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Antibacterial and antifungal activity of red rice obtained from *Monascus purpureus*

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The paper presents the study of the antimicrobial activity of red yeast rice obtained from a *Monascus purpureus* 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 rice.
Antibacterial activity was analysed against strains of *Streptomices sp*., *Pseudomonas aeruginosa* and *Bacillus subtilis* using paper discs, 6 mm diameter, placed on nutritive agar surface. The petri dishes were inoculated in surface with 1 mL of suspension of bacterial cells and then the paper discs were applied. Each disc contained 15 μL of extracts in ethanol or n-hexan from red rice, for a 1:10 extraction ratio.
Antifungal properties of red rice obtained from *Monascus* were estimated against several mold strains of *Trichoderma viridae*, *Fusarium oxysporum*, *Fusarium roseum*, *Mucor*, *Aspergillus ochraceus, Aspergillus oryzae*, *Penicillium roqueforti* and *Aspergillus usamii* 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 μL 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 1% red rice powder and there were calculated the average growth rates.
Antimicrobial activity was analysed at the same time against strains of *Rhodotorula sp*., *Candida utilis*, *Torulopsis utilis*, *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*, *Saccharomyces fibuligera, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptomyces*, *Fusarium oxysporum*, *Fusarium roseum*, *Alternaria alternata*, *Penicilum camemberti*, *Penicilum hirsutum*, *Trichoderma viridae*, *Mucor pusillus*, *Aspergillus oryzae* by streak method.

1. Introduction

Monascus purpureus is a red mold species that can be grown on the substrate containing starch.
Monascus purpureus is used to obtain products with many different uses which led to a broader use - especially in the Orient; In Asia this product has a big tradition in southern

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China, *Monascus purpureus* is used for 2000 years in medicine and food (Carvalho et al., 1995);
The first writing about this fungi appeared in 1590: "Ben Cao Gang Mu - Dan Shi Bu Yi" (Chinese Pharmacopoeia) which states that *Monascus purpureus* is used to treat various diseases: infections, diarrhea and indigestion. This information has occurred since ancient times, from Ming Dynasty (1368 - 1644), and the qualities of traditional use of *Monascus* in Chinese cuisine but also in some medical books, scientific treated in Japan, China, Korea, India, USA and Germany; In China it is known as "Ang kak" or "Hong Qu" in Japan "Koji" (Dominguez-Espinosa and Webb C., 2003).
In Europe the *Monascus* pigments have become known because of germans scientists' research (Erdogrul and Azirak, 2004)
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 antihyper cholesteromic agents, including monakolin K (Li et al, 2004).

2. Materials and methods

2.1 Strains

Bacteria: *Bacillus subtilis, Pseudomonas aeruginosa, Streptomyces albus*
Yeasts: *Rhodotorula sp*., *Candida utilis*, *Torulopsis utilis*, *Saccharomyces cerevisiae*, *Saccharomyces fibuligera*
Molds: *Fusarium oxysporum*, *Fusarium roseum*, *Alternaria alternata*, *Penicillium roqueforti*, *Penicillium camemberti*, *Penicillium hirsutum*, *Trichoderma viridae*, *Mucor pusillus*, *Aspergillus ochraceus*, *Aspergillus oryzae*, *Aspergillus usamii.*
Monascus purpureus strain was cultivated in tubes on potato-dextrose-agar (M3) at 30 ^{o}C for a week.

