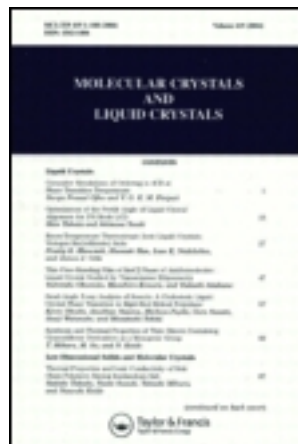


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Biostimulatory Properties of *Monascus sp.* Bioproducts

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The therapeutic metabolites properties of microorganisms obtained from Monascus sp in solid state biosynthesis in submerged media were studied and are presented. Some products isolated from solid state biosynthesis or from submerged media, were investigated in order to establish their biological or physico - chemical properties. The primary tests performed in order to establish the antioxidant properties revealed a strong antioxidant capacity situated in the range (1420 ÷ 3700) mMol Trolox Equivalent/mg product for intracellular metabolites obtained in submerged media or in solid state biosynthesis. The immunomodulatory tests performed in vitro on a monocyte human cell line with two products isolated from solid state biosynthesis show the stimulation of human macrophage (by induction of the secretion of TNF- α and IL-6 with differentiate cell THP-1 type). The physico-chemical characteristics of investigated compounds performed by FT-IR spectroscopy show the presence of N glucosylrubropunctamine and N-glucosylmonascorubramine in biomaterials obtained by solid biosynthesis.

Keywords Antioxidant properties; biostimulators; *Monascus sp.1*; *Monascus sp.2*

Introduction

Monascus sp. is able to biosynthesized therapeutically metabolites, in submerged media or in solid state. The most common bioproducts are obtained in solid state biosynthesis, and in worldwide are used as raw materials in food preservation, in beverage production or as food supplement, with therapeutic action in hypercholesterolemia, blood pressure and so on [1–5]. The aim of this work is to establish the biological and physical-chemical properties of two bioproducts obtained in solid state biosynthesis: *Monascus sp.1* and *Monascus sp.2*.

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Table 1. Cytokine results, monitorized during the immunomodulatory tests of bioproducts obtained from *Monascus* sp. 1 and *Monascus* sp.2

Type cytokine measured	Cell producers	Target cells	Functions
IL-6	Lymphocyte T	Lymphocyte T	Co-stimulation
	Macrophage	Lymphocyte B	Proliferation'
	Fibriblast	Old Lymphocyte B	Protein synthesis
		Liver Cell	with acute phasis
TNF- α	Macrophage	Lymphocyte T	Cytotoxic effect
	Lymphocyte T	Lymphocyte B	Co-stimulation
	Mastocyte	Endothelial cells	Activation
	NK cell	Hypotalamus	(inflammation)
		Liver	Fever
		Tumoral cell	Protein synthesis with acute phases

Methodology

In this study, two species of *Monascus* were used: *Monascus* sp.1 and *Monascus* sp.2. Bioproducts are obtained in solid state biosynthesis, on rice, according to Ref. [6]. Biological tests like viability and immunomodulatory activity were performed in vitro according to method indicated in Refs. [7,8], using THP-11 differentiate cells. Solid biomaterial obtained with *Monascus* sp. 2, contain 50% monascorubramine and 145 ppm citrinin. The biomaterial obtained from *Monascus* sp.1 contains 5% monascorubramine without citrinin. The two bioproducts were tested in dimethyl sulfoxide (DMSO) solution, because they contain intracellular metabolites which are no soluble in water. Lipopolysaccharide isolated from *Escherichia coli* (LPS) was used as positive witness for all immunomodulatory tests.

During the tests, two type of cytokine measurements were monitored: IL-6 and TNF- α . The results are presented in the Table 1. The antioxidant properties were established in comparison with Trolox, using ORAC method (Oxygen Radical Absorbance Capacity) with a chemofluorimeter type 6270 [7]. The FT-IR spectra were recorded using the FT-IR Spectrum GX Perkin Elmer with ATR (attenuated total reflectance) spectrometer.

