

## Antimicrobial effect of *Monascus purpureus* red rice against some bacterial and fungal strains

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The aim of this study is to evaluate the antimicrobial activity of red yeast rice obtained from a *Monascus purpureus* mutant strain. There is at present a growing interest in evaluating red rice for use as a natural food dye and/or preservative. The fungus was grown over PDA in tubes at 30°C and the spores suspension was used to inoculate ground steamed rice to produce the red colorant. The antimicrobial effect of *Monascus* culture, due of monascidin A, confirmed by scientific investigations, was proved against some bacterial and fungal strains. The brut pigment obtained by growing the *Monascus* strain in surface culture had a 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*. The observation of bacteriostatic and antifungal effects has lead to the consideration that besides the tinctorial properties, the pigments of *Monascus purpureus* have a preservative value.

### 1. Introduction

Among pigments produced by fermentation, one of the most important are *Monascus* pigments, which have been used for centuries as food colors in eastern countries, and which present potential use in meats, beverages, sauces and soups. *Monascus purpureus* is a red mold which may be cultivated on starch containing substrates (Carvalho et al, 1995 ; Dominguez-Espinosa et al, 2003). Red yeast rice obtained with *Monascus* fungus has been used in China as both a food and as a medicinal substance and is also mentioned in an ancient Chinese pharmacopoeia of medicinal foods and herbs, the Ben Cao Gang Mu of Li Shi-zhen published in 1590. Manufacturing procedures of red rice were described in addition to the therapeutic activities, including the bettering of digestion and revitalizing the blood (Erdogrul et al. 2004). The traditional indications are diarrhea and intestinal troubles, muscular contusions, dyspeptic colics in children and circulatory diseases.

Recently, it has been discovered that the red yeast rice contains substances that are similar to prescription medications that lower cholesterol, such as a group of

antihypercholesteromic agents, including monakolin K and the hypotensive agent  $\Gamma$ -aminobutyric acid and antibacterial compounds (Li et al, 2004).

The antibacterial activity of *M. purpureus* was first reported in 1977 (Wong et al, 1981, Wang et al, 2002). Several wild and mutant strains of *Monascus purpureus* were shown to produce antibiotics active against *Bacillus*, *Streptococcus* and *Pseudomonas*, three genera of bacteria generally found in spoiling foods.

## 2. Materials and methods

### 2.1 Fungal strain

*Monascus purpureus* M5 strain which was induced by electron beam irradiation for higher pigment productivity was used for the study of red mold rice antimicrobial properties. The mutant strain was cultivated in tubes on potato-dextrose-agar at 30 °C for a week.

### 2.2 Pigments biosynthesis

The cultivation of *Monascus* M5 was carried out in solid-state fermentation on moist rice, in 1500 mL Erlenmeyer flasks, at 30 °C, for 14 days, in darkness. The culture medium was dried, ground and treated to reduce microbial load. The final product was a dark-red powder with tinctorial properties.

### 2.3 Bioassay of antibiotic

#### 2.3.1. Antibacterial activity

Antibacterial activity was analysed against 2 strains of *Bacillus subtilis*, 2 strains of *Pseudomonas aeruginosa* and 1 strain of *Escherichia coli* using paper discs, 6 mm diameter, placed on nutritiv agar surface. The petri dishes were inoculated in surface with 1mL of suspension of bacterial cells and then the paper discs were applied. Each disc contained 15  $\mu$ L of extracts in ethanol or n-hexan from red rice, for a 1:10 extraction ratio. The data presented are average of four replicates.

#### 2.3.2. Antifungal activity

Antifungal properties of red rice obtained from *Monascus* were estimated against several mold strains of *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Alternaria* and *Botrytis* genus. The analyze of antifungal activity of red rice was carried out using potato-dextrose-agar medium in petri dishes inoculated in central zone with 2  $\mu$ L suspension of conidiospores from each mold. The diameters of cultures were measured in control dishes and in the experimental plates containing culture medium supplemented with 0.5% red rice powder and there were calculated the average growth rates. The inhibition ratio was estimated using the formula:

$$\text{inhibition ratio (\%)} = \frac{C - E}{C} \times 100$$

where C is the diameter of mold colony from control plate and E is the diameter of the mold colony growth in experiment plate which contains the medium with red rice.

### 3. Results and discussion

The *Monascus purpureus* strain has been selected because of the traditional use of this mold in Asian countries, as well as of its lack of toxicity and higher pigment productivity. This mold has been characterized from its morphocolonial microscopic aspect point of view, and for its pigment and antimicrobial substances production.

