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Cytotoxicity Study Regarding Some Products Derived from *Monascus sp.*

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Several species of the genus Monascus *produce citrinin, a mycotoxin, are harmful to the hepatic and renal systems. For this reason, the citotoxicity study is necessary to evaluate the toxicological effect of Monascus products obtained from solid state or submerged biosynthesis. In our study we evaluate the content of mycotoxin in some Monascus products and the cytotoxicity effect of them. The study regarding citrinin content revealed a content of 145 ppm citrinin in the product obtained by solid state biosynthesis, a maximum content. In the case in which the biomaterials are obtained in submerged media, in the intracellular materials, the citrinin concentration was 82,71 mg/L (or 0,827 mg citrinin/g wet Monascus biomass). In the case of extracellular product obtained in submerged media, the mycotoxin content fluctuate in the range (7 ÷ 24,5) mg/L. Study performed "in vitro" with product obtained in submerged media on fibroblast murine cell lines, reveals a cytotoxicity effect for concentration larger than 60 μg/ml.*

Keywords Cytotoxicity; fibroblast murine cell; monascus metabolites

Introduction

Several species of the *Monascus sp.* produce citrinin, a mycotoxin are harmful to the hepatic and renal systems.The main toxic metabolite of *Monascus sp.* is mycotoxin citrinin, which appears in different biomaterials used as food or as raw materials in food industry [1]. The maximum content of this compound in bioproduct from different *Monascus sp.* strain is shown in Fig. 1. Another compound, appearing in the Monascal metabolites, is the monapurones A-C [2] with the structure shown in Fig. 2, which shows a selective cytotoxicity against human cell lineA549, with IC 50 value situated under 4 μM, and with no significant toxicity to normal MRC-5 and WI-38 cell at the same concentration [2]. For this reason, the cytotoxicity study is necessary in order to evaluate the toxicological

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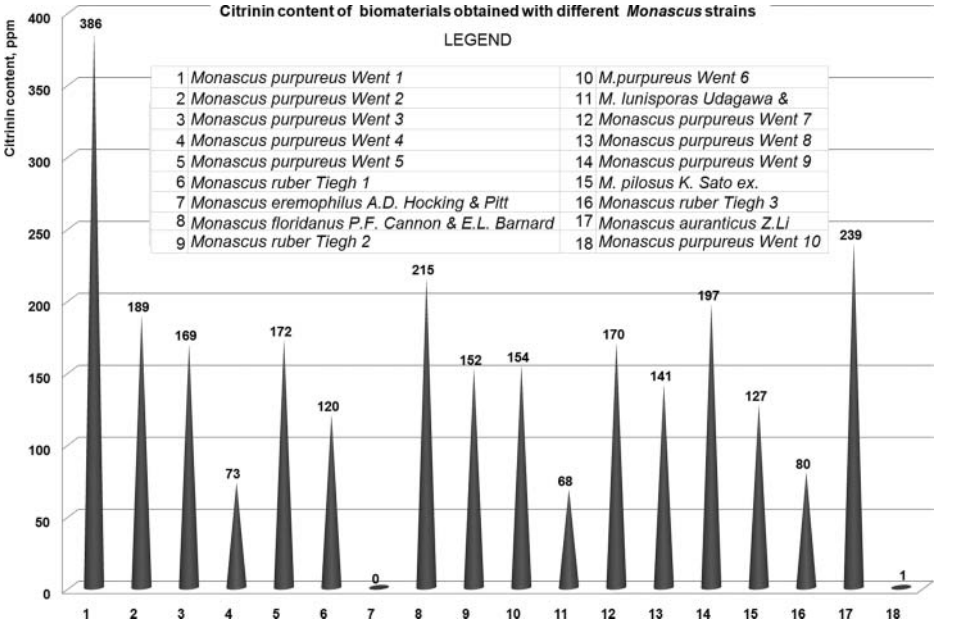

Figure 1. Maximum concentration in mycotoxin citrinin, appear in different *Monascus sp.* bioproducts.

effect of *Monascus sp.* products obtained from solid state or submerged biosynthesis, before performing the "in vivo" studies.

