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INFLUENCE THE DIFFERENT SUBMERGED MEDIA BY THE PRODUCTION OF YELLOW AND RED *MONSACUS SP.* PIGMENT

NICOLETA RADU*, MARIANA FERDES, OANA COMAN***, ISABEL GHITA CRISTESCU***, ALINA MIHALCEA**, CAMELIA UNGUREANU****

*I.N.C.D.C.P ICECHIM of Bucharest
**Politehnica University of Bucharest
***University of Medicine and Pharmacy Carol Davila of Bucharest

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Abstract

The production of red and yellow pigments by Monascus sp. is influenced by the composition of culture media that determine the quantity and the quality of the colorant. The Monascus sp. strain was cultivated in submerged, shaken culture, using media containing different carbon and nitrogen sources. The optimal cultivation conditions for this strain require glucose and $NaNO_3$ to produce the highest quantity of red pigment.

INTRODUCTION

The production of synthetic colouring agents and other chemicals used as food additives is under increasing pressure due to a renewed interest in use of natural products in food formulation and the strong interest in minimizing the use of chemical processes to produce food ingredients. By fermentation it is possible to develop processes for the production of environmentally friendly colouring and dyes intermediates than can be used as food constituents. With the cultivation of different strains of *Monascus species*, it is possible to obtain coloured polyketide compounds in varying shades such as purple, red, orange and yellow [1, 2]. Pigment yields in submerged cultures are highly affected by the nitrogen and carbon sources [1]. In this work we report some of key parameters, which determine Monascus pigment production in submerged fermentation using different carbon and nitrogen as nutrient source

MATERIALS AND METHODS

Microorganism

Monascus purpureus Went was obtained from Politehnica University of Bucharest, Biotechnology and Bioengineering Department.

Culture media

Maintenance ($g \cdot L^{-1}$): meat extract 3; peptone 5; glucose 10; agar 20 (pH=5.5). Different carbon sources were added in quantity of 1% into culture media with the following composition: KH_2PO_4 0.1%; $MgSO_4 \bullet 7H_2O$ 0.05%, NaCl 0.05%, $FeSO_4 \bullet 7H_2O$ 0.01. As carbon sources was used glucose, fructose, galactose, mannose, lactose, zaharose, celobiose, maltose, gentiobiose. The effect of different N source was used in the same conditions, using the culture media which contain: glucose 1%; KH_2PO_4 0.1%; $MgSO_4 \bullet 7H_2O$ 0.05%; NaCl 0.05%; $FeSO_4 \bullet 7H_2O$ 0.01% and different nitrogen source added into this media in the quantity of 0.3%. As nitrogen source was used NH_4Cl, NH_4NO_3, diammonium citrate, $CO(NH_2)_2 \bullet HNO_3$, $NaNO_3$, casein and peptone.

Procedure

In 250 ml Erlenmeyer flask was added 50 ml culture media, which subjected to sterilisation. The inoculation was made with 5 ml conidosphores in sterile water, which contain $2x10^5$ spores/ml. The following operational condition were kept constant by means of shaker controllers: temperature 30°C, agitation speed 300 rpm. The experiments was realized during 7 day.

Analytical methods

Dry cell weigh

Duplicate samples were weighed on an analytical scale, vacuum filter through pre-weighed membrane filters (cellulose ester membranes, 1.2 µm) washed with distilled water, dried in oven at 60°C and cooled in a desiccators before weighing for dry cell weigh. The results were expressed in grams per litter.

Extra cellular pigments

Absorbance measurements were performed in the filtrate obtained from dry cell weight, with a scanning spectrophotometer (TD60 UV/VIS Spectrophotometer, USA). The absorbance value measured at 400 and 510 nm was referred as the yellow and red color of extracellular pigments.

Intra cellular pigments

A two step procedure was employed. a) Cell disruption by sonification: a given mass of cells separated through filtration was suspended in 50 ml of ethanol 70% (v/v) and subjected to sonification for 40 min at 120 W (Jasco) b) Extraction: the sonified suspension was placed in a water bath (60°C) for 2 h and then, vacuum filtrated (cellulose ester membranes, 1.2 µm). The absorbance value of the filtrate between 400 and 510 nm was determined on a scanning UV-VIS spectrophotometer, multiplied by the dilution factor in ethanol 70% and referred as absorbance of intracellular pigments.

RESULTS AND DISCUSSION

Effect of carbon source of the pigment production

Measurement performed at 400 nm indicated no yellow pigment in the presence of different carbon sources. Productions of red pigments (measurement performed at 510 nm) are influenced by nitrogen and carbon source. Using a different monosaccharide sources revealed the possibilities of fugal strain to metabolised it, but the best results are obtained when the mould are developed in the presence of fructose and glucose (figure 1), except lactose, where under most results are obtained. Pigment production is under favour of disaccharides presence in the culture media, but after long time, due to both accommodated of microorganism to other source of carbon and production of other enzyme which are necessary to metabolised of the new source of carbon. During the monitoring trials the reduction of pigments produced was observed after 4-5 day.