2.2 Pigments biosynthesis

The cultivation of *Monascus* was carried out in solid-state fermentation on moist rice, in 1500 mL Erlenmeyer flasks, at 30 ^{o}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 analyzed against 2 strains of *Bacillus subtilis*, 2 strains of *Pseudomonas aeruginosa* and 1 strain of *Streptomyces albus* using paper discs, 6 mm diameter, placed on nutritive 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 μL 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 *Fusarium oxysporum*, *Fusarium roseum*, *Alternaria alternata*, *Penicillium roqueforti*, *Penicillium camemberti*, *Penicillium hirsutum*, *Trichoderma viridae*, *Mucor pusillus*, *Aspergillus ochraceus*, *Aspergillus oryzae*, *Aspergillus usamii* 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 μL 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 (M5) and there were calculated the average growth rates. The inhibition ratio was estimated using the formula:

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

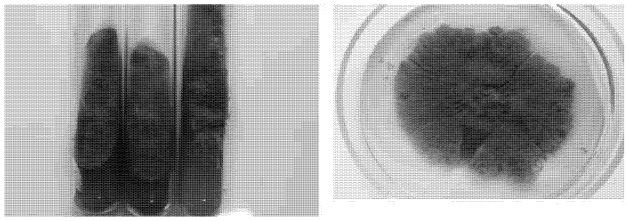
2.3.3. Antimicrobial activity

In Petri dishes containing potato-dextrose-agar medium there was drown a streak from the *Monascus purpureus* culture and after 6 days another perpendicular lines from the *Rhodotorula sp.*, *Candida utilis*, *Torulopsis utilis*, *Saccharomyces cerevisiae*, *Saccharomyces fibuligera, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptomyces*, *Fusarium oxysporum*, *Fusarium roseum*, *Alternaria alternata*, *Penicilum camemberti*, *Penicilum hirsutum*, *Trichoderma viridae*, *Mucor pusillus*, *Aspergillus oryzae* cultures. The distances between streaks were observed.

3. Results and discussion

Morphological characterization of *Monascus purpureus* strain

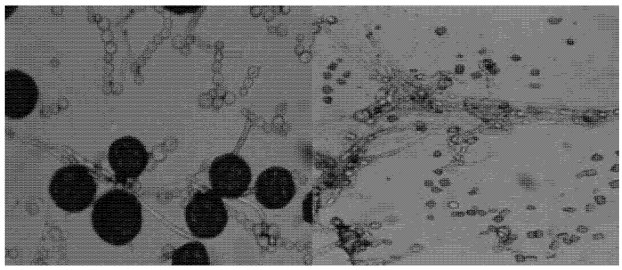
Monascus purpuresus strain has been characterized from its morphocolonial microscopic aspect point of view, and for its pigment and antimicrobial substances production. On potato-dextrose-agar medium in petri dishes, *Monascus purpureus* forms fluffy, red colored with orange reverse colonies which present an average growth rate of 0.20 mm/hour at 30°C.



a) *b)*

Figure 1. Culture of Monascus purpureus on the potato-dextrose-agar: a) in tubes; b) in petri dishes

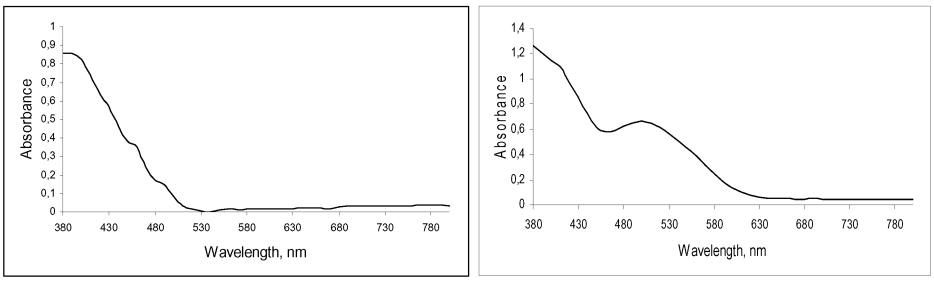
On microscopic slides there could be observed septate hypha, with hyaline walls and 3-5 μm diameter. These hypha presents sexuate asca with ascospores, closed in ascocarpe, and asexuate conidospores. The ascocarpe, of globular shape with the diameter of 20 - 70 μm, forms yellowish ascospores, presented 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 succession, 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.



a) b)

Figure 2. Microscopic appearance: a) the culture of Monascus purpureus; b) crystals of pigment visible on microscopic preparation

The mixture of pigments synthesized by *Monascus* mold grown on rice culture medium, has been extracted on 96% 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).



a) b)

Figure 3. The visible spectrum for the extract in a) n-hexane and b) 96% ethanol

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. The values of diameters for the inhibition growth zones against 2 strains of *Bacillus subtilis*, 2 strains of *Pseudomonas aeruginosa* and 1 strain of *Streptomyces albus* are presented in table 1.

