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Antioxidant Properties of Fungal Biomaterial

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The antioxidant properties of methanolic extracts from extract of parental and modified Monascus sp were studied and evaluated. The fungus was inoculated into different submerged media and the produced red pigment was separated by filtration and methanol extraction from the filtrate. The antioxidant or prooxidant properties of ethanolic extract was evaluated by measuring the quenching ratio of chemiluminescence intensity. The results obtained showed a quenching ration of 87.75% for parental strain, growth 96 h in culture media with 50% maltose. The mutant strain reveals less antioxidant properties, the value of quenching ratio being 4.91%. If the growth time increased, the extract showed a prooxidant activity. At the same time the extract of mutant strain grown 96 H in the culture media containing meat extract reveals excellent antioxidant property, quantified by quenching ratio of 93.36%. When the mutant strain is grown 96 H in a culture media, which contain 30% dextrose, the methanolic extract presents an antioxidant effect with quenching ratio of 98.55%.

Keywords Antioxidant properties; biopigment; solid state submerged biosynthesis

Introduction

The fungus *Monascus sp.* possesses functional components effective in improving human health. The components isolated from the fungus exert several biological actions and produce hypocholesterolemic liver-protective and antitumor effects. Phytochemicals such as phenolics and flavonoids, which are present in raw material (rice grains), are associated with reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and certain cancers. Antioxidant components, like ascorbic acid, tocopherols, and total phenols, were found in methanolic extracts from various rice products, (see Table 1) [1–2]. β -Carotene was not found whereas the contents of ascorbic acid and tocopherols were in the range of 0.05–0.25 mg/g. However, total phenols were the major naturally antioxidant compounds found, as well as the methanolic extracts from monascal rice. These products contain higher amount of polyphenols than uninoculated rice products.

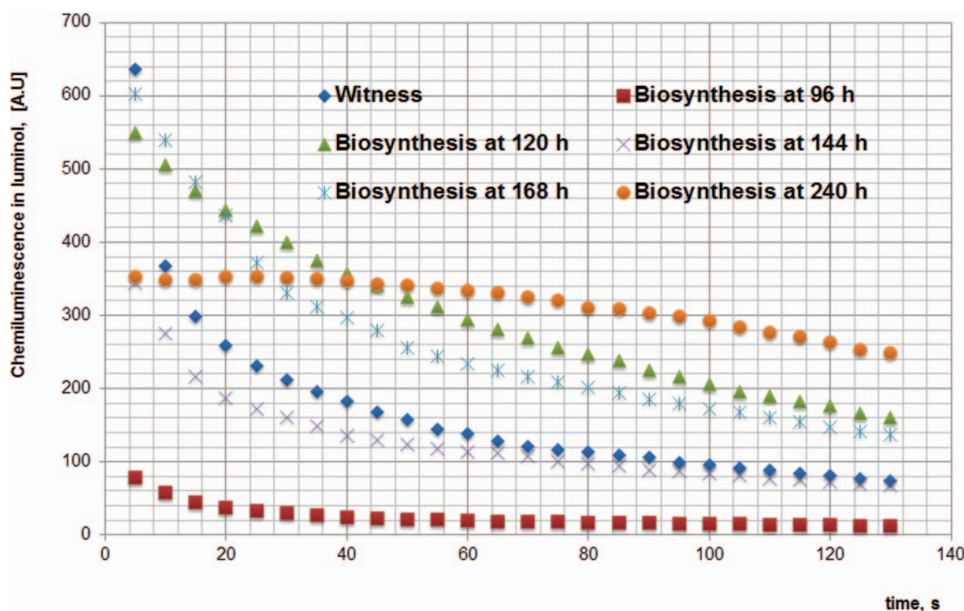
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Table 1. Contents of ascorbic acid, β -carotene, tocopherols and total phenols of methanol extracts from various rice products [1]

| Compound | Content, (mg/g) | | | |
|----------------------|-----------------|------------------|------------------------|------------------------|
| | Polished rice | Dehulled rice | Monascal polished rice | Monascal dehulled rice |
| Ascorbic acid | 0.25 ± 0.04 | 0.06 ± 0.03 | 0.23 ± 0.02 | 0.12 ± 0.02 |
| β -Carotene | Not detected | Not detected | Not detected | Not detected |
| α -Tocopherol | 0.08 ± 0.03 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.12 ± 0.03 |
| γ -Tocopherol | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.04 ± 0.01 |
| δ -Tocopherol | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| Total phenols | 4.04 ± 0.13 | 11.68 ± 0.18 | 40.39 ± 0.03 | 15.41 ± 0.3 |

Objectives And Methodology

Our objective was to evaluate the antioxidant properties of methanolic extracts from the extract of parent and modified *Monascus sp.* The fungus was inoculated into different media [2] (solid state on rice or submerged media) and the produced red pigments were separated by filtration or methanol extraction. The antioxidant or prooxidant properties of methanolic extract was evaluated by measuring the quenching ratio of chemiluminescence intensity [3], using a Turbo Design chemoluminometer TD 20/20. Quantitative measurement of antioxidant capacity was performed using Oxygen radical absorbance capacity (ORAC method), with TROLOX as reference, using a JASCO fluorimeter FP 6500.

