3rd International Conference
"Research & Innovation in Engineering"
COMAT 2014
16-17 October 2014, Braşov, Romania

INFLUENCE OF CULTIVATION CONDITIONS ON BIOSYNTHESIS OF RED RICE – A COLORING BIOMATERIAL PRODUCED BY MONASCUS

Mariana Ferdes[1], Mirela Dinca[1], Gheorghe Voicu[1], Gigel Paraschiv[1], Georgiana Moiceanu[1], Gabriel Muşuroi[1]
[1]University Politehnica of Bucharest, Bucharest, ROMANIA, e-mail: marianaferdes@yahoo.com

Abstract: *A mutant strain of Monascus purpureus was cultivated in SSF system on ground rice to produce red and yellow pigments with tinctorial, antimicrobial and dietary properties. The biosynthetic process was carried out at different temperatures, humidity values, amounts of inoculum and concentrations of Tween 80. After 5 days of biosynthesis the pigments were extracted in Et-OH and the characteristic absorbances for yellow and red pigments were determined at 400 and 510 nm.*
Keywords: *red rice, biomaterial, Monascus, pigments*

1. INTRODUCTION

Red rice represents a traditional medicinal and tinctorial biomaterial used in Est Asian for centuries. The red rice is formed during the fermentation of rice with the fungus *Monascus* and it is called as Ang Khak or Hong Qu in China and Red Koji, Beni Koji in Japan [2,3]. In Chinese medicine red rice is used to promote blood circulation, in some diseases of body organs, for bruised muscles, indigestion and colics in infants. In recent years it has been discovered that the red rice inhibits the action of a body enzyme called HMG-CoA reductase and is considered an cholesterol lowering agent because this material reduce and maintain healthy cholesterol levels and promote blood circulation, thereby lowering the risk of heart disease [5, 7, 8]. Scientific investigations have confirned pharmacological effects of *Monascus* fermentate. Some studies demonstrated that the red rice extract decreased insulin and blood glucose levels in type II diabet. Recent discoveries suggest that the red rice can prevent cancer in laboratory animals [9, 10].
The main application is however as a food aditive, in particular to meat as a preservative and condiment. Red rice contains starch, protein, fibre, sterols, unsaturated fatty acids that may help reduce serum lipids, but also numerous active constituents such as monacolin K that inhibits the production of cholesterol, sterols such as beta-sitosterol and campesterol which interfere with the absorbtion of cholesterol in the intestines. Red rice demonstrates inhibitory effects against some spoilage bacteria and molds and also against some pathogens: *Escherichia coli, Staphilococcus aureus, Pseudomonas sp., Bacillus subtilis, Mucor, Aspergillus* and *Fusarium* [2, 3, 4, 6].
The species of *Monascus* produce at least 6 pigments belonging of group of azaphilone: monascin, ankaflavin (yellow), rubropunctatin and monascorubrin (orange) and rubropunctamine and monascorubramine (red). Another important characteristic of *Monascus* fungi is their capability for production of proteases and amylases, so they are used for obtaining these enzymes by cultivating them on different natural substrates.
Monascus was classified and named in 1884 by the scientist van Tieghem. In 1895, Went published a careful study on *Monascus purpureus*, a species discovered from the samples collected by Dutch scientists in Java, where it was used largely for coloring rice. The genus *Monascus* is considered to belong to the family *Monascaceae*, the order *Eurotiales*, the class *Ascomycetes*, the phylum *Ascomycota*, and the kingdom *Fungi* [7].
Solid-state fermentation (SSF) is a traditional method of fermentation, used since centuries in the production of traditional foods in Orient, such as ang-kak itself. Although liquid fermentation is used in most industrial fermentative processes, there are advantages for the production of *Monascus* pigments by SSF, with high

productivity of pigments at a relatively cheap cost. Furthermore, the extraction of pigments from SSF matter seems to be more efficient, since the production in solid media is in concentrated form [1].

