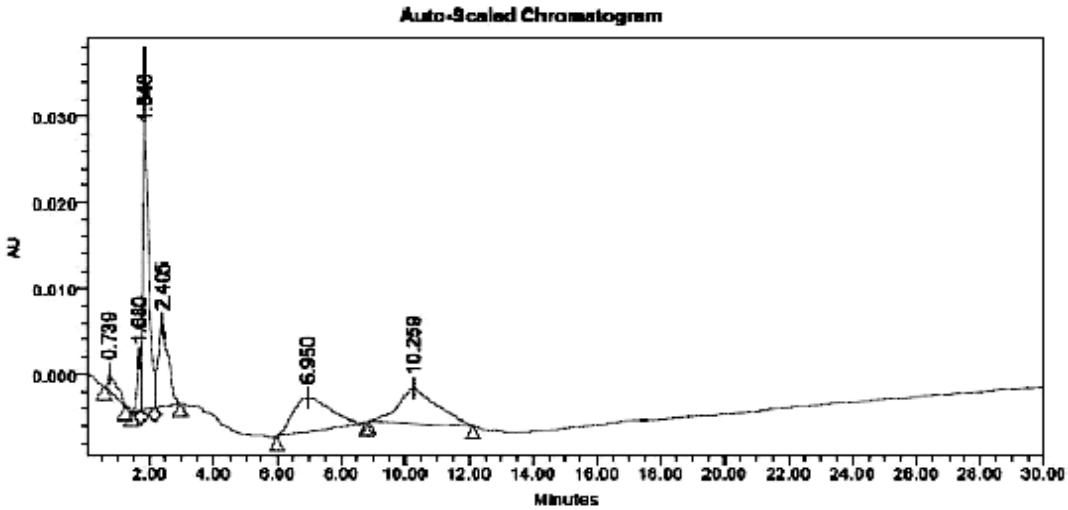

Fig. 6.

CONCLUSION

The most significant outcome of this study was the high yield of red pigment by *Monascus purpureus* using the cheap and easily available rice malt. The process of preparation of pigment is also simple and cost effective, since rice malt is common waste in many aspects, can be utilized as substrate for pigment production. This novel experiment has provided the eco-friendly method for the production of bio pigments. The method can be utilized to meet the future demand of bio pigment as well as can help to reduce the usages of synthetic pigments. Stability of the color of the pigment over wide range of acidic pH aids the buffering nature of the substrate aids future scope of this pigment in food applications.

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