**Figure 1.** Antioxidant properties of *Monascus* pigment in alcoholic media (parental strain).

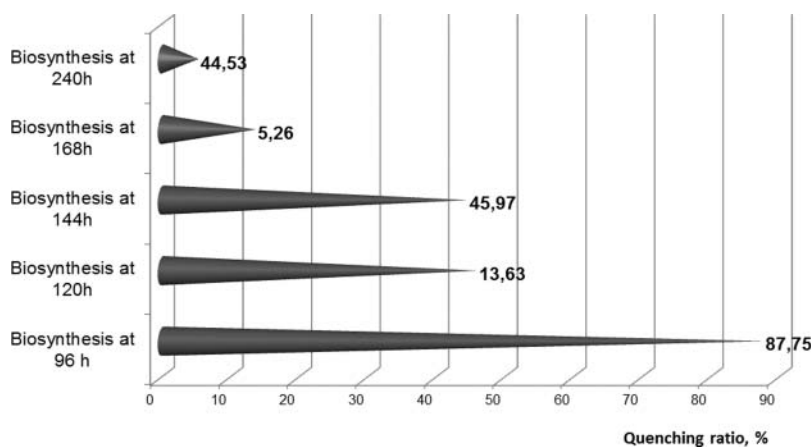


Figure 2. Quenching ratio of alcoholic pigment solution at different time of biosynthesis.

Results And Discussion

The qualitative evaluation of antioxidant properties of biomaterials obtained in submerged media, at different time of biosynthesis, shows in the case of parental strain good antioxidant properties as it can be seen in Fig. 1. Although the chemiluminescence signal for the parental strain extract (96 h) is smaller than for the witness, the quenching ratio is 87.75% (Fig. 2). The biomaterial obtained after 120 h and 144 h shows a similar quenching ratio of chemiluminescence, respectively 45.97% and 44.53%, which is smaller than the quenching ratio of chemiluminescence obtained for the biomaterials obtained after 96 h (87.75%). Higher is the biosynthesis time, smaller is the quenching ratio of biomaterials. Thus for a biosynthesis time of 168 h the quenching ratio of chemiluminescence of obtained biomaterials was 13.65% while for a biosynthesis time of 240 h the quenching ratio of chemiluminescence is 5.26%. Among the bioproducts separated from modified strain developed in the same conditions only the bioproduct separated at 96 h shows a small antioxidant properties, (results presented in Figs. 3 and 4) having a quenching ratio of chemiluminescence of 4.91%. If the time of biosynthesis increases the separated bioproducts reveal prooxidant properties because the quenching ratio of chemiluminescence is negative [$-17.8\% \div -138.52\%$]. The culture medium determines the antioxidant properties of bioproduct. For intracellular pigment obtained from *Monascus* modified strain growth on modified Lin media (rice and dextrose) and for the extracellular pigment obtained from the same strain in the culture media which contain yeast extract the chemiluminescence signal is situated under the signal for witness with a quenching ratio of 98.66% and 93.37%, as it can be seen in figure 5. The powerful antioxidant properties of biomaterials obtained on YDM media (yeast extract and dextrose culture media) are predictable, due to the complex composition of yeast extract: protein (70–75%), polysaccharidic compounds type mannan and glucans (5%), similarly as for oligosaccharidic compounds and vitamins like thiamine (B10), riboflavin (B2), B6, folic acid, Ca pantothenate, biotin and glutamic acid. At the same time the Lin modified media contain glutamic acid, which is a powerful hydrogen donor, enhancing also the antioxidant properties. Moreover during the biosynthesis, the glutamic acid and glucose were transformed into proline, a nonessential aminoacid with antioxidant properties [4]. The quantitative evaluation of antioxidant capacity of *Monascus* bioproducts presented in Fig. 6 shows that the best antioxidant capacity ($483.41 \mu\text{mol}$ of Trolox equivalent/mg