2. MATERIALS AND METHODS

In the process of biosynthesis, the fungal strain of *Monascus purpureus* MUCL 28 962 was used. To emprouve the ability of pigments production, the conidiospores of this mold were irradiated with electron beam at the dose of 2 KGy and the mutant strain namely M5 was selected and izolated. M5 was used in conducted studies to obtain red rice in SSF (solid state fermentation) system.
Monascus purpureus M5 was grown on Potato Dextrose Agar at 30^{o}C for a week and preserved at 4 ^{o}C. The culture medium was the ground rice, 100 g per Erlenmeyer flask and autoclaved. The rice was inoculated with 10^{6} conidiospores in sterile water and the initial moisture content of culture medium was about 8%. The moistrure content was analyzed on a termobalance Kern, at 105 ^{o}C and the absorbance was determined using a T 92+ UV-VIS spectrophotometer.
The cultures were grown at 30 ^{o}C for 14 days, shaking vigourously to omogenise and to ensure the release of CO_2 during the biosynthesis, which negatively influence the production of red pigments. After the biosynthesis was finished, the red rice was dried at 60 ^{o}C and the red and yellow pigments were spectrophotometricaly analyzed, at 400 nm and 510 nm respectively.

3. RESULTS AND DISCUSSION

The *Monascus purpureus* M5 radioinduced strain was cultivated on Potato Dextrose Agar in tubes and Petri dishes and the main characteristics of fungal colony were described. The studied strain forms pink colonies on PDA medium, with a diameter of 3-5 cm after a week at 30 ^{o}C. On the microscopic slides the M5 strain forms septate hyphae and chains of red conidiospores, as shown in figure 1 a, b.

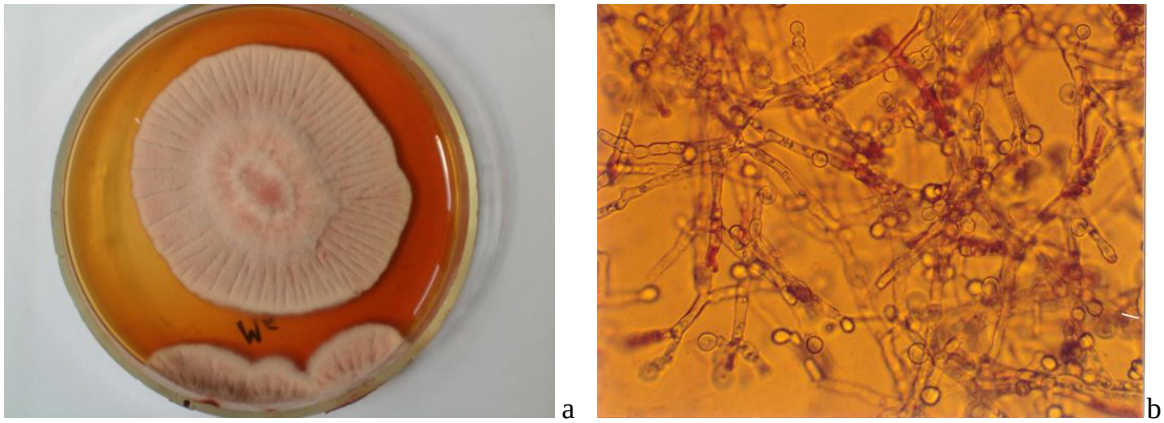
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Figure 1: The M5 *Monascus* colony on Potato Dextrose Agar a) and the microscopic aspect of culture b)

The influence of moisture content of culture medium was studied using a rice substrate with different moisture contents, as follows: 8% (initial humidity of rice after autoclaving), 16%, 23%, 34% and 43%, obtained by adding sterile water in the culture medium. The fungal growth is strongly influenced by moisture and the red rice color depends on the development of Monascus mycelium.
In the first 3-4 days of incubation, the germination of conidiospores has occured. For the begining a white hyphal system has grown, transforming the initial rice into a compact mass, difficult to homogenize. After the first week, the synthesis of red and yellow pigments begins and the culture becomes pink and red.
The moisture content of culture media has a major influence on the substrate consumption by the fungus due to respiration and transformation of nutrients. The substrate consumption is illustrated in figure 2.
As can see in figure, the highest value of substrate consumption is determined for samples 3 and 4 coresponding at 34% and 43% humidity. In this case the cellular respiration is more intense than the other 3 samples and the Monascus M5 strain has grown the most. The maximum of substrat consumption was registered between the 6 and 10 days.