On potato-dextrose-agar medium in petri dishes, *Monascus purpureus* forms fluffy, red-coloured with orange reverse colonies which present an average growth rate of 0.20 mm/hour at 30°C.

On microscopic slides there could be observed septate hypha, with hyaline walls and 3-5 µm diameter. These hypha presents sexuate asca with ascospores, closed in ascocarpe, and asexuate conidiospores. The ascocarpe, of globular shape with the diameter of 20 - 70 µm, forms yellowish ascospres, presented in oval-elypsoide shape, often with hyaline wall, with 5-6 x 3-4 µm as dimensions. In the case of asexuate reproduction, the conidiospores are chained, in basipetale sucesion, and presenting an ovate to pyriform shape and dimensions of 6-8 x 5-6 µm. In most of the cases the conidiospores presents thin walls, but they could function as chlamidospores too, when the walls become thicker.

The mixture of pigments synthetized by *Monascus* mold grown on rice culture medium, has been extracted on 95% ethylic alcohol to an extraction ratio of 1:2000 and presents for its visible spectra an absorption value of 1.14 at 400 nm (corresponding to yellow components) and a value of 0.649 at 510 nm (that correspond to red pigments).

#### *Antibacterial activity*

The study of antibacterial activity of *Monascus* red rice powder was performed effected using 2 types of extracts, obtained in ethanol and n-hexane (extract ratio 1:10). The values of diameters for the inhibition growth zones against 2 strains of *Bacillus subtilis*, 2 strains of *Pseudomonas aeruginosa* and 1 strain of *Escherichia coli* are presented in table 1.

*Tabel 1. The values of diameters of the inhibition zones for bacterial strains*

| Bacterial strain        | Inhibition zone diameter, mm |   |   |   |                  |    |    |    |
|-------------------------|------------------------------|---|---|---|------------------|----|----|----|
|                         | ethanol extract              |   |   |   | n-hexane extract |    |    |    |
| <i>B. subtilis B1</i>   | 9                            | 9 | 9 | 8 | 11               | 11 | 12 | 11 |
| <i>B. subtilis B2</i>   | 9                            | 9 | 9 | 9 | 12               | 12 | 12 | 12 |
| <i>P. aeruginosa P1</i> | 8                            | 8 | 8 | 8 | 9                | 9  | 9  | 9  |
| <i>P. aeruginosa P2</i> | 8                            | 8 | 8 | 8 | 9                | 9  | 9  | 9  |
| <i>E. coli</i>          | 8                            | 8 | 8 | 8 | 9                | 9  | 9  | 9  |

The values obtained for the inhibition zone diameters has demonstrated that the extracts obtained on fermented red rice presented a low antibacterial activity, but different as function of tested strain type. The two strains of Gram (+) bacteria belonging to

*Bacillus* genus has been more sensitive to the action of the extract containing *Monascus* pigments whereas the *Pseudomonas* Gram (-) strains has been less influenced by the extract presence. Despite the inhibition zone has been minimal, around the disc impregnated with pigment extract, the culture formed smaller colonia, with a slightly transparent aspect. Also, it has been demonstrated that the n-hexane extract presented an increased antibacterial activity, comparing with the alcoholic extract.

#### *Antifungal activity*

Antifungal activity was estimated using 10 fungal strains as indicator microorganisms, cultivated on potato-dextrose-agar (control plates) and on the same medium supplemented with 0.5% red rice powder (experiment plates). The diameters of colonies were measured and the growth rates and inhibition ratio values were calculated for each fungal strain.

Table 2. Values of diameters of fungal colonies and average growth rates for fungal strains

| Fungal strain                | Growth time, hours | Colony diameter,mm |            | Average growth rate, mm/h |            |
|------------------------------|--------------------|--------------------|------------|---------------------------|------------|
|                              |                    | control            | experiment | control                   | experiment |
| <i>Aspergillus niger</i>     | 120                | 66                 | 65         | 0.55                      | 0.54       |
| <i>Aspergillus ochraceus</i> | 120                | 69                 | 62         | 0.57                      | 0.52       |
| <i>Aspergillus terricola</i> | 72                 | 62                 | 56         | 0.86                      | 0.77       |
| <i>Aspergillus flavus</i>    | 72                 | 68                 | 54         | 0.94                      | 0.75       |
| <i>Fusarium oxysporum</i>    | 120                | 82                 | 59         | 0.68                      | 0.49       |
| <i>Fusarium roseum</i>       | 168                | 89                 | 66         | 0.53                      | 0.39       |
| <i>Alternaria alternata</i>  | 168                | 59                 | 41         | 0.35                      | 0.24       |
| <i>Penicillium hirsutum</i>  | 120                | 47                 | 35         | 0.39                      | 0.29       |
| <i>Botrytis cinerea</i>      | 120                | 89                 | 60         | 0.74                      | 0.50       |
| <i>Mucor sp.</i>             | 72                 | 60                 | 34         | 0.83                      | 0.51       |