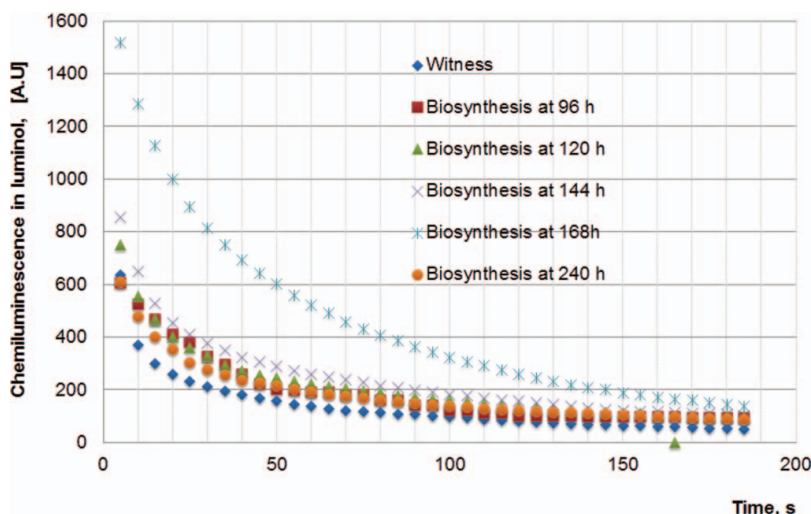


Figure 3. Antioxidant or prooxidant properties of *Monascus* pigment in alcoholic media (modified strain).

sample ($\mu\text{mol T.E./mg}$) is obtained for the solid state biosynthesis on polished rice or brown rice. When the biosynthesis was performed on the white rice (polished rice) with the same strain, the antioxidant capacity of resulted biomaterial was of $100 \mu\text{mol T.E./mg}$. This result is due to the rice content in phytic acid which exhibits an antioxidant effect (brown rice contain 0.84–0.99%, while white rice 0.14–0.5% [5–7]). Similar values were obtained with intracellular pigment obtained from *Lin* modified media with the same microorganisms. Parental strain growth on the white rice reveals a good antioxidant capacity of $321.19 \mu\text{mol T.E./mg}$. These results are in agreement with other studies [8] in which the phenolic and flavonoid compounds from rice grains (raw material in the solid state biosynthesis) contribute to the antioxidant activity. Tandem mass spectrometric techniques revealed the antioxidants identified in wild rice to be flavonoid glycosides (diglucosyl apigenin, glucosyl-arabinosyl epigenin, and diarabinosyl epigenin) and flavan-3-ols (catechin, epicatechin, and oligomeric procyanidin) [8]. For dark (brown) rice samples, DPPH radical

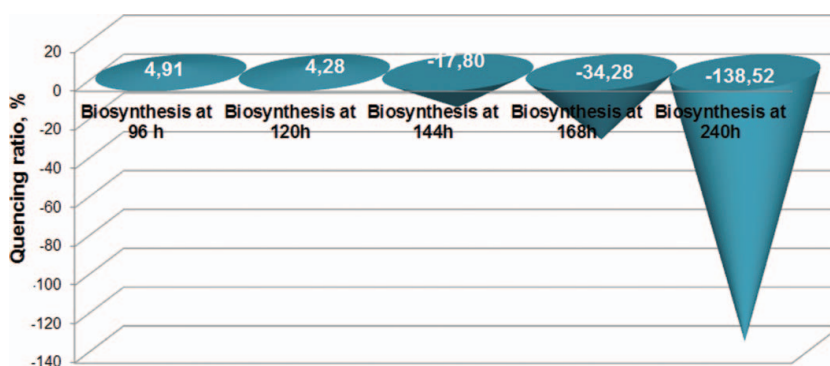


Figure 4. Quenching ratio of alcoholic pigment solution at different time of biosynthesis (modified strain).

