PRODUCTION OF POLYKETIDE COLORANTS BY ASCOMYCETE FUNGI: A BIOPROCESS APPROACH.

Nathália R. C. M. Castanho[1], Denise Grotto[2], Angela F. Jozala[2] & Valéria C. Santos-Ebinuma[3]*

[1]PhD in Biosciences and Biotechnology applied to pharmacy. Department of Bioprocess Engineering and Biotechnology. Universidade Estadual Paulista "Júlio de Mesquita Filho". Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculty of Pharmaceutical Sciences - Araraquara Campus, Brazil. [2]University of Sorocaba, Sorocaba / São Paulo, Brazil.

[3] Department of Bioprocess Engineering and Biotechnology. Universidade Estadual Paulista "Júlio de Mesquita Filho", Araraquara, Brazil.
** nathalia.castanho@unesp.br and valeria.ebinuma@unesp.br*

ABSTRACT

Several fungi are able to produce colorants, which can belong to several classes of natural compounds. Among them, there are the polyketides which are the most abundant secondary metabolites in fungi. Generally, these compounds present biological activity besides exhibiting varied colorations according to their molecular structures. Microbial colorants offer sustainable alternatives to synthetic counterparts since they exert minimal environmental impact in comparison to synthetic ones. Investigations involving fungi such as *Fusarium oxysporum* are therefore of great scientific interest, particularly concerning the optimization of culture medium, pH levels, and potential supplements. So, the aim of this work was to understand these variations in culture medium and the correlation regarding biomass and colorant production. Thus, the fungus *F. oxysporum* was cultivated in different rice broths, supplemented or not with Dextrose anhydrous. Results were monitored for pH variation, sugar consumed and biomass production. In addition, studies of colorants extraction using acetone were also performed. The results showed that supplementation with 5 g/L of anhydrous dextrose positively influenced biomass production obtaining 18.7g/L of biomass and extracting bikaverin 34.18 $UA_{500nm}/g_{biomass}$. This study contributes to the development of sustainable colorants production with diverse applications.

Keywords: Colorant. *Fusarium oxysporum*. Submerged Culture. Rice Broth.

1 INTRODUCTION

Colorants have historically been used for several purposes, encompassing aesthetics, religion practices and artistic expression (from indigenous paintings to Egyptian ones, for example). The basis for the extraction of colorants began with plants and animals, however, at the end of the 19th century, these molecules began to be chemically synthesized. These molecules gained the market because of they homogeneity, greater speed of manufacture and lower production costs when compared to natural colorants ([1,2,3]). However, the main disadvantages of synthetic colorants occur after their application/use because research claims that these compounds can cause allergies, be carcinogenic and pollute the environment ([4,5]).

According to Dabas ([6]), natural colorants are molecules synthesized and excreted by living cells such as algae, animals, microorganisms and plants, Colorants from microorganisms have an advantage in their production compared to the other sources since the process can be monitored and replicable, and generally the colorants derived from microorganisms are more stable than those from animals and plants ([4, 7, 8]).

The applications of microbial colorants are diverse, and they can be used in the paper, textile, wood, food, and pharmaceutical industries. Filamentous fungi have been gaining ground in these areas, as the colorants produced by these microorganisms can have antioxidant, anticancer and antimicrobial activity, and be non-toxic, which leads to environmentally friendly disposal and easy degradation ([4, 9, 10]).

Our research relates the production of colorants by submerged culture of the ascomycete fungus *Fusarium oxysporum*. The literature shows the widespread production of quinones by the genus of this fungus, making it one of our biomolecule classes of interest ([11, 12]). This class is also known for its great color variability, giving a fungal bioproduct several commercially valuable uses.

2 MATERIAL & METHODS

Pre-inoculum and Inoculum: the fungus *F. oxysporum* was inoculated in the media Sabouraud Dextrose Agar at pH 4.5 in agar plate and kept in a refrigerated incubator for 120 hours at 30ºC. To prepare the inoculum, 25 mL of Sabouraud Dextrose broth was inserted in 125 mL Erlenmeyers type flasks, along with five plugs of the apical mycelium, which were kept under orbital shaker for 72 hours at 200 rpm and 30ºC for cell growth, to be used as an aliquot for cultivation.

Cultivation in rice broth with or without anhydrous dextrose supplementation: Four cultivation were carried out as follows: the comparative one with only Rice Broth 50 g/L (cooked and crushed medium), Rice Broth + 5g/L Dextrose, Rice Broth + 10g/L Dextrose and Rice Broth + 15g/L Dextrose, all with 5% inoculum, totaling 25mL in each 125mL Erlenmeyer type flask, initial pH at 3.5. The triplicates were kept shaking in an orbital shaker for 120 hours at 200 rpm at 30ºC. After the cultivation time, the

samples were centrifuged for 20 min at 13.000xg, the supernatant was vacuum filtered using a qualitative filter paper 80g, and the biomass was dried for 24 hours in a stove at 30ºC.

Biomass, pH and percentage of sugar consumed: Biomass was counted by dry weight, using the average between the triplicates in each of the cultures, which was used for intracellular extraction. The supernatant was filtered and used to measure the pH and sugar consumed. The pH was measured to observe changes in each culture, while the percentage of sugar consumed took into account the initial amount of starch (5g/L) and anhydrous dextrose in each of the cultures. All the analyses were carried out in triplicate.

Extraction of intracellular colorant: For the extraction, it was used the following process: i) biomass was washed with Milli-Q water in a ratio of 1:5 ratio of biomass to water, followed by 30 seconds of vortex agitation and 30 seconds of standby, 5 times. After this, the solutions were centrifuged at 2,740xg for 20 minutes. The supernatant was discarded. ii) the extraction cycles with Acetone were started in the same way as mentioned above. The aliquots from the extractions were read on a plate reader by scanning between 300 and 600 nm. The Absorbance Unit (AU) per gram relationship was calculated using scan data at 500 nm and biomass production.