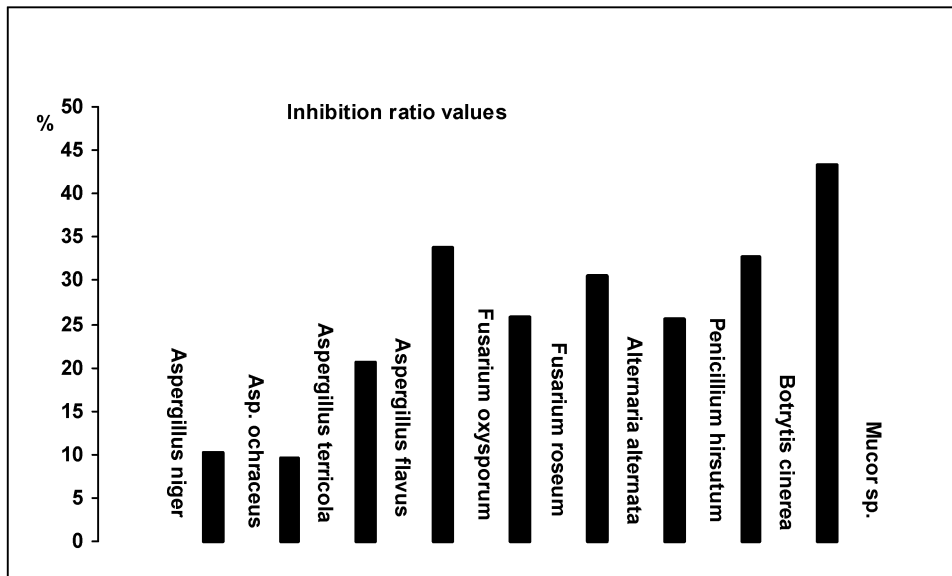


Figure 3. Comparative values of inhibition ratio for the mold colonies grown on media with *Monascus red rice*

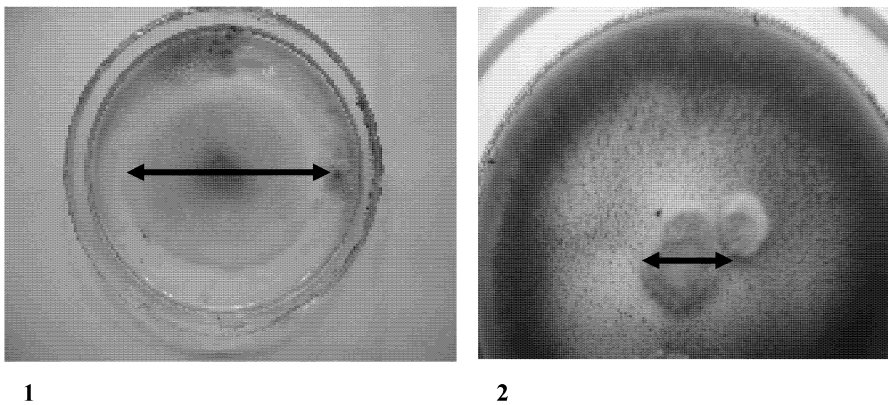


Figure 4. *Mucor* colonies grown on potato-dextrose-agar (1) and on medium supplemented with red rice powder (2)

The assay of antifungal activity by using the culture medium with 0.5% red rice powder demonstrated that, excepting the *Aspergillus niger* strain, all fungal strains were more or

less inhibited in presence of this natural food dye. These fungal colonies showed values of inhibition ratio higher than 10% and a maximum value of 43% for *Mucor sp.* Moreover, the culture medium with red mold rice powder altered the aspect of some fungal colonies comparatively with the control colonies: the *Fusarium* and *Alternaria* strains are less grown and the production of spores is diminished, while the *Aspergillus* colonies have the same aspect.

#### 4. Conclusions

The antibacterial and antifungal activity of red rice obtained from *Monascus purpureus* M5 strain against some microbial strains is due of monascidin A, the main compound responsible for the inhibitory activity. Antimicrobial properties of red rice was determined using 2 strains of *Bacillus subtilis*, 2 strains of *Pseudomonas aeruginosa*, a strain of *Escherichia coli* and many species of genera *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Alternaria* and *Botrytis*. Antimicrobial activity of *Monascus* pigments proved the preservative characteristics of this natural food colorant.

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