Materials and Methods

In our study we used the powder biopigment obtained with 2 strain *Monascus sp. 1* and *Monascus sp. 2* on rice [3] and the solution of monascorubramine obtained in submerged culture media, using enriched maltose media (DNA) [4] and YDM media [5], with 2 *Monascus sp*. strains. The etalon mycotoxin used was Citrinin powder, from Sigma Aldrich. Citrinin measurements was made with a HPLC Agilent 1100 with Diode Array detection. For the cytotoxicity tests we used the fibroblast L929 murine cell. For toxicity essay, we

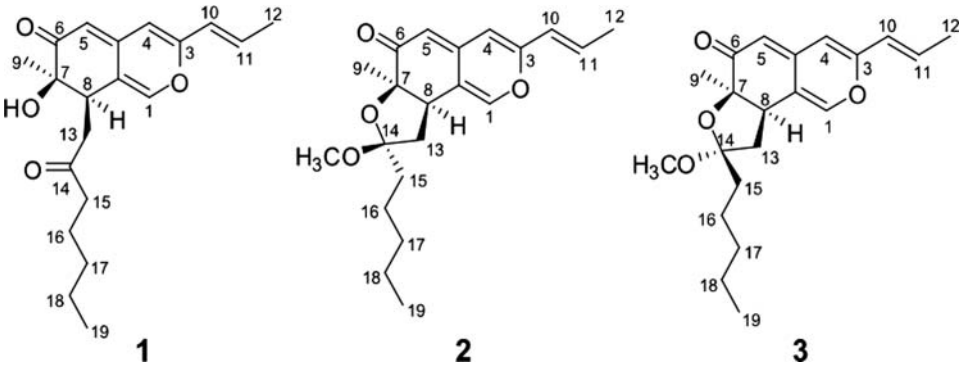

Figure 2. Structure of cytotoxic azaphilone, designated monapurone A-C (1–3).

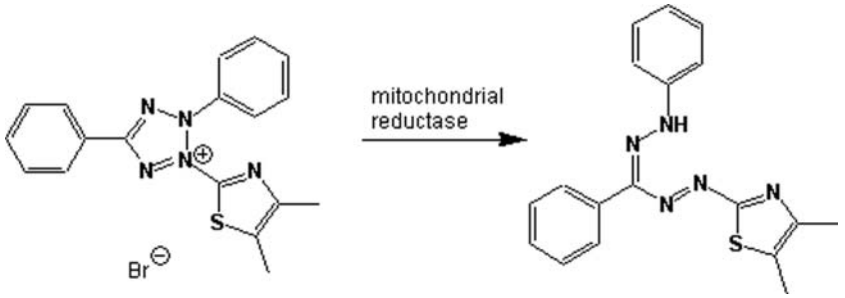

Figure 3. Reaction involviong in the reduction of tetrazolium salt to formazan.

used the method based on reduction of tetrazolium salt to formazan [6] using the specific reagent type 3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl tetrazolium bromide (MTT). In this method the **MTT**, a yellow tetrazole, is reduced to insoluble purple formazan with the living cells. The insoluble formazan is dissolved with ethanol, and the coloured solution which results after solubilization is measured spectrophotometrically at 560 nm, using an Elisa reader plate. The intensity of purple formazan solution is proportional with the live cells. The reaction involved in this process is presented in Fig. 3.