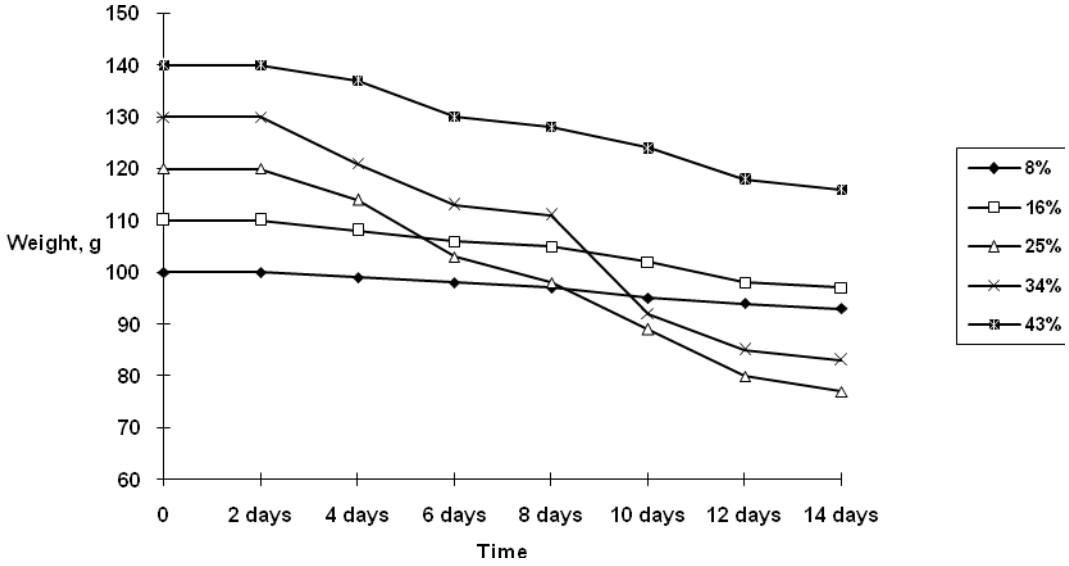


Figure 2: The substrate consumption in the culture medium for different moisture contents

After the time growth the colors of cultures in the 5 flasks were considerably different: for the samples 1 and 2, the color of rice with low humidity was pink, for the samples 3 and 4 the color was dark red and the sample number 5 was yellow. To compare the absorbance of red and yellow pigments, the culture medium was dry at 50 ^{o}C and the Et-OH extract was analyzed. The highest values of absorbances at 400 nm and 510 nm were recorded for the sample 3, with the value of humidity of 25%. The results were illustrated in figure 3.

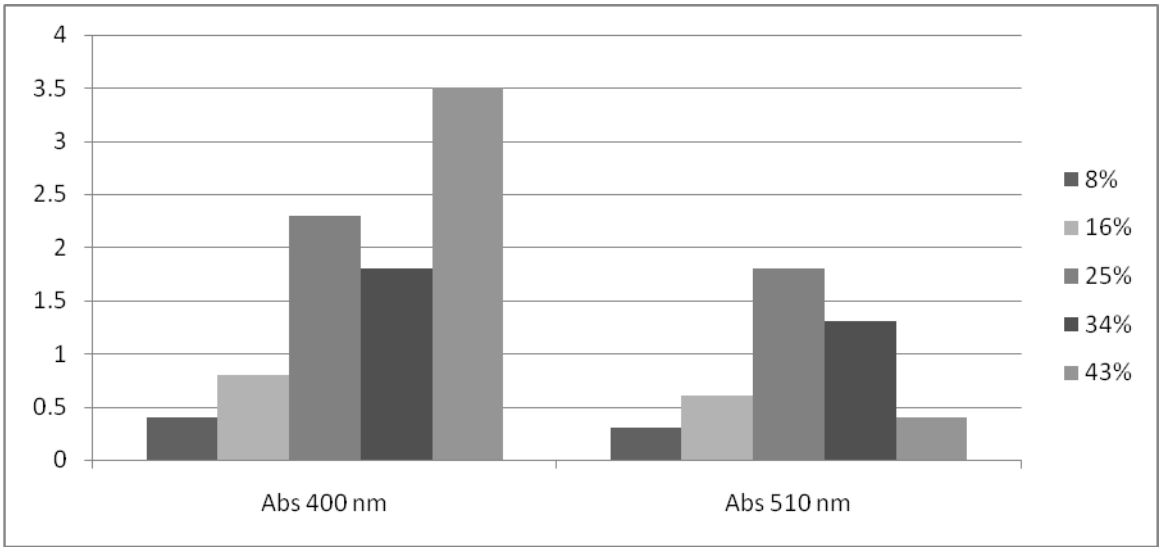


Figure 3: The absorbances values at 400 nm and 510 nm for different moisture contents in culture media

From experimental data results that the optimal moisture content was 25% which induces a rapid grown of microorganism but a good red and yellow pigments production. Lower moistures than 25% did not favor the fungal growth and a large amount of water causes yellow colour and inhibits red pigments synthesis.