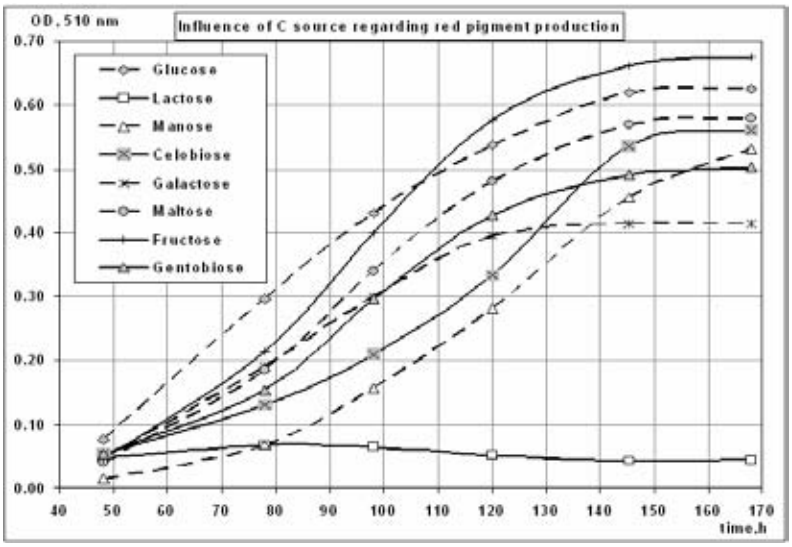

Fig. 1. Influence of different carbon source of the red pigment biosynthesis during 7 days (measurement made at 510 nm)

Get off the pigment from the inside microorganisms indicate that the best results are obtained when the moulds are developed in the medium containing glucose and fructose (figure 2); in the same time the biomass production is shrike in the following order: glucose > fructose > mannose > celobiose > gentobiose > galactose > maltose (figure 3).

Effect of nitrogen source of the pigment production

The best results regarding pigment production was obtained using $NaNO_3$ as single nitrogen source (figure 4 and 5), compound which favorised the formation of monascorubramine and rubropuntctamine [3].

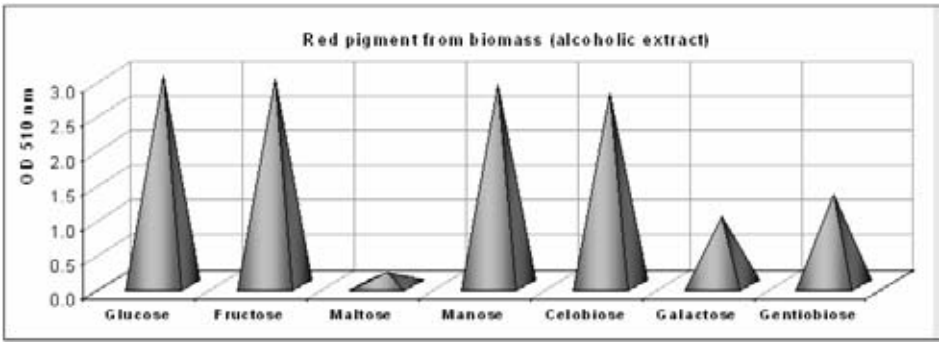

Fig. 2. Influence of different carbon sources regarding intracellular red pigment accumulation

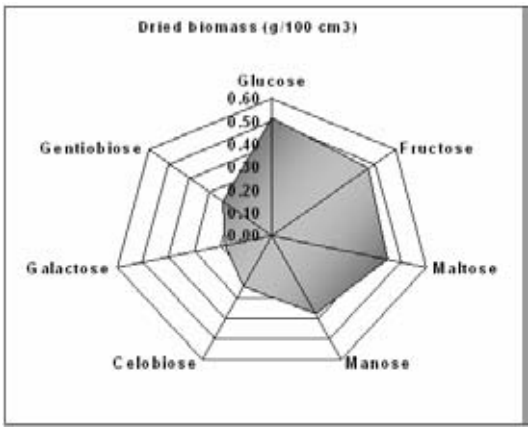

Fig. 3. Influence of different carbon sources regarding biomass accumulation

The pH of culture media is high influenced with ammonium compound by unbuffered culture media, when the pH has the value below 5. At this pH, orange pigments cannot react with ammino group in order to transform it into red pigments, but these are reduced to Monascin and Ankaflavin which represent in fact yellow pigments [3]. The measurement performed on dried biomass, indicate the positive effect regarding the biomass formation, when the greatest quantities of biomass are obtained when the casein and peptone are used as nitrogen sources (figure 6). At the same time in the presence of inorganic compound the biomass formation is slightly inhibited (figure 6). The value of alcoholic absorbance indicate the $NaNO_3$ induced the intracellular red pigment production (figure 7) and the casein and peptone induced the production of yellow pigment, whereas in the presence of the ammonium ions (nitrate of ureea) intracellular yellow pigments are mainly produced (figure 8).