Results and Discussion

In the first step of the study we evaluated the content of mycotoxin in some Monascus products, as shown in Fig. 4. The obtained results indicated a content of 145 ppm in bioproduct got in solid state with *Monascus sp. 2* (M.sp.1) in comparison with the bioproduct obtained with *Monascus sp. 1* (M.sp.2) which are free of citrinin. In the case of bioproducts obtained in submerged media, the content of citrinin increased in the following order: bioproduct obtained with M.sp. 2 grown on yeast extract contains 3.15 ppm, bioproduct

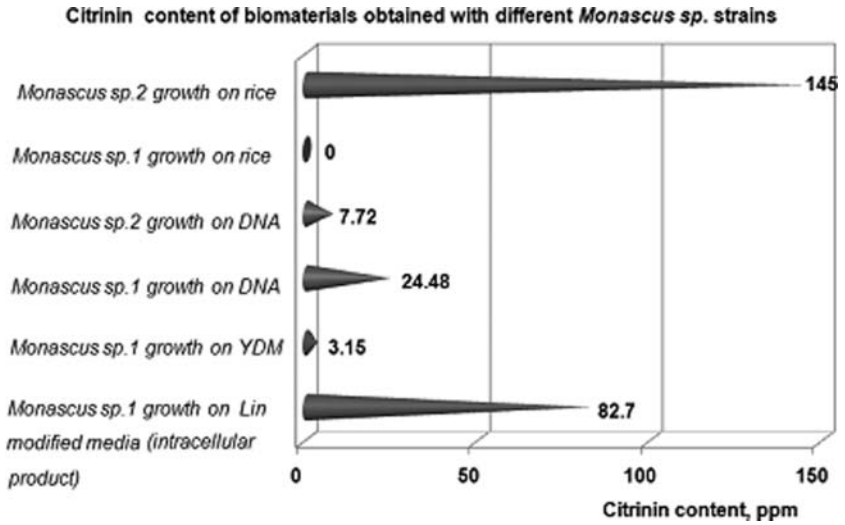

Figure 4. Citrinin content in some bioproducts obtained with two strain of *Monascus*, in solid state biosynthesis or in submerged media on rice, DNA(dextrose), YDM (yeast extract) or Lin media.

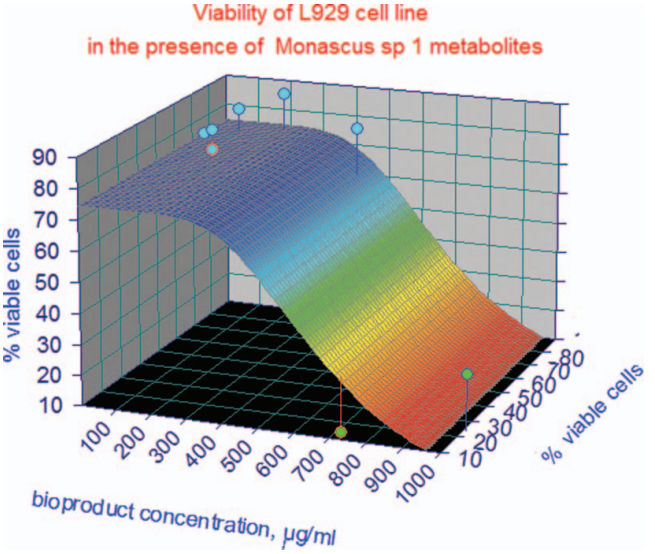

Figure 5. Cell viability L929 treated 24 h with *Monascus* bioproduct obtained from *Monascus sp. 1* growth in solid state, on rice).

from *M. sp. 2*. grown on dextrose media contains 7.72 ppm, bioproduct grown on the same media but with *M. sp. 1* contains 24.48 ppm citrinin, and the bioproduct obtained on Lin media with *M. sp.* 1 contains 82.7 ppm. From all bioproducts tested, the citrinin content is comparable with maximum citrinin concentration found in the main monascal bioproducts. The second step of research consists in determination of the cytotoxicity of bioproducts obtained in solid state biosynthesis or in submerged media. The tests were performed "in vitro" using murine line cells L929. First was tested the bioproduct derived