The influence of amount of inoculum on pigments biosynthesis has been studied using different volumes of fungal cells suspensions (conidiospores, ascospores, hyphae) in sterile water. The culture medium (100 grams rice) was inoculated with 5 ml, 10 ml, 15 ml, 20 ml and 30 ml from the suspension of fungal cells with a concentration of 10^6 CFU/ml. After 2 weeks, the culture was dried at 60^{o}C and the pigments were extracted in Et-OH (1:1000 extraction ratio). The obtained results are shown in figure 4.

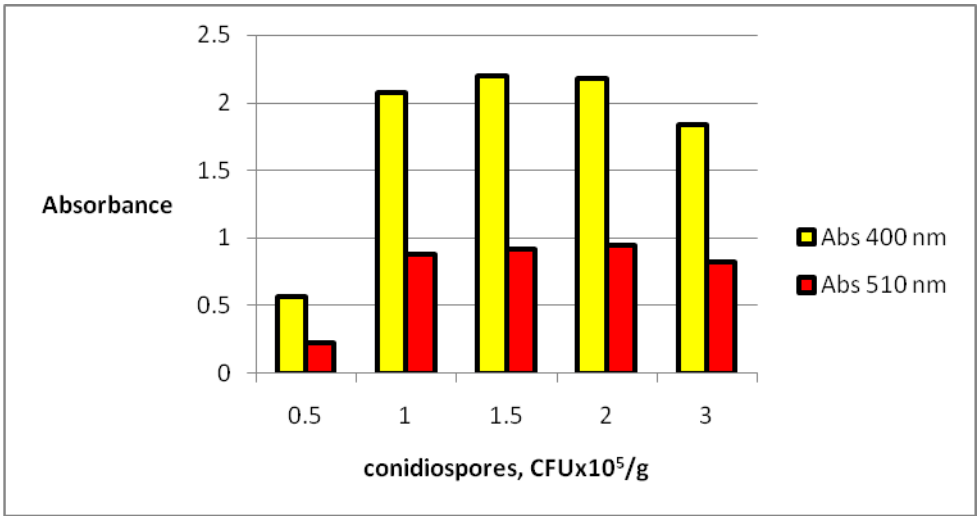


Figure 4: The absorbance values of red rice extract for culture media with different volumes of inoculums

The diagram suggests that the optimal fungal cells concentration is between $1x10^5 – 2x10^5$ CFU/g medium to produce the red rice; smaller amounts of inoculum do not ensure the growth of *Monascus* strain and larger amounts cause a rapid growth of mycelium at the expense of pigments synthesis and red rice production. An important aspect of inoculation is represented by the requirement of an uniform distribution of cells in the medium.

Tween 80 (polysorbate 80) is a hydrophilic nonionic surfactant and emulsifier used in foods and medicine. The supplementation of culture media with this substance was studied in order to improve the pigments production because the extrusion of the pigments through the hyphal walls is known to be facilitated by Tween 80. This compound increases the permeability of cellular membrane to the nutrients and metabolic products. The analysis of pigments demonstrates that the addition of 0,1 g of Tween 80 in 100 g culture medium determines a good production of red rice. For larger quantities of this surfactant it occurs rather an accumulation of yellow pigments. Another avantage of Tween 80 is this substance prevent the particles of rice to become adherent and sticky.