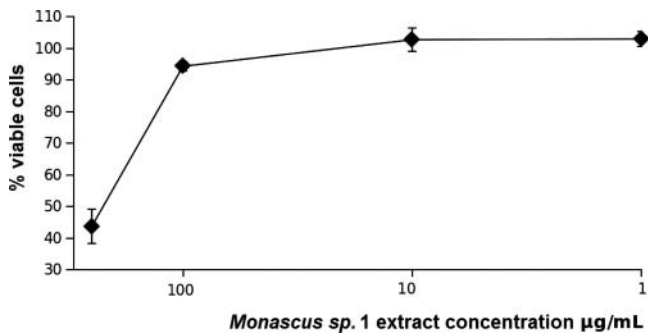


Figure 1. Viability of THP-1 cells treated with different concentrations of *Monascus* sp1. extracts (bioproduct dissolved in DMSO), during 24 h.

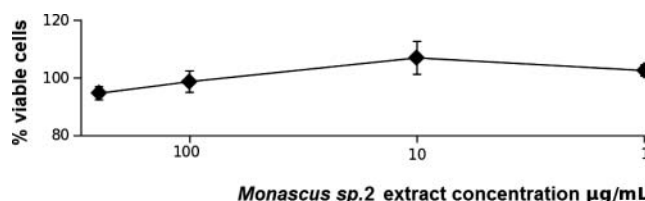


Figure 2. Viability of THP-1 cells treated with different concentrations of *Monascus sp.2* extracts (bioproduct dissolved in DMSO), during 24 h.

Results and Discussion

1. Cytotoxicity Effect

In the first step, we initiated the studies on cytotoxicity of both bioproducts, on the THP-1 differentiated cells, during 24 h. The obtained results indicate no cytotoxic effect for each product and for concentrations smaller than 100 µg/mL (Figs. 1 and 2). It should be underlined that the carrier (DMSO), used to solubilise both *Monascus* biomaterials, has no cytotoxic effect.

2. Regarding Cytokine Secretion

We observe significant differences between the two biomaterials, concerning secretion of IL-6. For biomaterials obtained from *Monascus sp.1*. THP-1 macrophage to produce IL-6 (Fig. 3) was not activated at any concentration. On the other side for the bioproduct obtained from *Monascus sp.2*, the IL6 secretion increased with increasing bioproduct concentration in the culture media. Important results were obtained in this case for a bioproduct concentration of 100 µg/mL in the culture media. The concentrations of IL-6 secreted by human macrophage is of 61.64 pg/mL (Fig. 4). All these results indicate that the bioproduct obtained from *Monascus sp.2* acts as a favourable factor in immune response of macrophage cells, because these are stimulated to produce IL-6 interleukin.

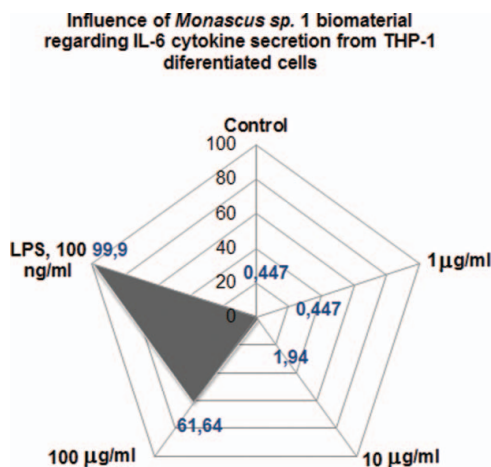


Figure 3. Effect of *Monascus sp.2* extract on IL6 cytokine secretion from THP-1 differentiated cells.

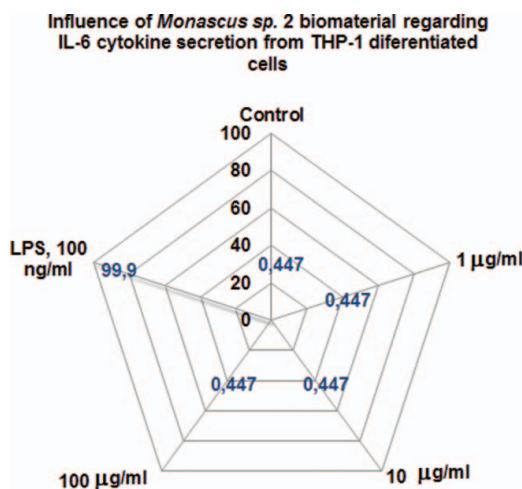


Figure 4. Effect of *Monascus sp.2* extract on IL6 cytokine secretion from THP-1 differentiated cells.