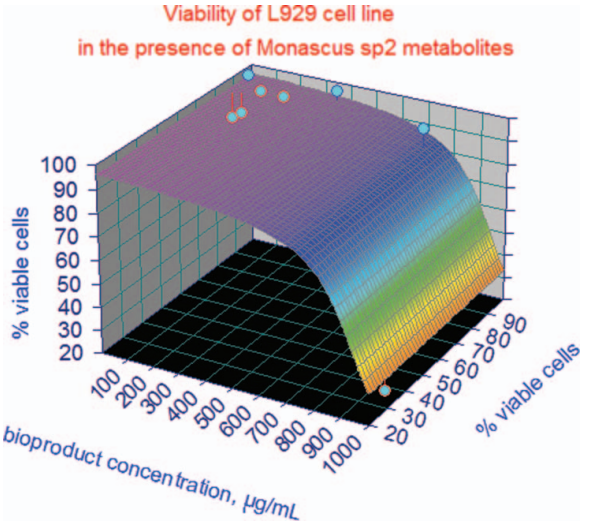

Figure 6. Cell viability L929 treated 24 h with *Monascus* bioproduct obtained from *Monascus sp. 2* grown in solid state, on rice.

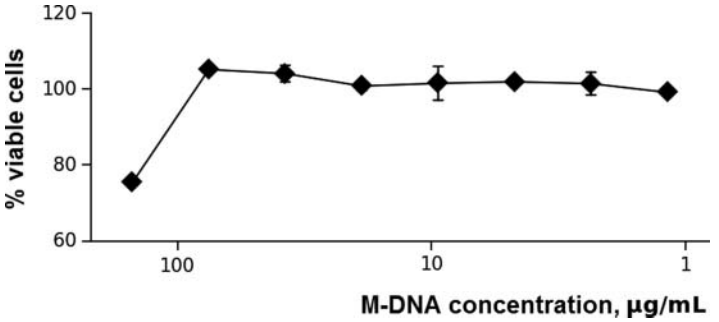

Figure 7. L929 cell viability treated 24 h with M-DNA bioproduct (M-DNA is bioproduct obtained when *Monascus sp. 1* was growth in submerged media with dextrose).

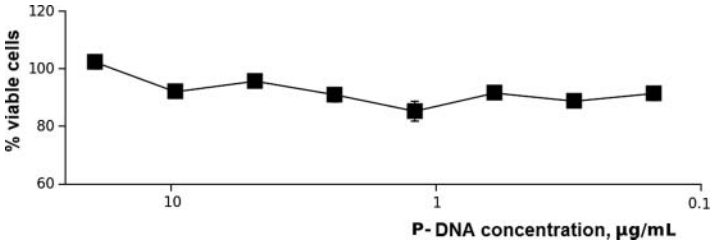

Figure 8. L929 cell viability treated 24 h with P-DNA bioproduct (P-DNA is bioproduct obtained when *Monascus sp. 2* was grown in submerged media with dextrose).

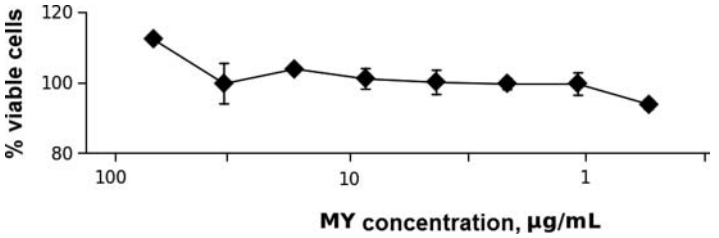

Figure 9. Cell viability L929 treated 24 h with MY bioproduct (MY is bioproduct obtained when *Monascus sp. 1 was* grown in culture media with yeast extract).