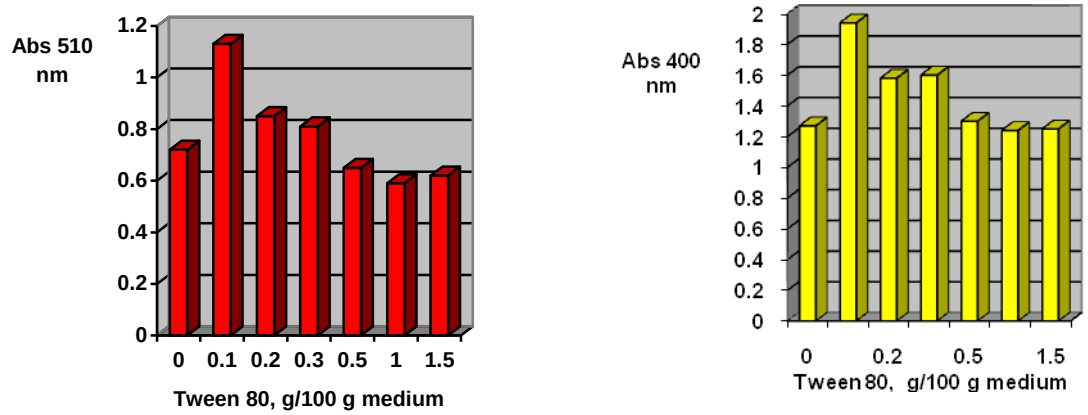
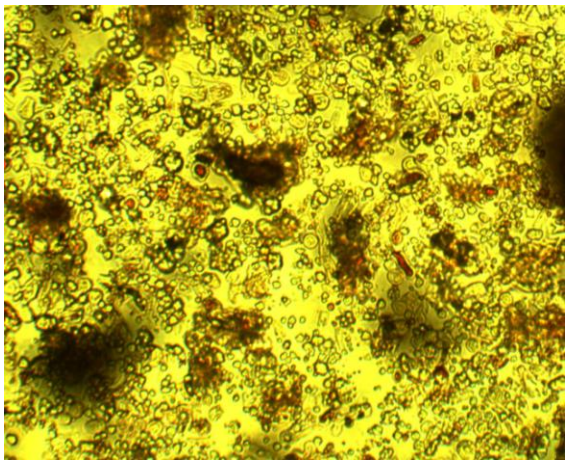


Figure 5: The absorbance values at 510 nm (red pigments) and 400 nm (yellow pigments) in culture media with different quantities of Tween 80

The influence of growth temperature on red rice production was studied at 20 ^oC , 30 ^oC and 37 ^oC. The cultures were dried at 60 ^oC and then the red and yellow pigments were extracted in Et-OH (extraction ratio 1:1000). The values of absorbances were 0,47 at 510 nm and 1,03 at 400 nm for the red rice obtained at 20 ^oC, that represent about 50% from the values of absorbances for the cultures grown at 30 ^oC (0,9 at 510 nm and 1,87 at 400 nm) and 37 ^oC (1,1 at 510 nm and 1,8 at 400 nm). Therefore, the optimum temperature range is between 30 ^oC and 40 ^oC, higher temperatures inhibiting the growth of *Monascus* strain.

Figure 6 illustrates the microscopic aspect and the visible absorbtion spectrum of red rice obtained with Monascus purpureus M5 mutant strain.



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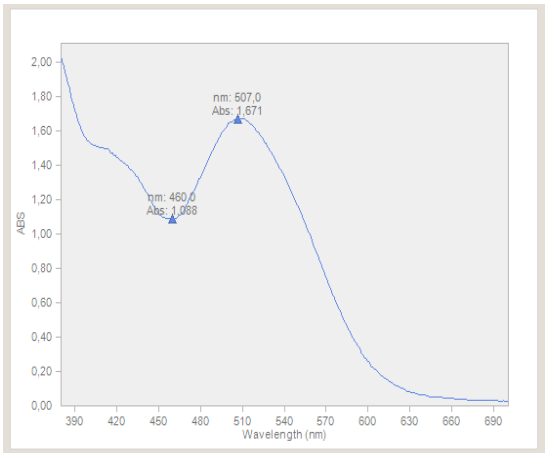

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Figure 6: Microscopic aspect a) and visible absorbtion spectrum b) of red rice

3. CONCLUSIONS

The red rice represents a multifunctional biomaterial used as a pharmaceutical compound, as food dye and preservative, known for centuries in Asian countries. In recent years the considerable properties of this material have been highlighted and valued.

The red mold rice was obtained using the M5 *Monascus* mutant strain isolated after electron beam irradiation at 2 KGy dose. The fungal strain was grown on ground rice in Solid State Fermentation system, in Erlenmeyer flasks for 2 weeks. The influence of moisture content of culture media, amount of inoculum, temperature and Tween 80 have been studied.

The optimal growth conditions were established by analysis of synthesized red and yellow pigments and the absorbances at 510 nm and 400 nm respectively were determined in the Et-OH extract (extraction ratio 1:1000). *Monascus purpureus* M5 strain grows best at 30 ^{o}C, on rice with moisture content of 25%, inoculated with 1,5x10^{5} - 2x10^{5} conidiospores/g medium supplemented with Tween 80 0,1 g/100 g culture medium.

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