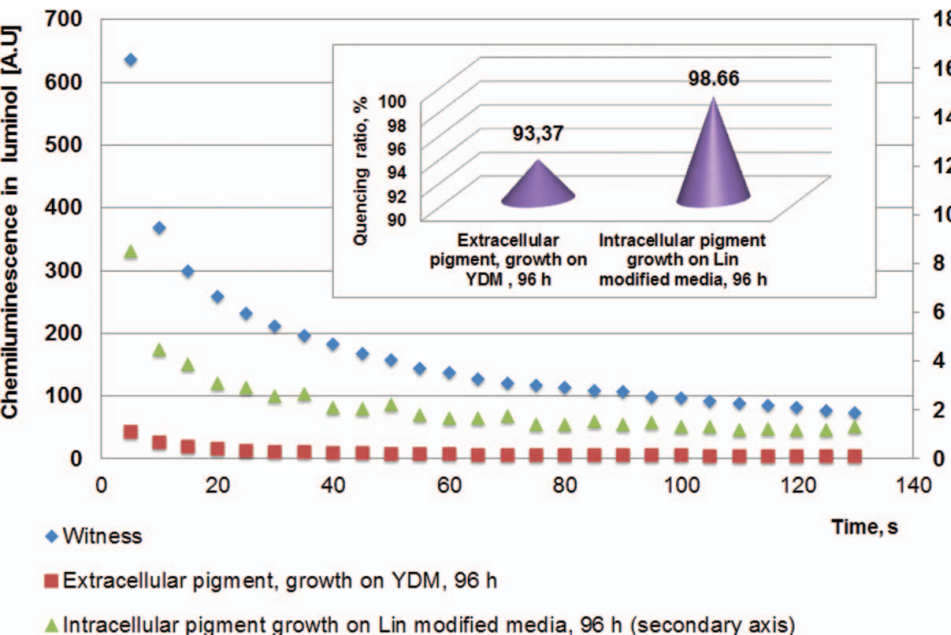


Figure 5. Antioxidant properties of intracellular or extracellular *Monascus* sp. pigments in alcoholic media (modified strain).

scavenging activities and ORAC values ranged from 611 to 917 μmol of Trolox equivalent (TE)/100 g and from 4069 to 6064 μmol of TE/100 g, respectively. For mixed and processed wild rice, DPPH radical scavenging activities were 373 and 441 μmol of TE/100 g, respectively. The corresponding ORAC values were 2284 and 2557 μmol of TE/100

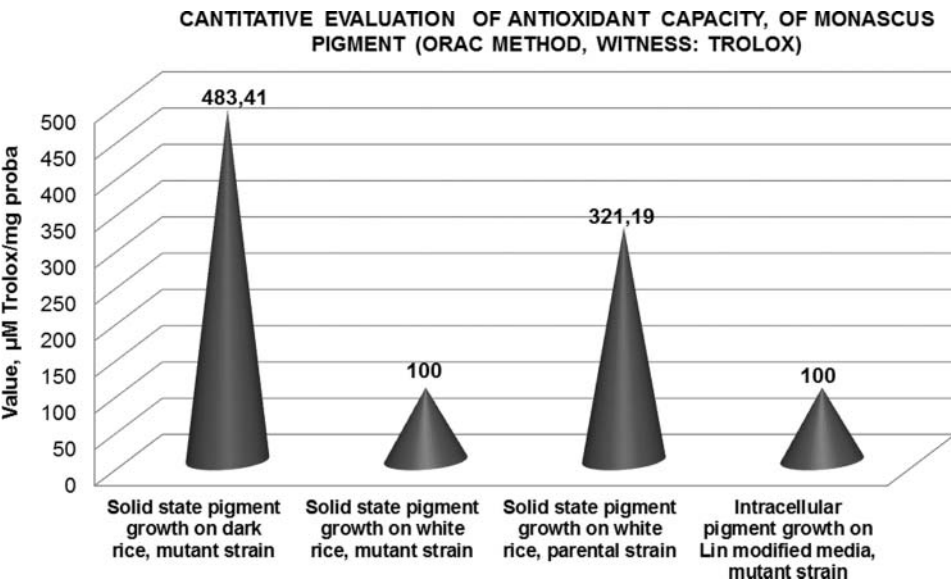


Figure 6. Quantitative evaluation of antioxidant properties of intracellular or extracellular *Monascus* sp. pigments in alcoholic media (modified strain).

g. Total phenolic content (TPC) of raw wild rice varied from 2472 to 4072 mg of ferulic acid equivalent (FAE)/kg, higher than that of the mixed sample (1460 mg of FAE/kg) and processed sample (2076 mg of FAE/kg) [8].

Conclusions

The studies on antioxidant properties of metabolite produced of two *Monascus* species (a parental and a modified strain) reveal the following: antioxidant activities were good for parental strain, grown 96 h in culture media with 50% dextrose, when the quenching ratio was 87.75%. The mutant strain reveals less antioxidant properties, the value of quenching ratio being of 4.91%. If the growth time is increased the extract reveals a prooxidant activity. At the same time the extract of mutant strain grown 96 h in the culture media containing yeast extract shows excellent antioxidant property, quantified by quenching ratio of chemiluminesce of 93.36%. If the mutant strain is grown 96 h in the culture media which contains 30% of dextrose, the methanolic extract gives the antioxidant effect with quenching ratio of chemiluminesce 98.55%.

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