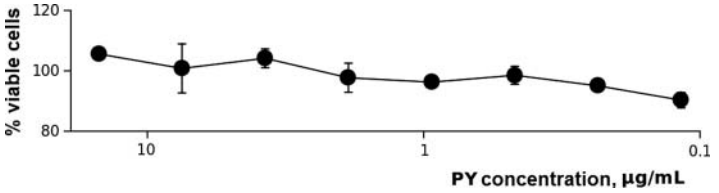

Figure 10. Cell viability L929 treated 24 h with PY bioproduct (PY is bioproduct obtained when *Monascus sp. 2 was* grown in culture media with yeast extract).

from solid state biosynthesis, as a diluted solution in DMSO (dimethylsolfoxide). The obtained results indicated a cytotoxic effect for a concentration larger than 250 μg/mL for M. sp 1 of solid bioproduct (Fig. 5) and higer than 500 μg/mL for M. sp. 2 solid bioproduct (Fig. 6). In the same conditions the solvent (DMSO) has no cytotoxicity. For bioproducts obtained in submerged biosynthesis (results shown in Fig. 7, 8, 9–10), the cytotoxicity study reveals the following: the bioproducts obtained from *Monascus sp. 2* on culture media, containing yeast extract or dextrose, has no cytotoxic effect for a concentration smaller than 20 μg/mL. In the same conditions, the bioproduct obtained with *Monascus sp. 1* in culture media which contain yeast extract, at the concentration less than 100 μg/mL has no cytotoxic effect but if the same strain is cultivated in submerged media which contains dextrose, the cytotoxic effect appears at concentrations larger than 68 μg/mL. The difference between concentration at which the cytotoxic effect appears is probably due to the difference between citrinin content in the two bioproducts obtained in submerged biosynthesis with *Monascus sp. 1*. In bioproducts obtained in culture media which contains dextrose, the citrinin concentration is 24.48 ppm.

Conclusion

For two Monascus strains: *Monascus sp. 1* and *Monascus sp. 2,* respectively, we have performed the tests regarding the content of citrinin in bioproducts obtained in solid state biosynthesis or in two different submerged media. The obtained results in the case of insoluble intracellular bioproducts in solid state biosynthesis, on rice, indicate a content of citrinin micotoxin situated at the same level like in the case of bioproducts obtained from another strain of *Monascus*. In the case of biomaterials obtained in submerged media (with dextrose or yeast extract), with two strains, the citrinin content is situated under citrinin level from another similar bioproducts. In our case (respectively in the case of extracellular product obtained in submerged media), the mycotoxin content varies in the range ($7 \div 24.5$) mg/L. The cytotoxic study performed on L 929 murine cell line with 6 bioproducts, from which 2 were obtained in solid state biosynthesis and 4 in submerged media, indicates the following: in the case of bioproducts obtained in solid state (rice) the cytotoxic effect appears at the concentration larger than 250 μg of bioproduct per mL for biomaterial obtained with *Monascus sp. 1* and at the concentration larger than 500 μg of bioproduct per mL in the case of biomaterial obtained with *Monascus sp. 2*.

The study performed with product obtained in submerged media on fibroblast murine cell, the cytotoxicity effect appears at the concentration larger than 20 μg/mL for bioproducts obtained with *Monascus sp. 1* in culture media, which contain dextrose or yeast extract.

In the case of submerged bioproducts obtained from Monascus sp. 2 the cytotoxicity effect appears at concentrations larger than 100 μg/L for products obtained in culture media which contain yeast extract or at 68 μg/L for biomaterials obtained in the presence of dextrose.

References

[1] Duliu, O., Ferdes, M., & Ferdes, O. (2000). *Radiation Physics and Chemistry*, *57*(1), 97–101
[2] Li, J. J., Shang, X. Y., Li, L. L., Liu, M. T., & Zheng, J. Q. (2010). *Molecules*, *15*, 1958
[3] Bum-Kyn L., Hai, Y. P., & Wook-Jin, C. (2002). *Biotechnol. Bioprocess Eng.*, *7*, 21
[4] Chattejen, S., & Maity, S. (2009). *J. Applied Sciences Research*, *5*, 2102
[5] Casaz-Lopez, J. L., & Sanchez Perez, J. K. (2003). *Enzyme and Microbial Technology*, *33*, 270.
[6] Mosmann, T. (1983). *J. Immunological Methods*, *65*, 55–63