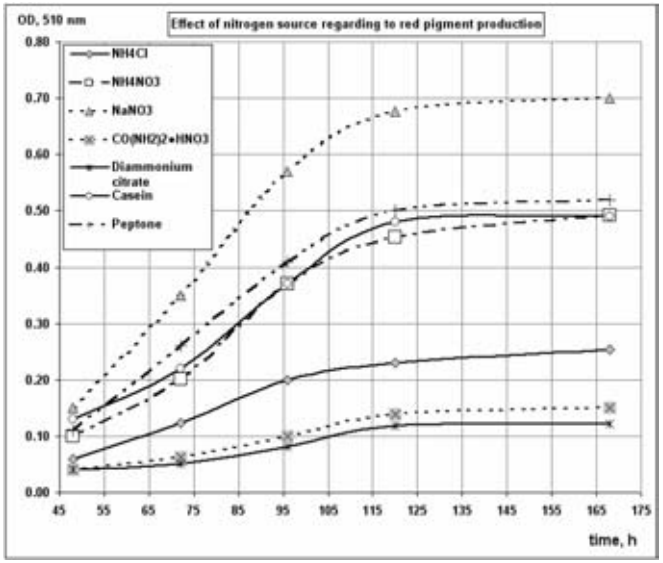

Fig. 4. Influence of different nitrogen sources regarding the production of red pigments

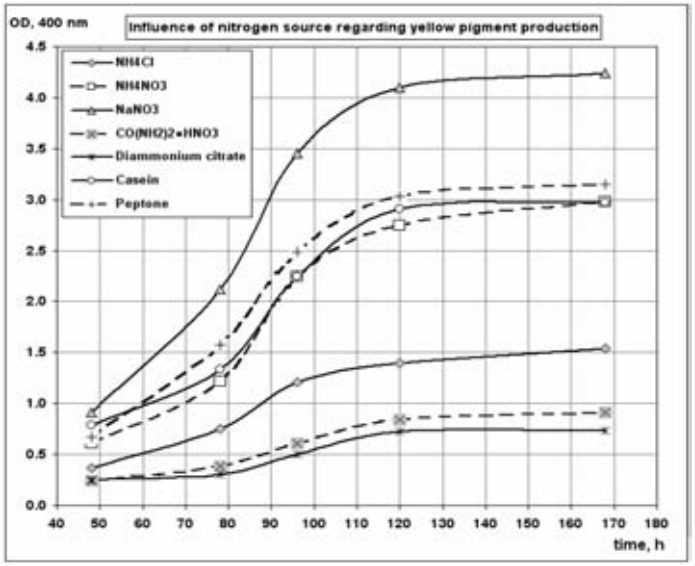

Fig. 5. Influence of different nitrogen sources regarding the production of yellow pigments

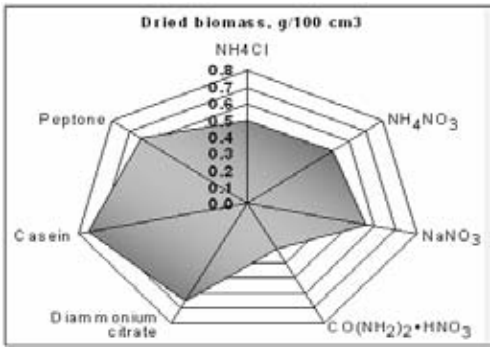

Fig. 6. Influence of different nitrogen sources regarding biomass production

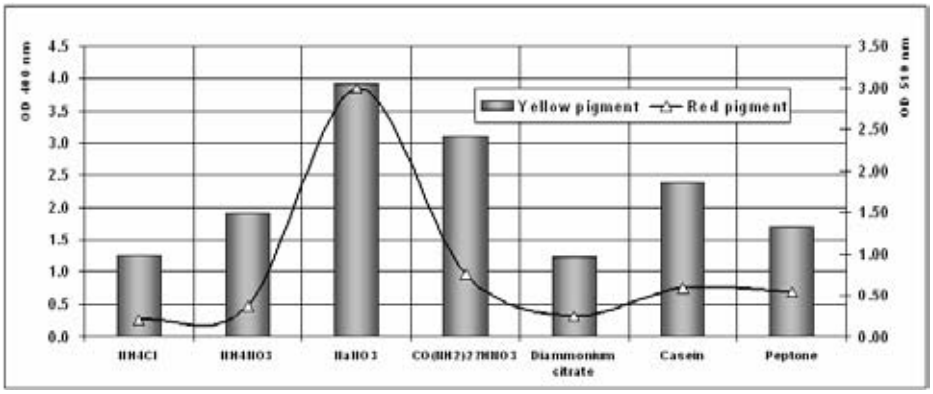

Fig. 7. Influence of different nitrogen sources regarding intracellular accumulation of red and yellow pigment

CONCLUSIONS

1. Influence of different sources of carbon and nitrogen regarding the red and yellow pigment production indicate that glucose is the best source for red pigment synthesis both extra or inside cell. The best nitrogen source for pigment production is $NaNO_3$ when the production of red pigments is favourised; in the same time, the ammonium ions favorised the yellow pigment production.

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