The results of experiments performed in order to establish the effect of both *Monascus* materials, regarding the cytokine secretion $\text{TNF-}\alpha$ have shown that:

- 1) the bioproduct obtained from *Monascus sp.1* induces the secretion of very small quantities of $\text{TNF-}\alpha$, in comparison with unstimulated cells (Fig. 5);
- 2) the bioproduct obtained from *Monascus sp.2*, at the concentration of 100 µg/mL, induces the secretion of great quantities of $\text{TNF-}\alpha$, larger than the cytokine secreted in the presence of LPS (Fig. 6): respectively 3731 pg $\text{TNF-}\alpha$ /mL at 100 µg/mL, in comparison with 1655 pg $\text{TNF-}\alpha$ /mL secreted in the presence of a powerful cytokine inducer like LPS.

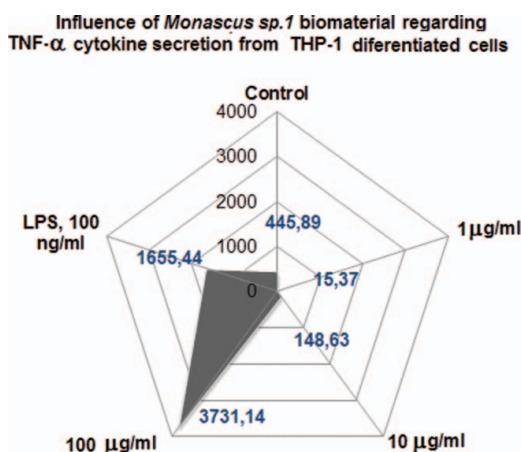


Figure 5. Effect of *Monascus sp.1* extract on $\text{TNF-}\alpha$ cytokine secretion from THP-1 differentiated cells.

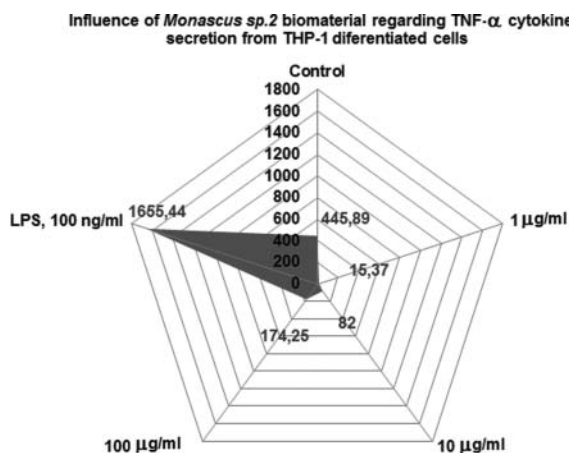


Figure 6. Effect of *Monascus sp.2* extract on TNF- α cytokine secretion from THP-1 differentiated cells.

Because the deregulation of TNF- α the cytokine secretion was involved in major human diseases, like Alzheimer or cancer, the bioproduct obtained from *Monascus sp.2* can be an important therapeutic bioproduct used in prevention of these kind of human diseases.

Basing on these results it is possible to recommend the use of bioproducts obtained from *Monascus sp.2* as inducers of TNF- α cytokine secretion, able to induce apoptotic cell death, to reduce inflammation and/ or to inhibit tumorigenesis and viral replication.

These results are confirmed by antioxidant properties of these biomaterials which increased with the quality of raw materials used in solid state biosynthesis. From this point of view, the best results are obtained for brown rice, for which we obtained the antioxidant capacity of 483.41 mmol TE/mg product, (TE–Trolox Equivalent) in comparison with the bioproduct obtained from white rice, for which the antioxidant capacity fluctuate in the range 285–321.19 mmol TE/mg product (Fig. 7).

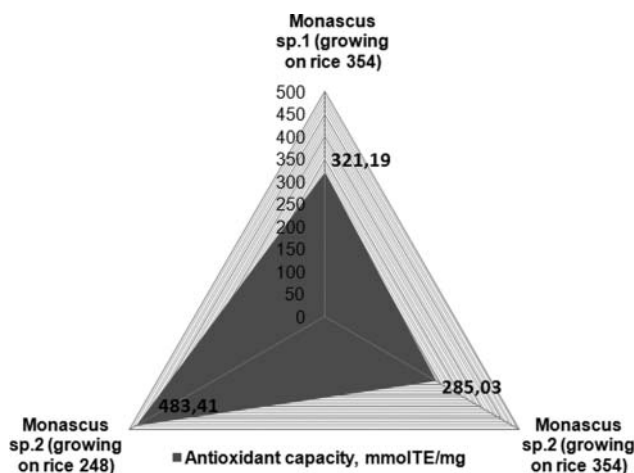


Figure 7. Antioxidant properties of biomaterials obtained from *Monascus sp.1* and *Monascus sp.2* in solid state biosynthesis.