Table 1. Results of the measurement of inhibition

	Inhibition zone [mm]	
Indicator strain	ethanol extract	n-hexane extract
Streptomyces albus	8	10
Bacillus subtilis	9	10
Pseudomonas aeruginosa	9	10

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 colonies, 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.

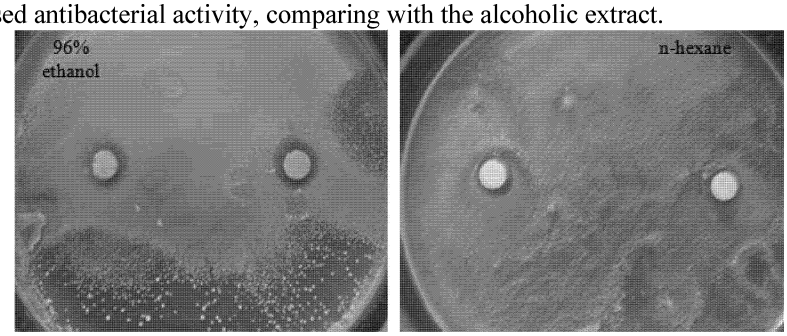


Figure 4. Culture of Bacillus subtilis inhibited by the extract in a) 96% ethanol and b) n-hexane

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

	hourly growth rate, [mm/h]						
	Trichoderma viridae	*Fusarium oxysporum*	*Fusarium roseum*	*Mucor pusillus*	*Aspergillus ochraceus*	*Aspergillus usamii*	*Penicillium roqueforti*
M3	0.53	0.37	0.18	0.220	0.220	0.176	0.184
M5	0.35	0.20	0.37	0.147	0.183	0.220	0.220

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.

3.1.1. Antimicrobial activity

In Petri dishes containing potato-dextrose-agar medium there was drown a streak from the *Monascus purpureus* culture and after 6 days another perpendicular lines from the *2.3.3* cultures. The distances between streaks were observed.

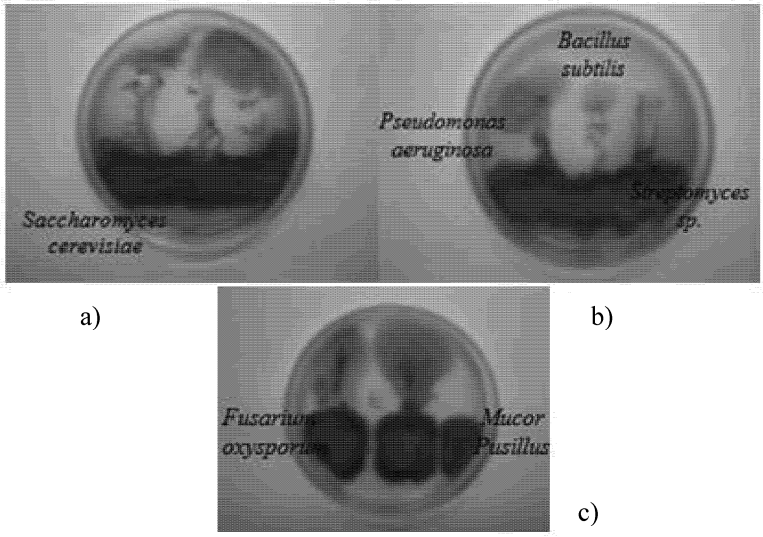


Figure 5. Antimicrobial activity for a) yeasts, b) bacteria and c) fungus

4. Conclusions

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 *Streptomyces sp*. 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.

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