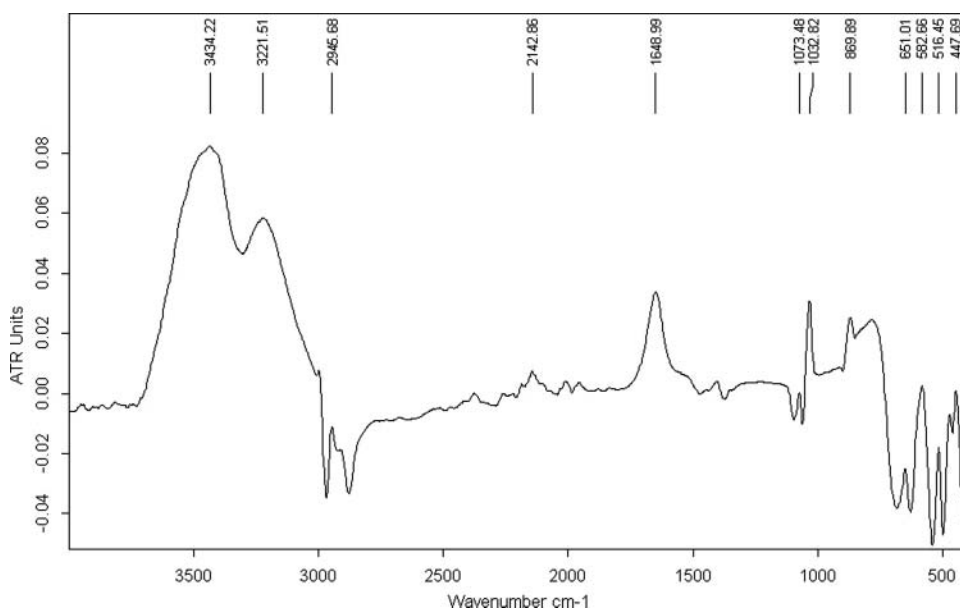


Figure 8. Infrared spectra for biomaterial obtained with *Monascus sp.2* on solid state biosynthesis.

The physico-chemical characteristics of biomaterials, investigated by FT-IR spectroscopy (Fig. 8), reveal the presence of monascorubrin, rubropunctatin, monascin and ankaflavin in the final product obtained by solid biosynthesis. It is seen by the presence of a band at 1073.48 cm^{-1} , characteristic for C-O-C binding [9]. The presence of yellow bioproducts is confirmed by the bands at 2142.86 cm^{-1} (C-N-C isocyanide) and the 3438.22 cm^{-1} (N-H secondary amide) [10]. The unsaturated ketone was identified in biomaterial by the band at 1648.96 cm^{-1} . The existences of alchil group in the chemical structure of the biomaterial is confirmed by the shoulder at 2945.68 cm^{-1} [9, 10]. The presence of the intense band at 3221.51 cm^{-1} reveals a great concentration of phenolic group derived from N-glucosylrubropunctamin and N-glucosylmonascorubramine, responsible of the antioxidant properties

Conclusions

The tests performed in order to establish the viability of THP-1 cells in the presence of biomaterials produced by two *Monascus sp.* strains reveal that their cytotoxic effects appear for concentrations higher than 100 mg/mL . The immunomodulatory tests performed in vitro on THP-1 human cells with two products isolated from *Monascus sp.* by solid state biosynthesis showed the stimulation of human macrophage by induction of the secretion of $\text{TNF-}\alpha$ and IL-6. The primary tests concerning the antioxidant properties revealed a good antioxidant capacity in the range $(285 \div 483)\text{ }\mu\text{mol TE/mg product}$. Physico - chemical characteristics of investigated compounds performed by FT-IR spectroscopy have shown the presence of rubropunctamine, monascorubramine, N-glucosylrubropunctamin and N-glucosylmonascorubramine in the biomaterials obtained by solid state biosynthesis.

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