3 RESULTS & DISCUSSION

Figure 1a shows the Erlenmeyer flasks after the cultivation process. As can be seen, the cultures with 5% inoculum in Rice Broth and Rice Broth supplemented with different concentrations of Dextrose. The coloration formed during the cultivation process is quite similar from the look of each Erlenmeyer flask, but we can already see a difference in the biomass growing. Other culture media have already been tested for the growth of colorant-producing filamentous fungi, such as Minimal Dextrose Broth, Casamino Dextrose Yeast Broth ([8]), peanut broth medium, and peanut shells in solid state ([10]) and grape residue ([11]). The results of extraction with acetone are already visible for the extraction of bikaverin, since the solvent turns completely red (figure 1b).

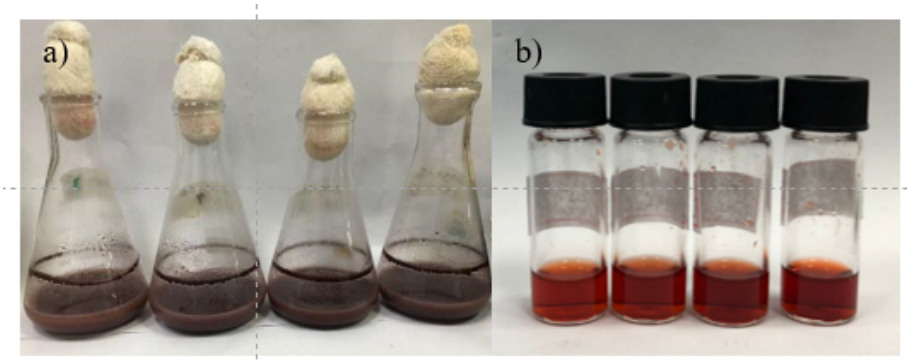

Figure 1. (a) Rice broth (RB) supplemented with dextrose. From left to right: RB only; RB + 5g/L dextrose, RB+ 10g/L dextrose, RB+ 15g/L dextrose, after five days of cultivation. (b)Acetone extraction. Always from left to right: No supplementation, 5g/L, 10g/L and 15g/L.

Figure 2 shows the numerical results of *F. oxysporum* cultivation in rice broth. As can be seen, the rice broth without supplementation obtained the lowest amount of biomass (15.67g/L), as well as the lowest unit of bikarevin absorbance per gram of biomass (33.13AU_{500nm}/g)), while the sugar was consumed with 79%. When we analyzed the second culture, rice broth + 5g/L dextrose, we obtained 34.18 AU_{500nm} /g and a higher biomass production, with 18.7 g/L and 76% of sugar consumed. The other two supplements (10g/L and 15g/L) also obtained good results, both in terms of biomass production (18.75g/L and 19.48g/L) and bicaverine absorbance unit in biomass (35.04 and 34.89), although the percentage of sugar consumed was 76.2% and 72.8% respectively. The pH in the standard medium dropped to 3.4, while in the supplemented media it dropped to 3.2.

In comparison with the literate, the work of Lebeau and colleagues ([8]), who used three different culture media, potato-dextrose broth (PDB), defined minimal dextrose broth (DMD) and teast-casamino acid-dextrose broth (YCD). What's interesting about the rice broth is the difference in biomass production: in our best condition, we obtained 18.7 g/L of biomass (without light), while the PDB produced 3.4, g/L, the DMD 3, g/L and the YCD 2 g/L.

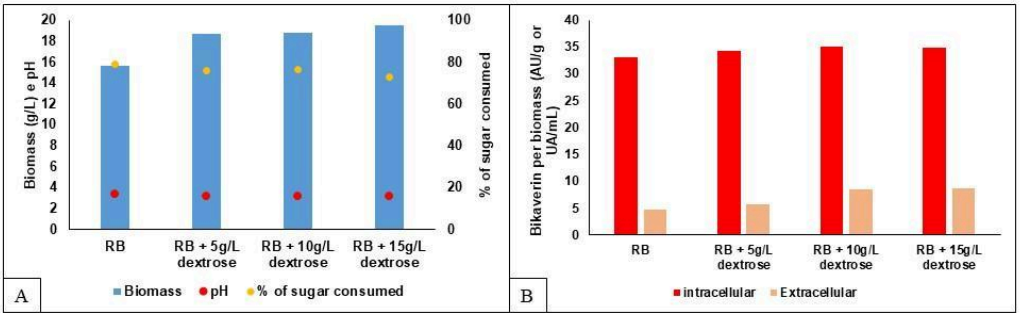

Figure 3.Cultivation with different concentration of dextrose supplementation in rice broth. A) In blue we have the representation of biomass (blue bars), pH (red point), sugar consumption (yellow point); B) intracellular bikaverin in AU_{500nm}/g (red bars) and extracellular bikaverin (light pink bars).

4 CONCLUSION

The cultures obtained a somewhat purple color at first glance in the Erlenmeyers flasks, separating into a light pink supernatant and a wine-coloured biomass. The supplemented media showed greater biomass production, as well as greater expression of biomolecules per gram of biomass. Economically, with a view to future production in a bioreactor, rice broth + 5 g/L dextrose medium is the best option. Since we used less dextrose, we obtained a quantity similar to the other supplements and higher than rice broth alone, producing 18.7 g/L of biomass and 34.18 UA/L. During the preparation of the scientific plan, we noted in the literature the presence of bikaverin as one of the main polyketide molecules present. This leads us to believe that the results expressed at 500 nm refer to this secondary metabolite, which will be confirmed in the future through NMR and HPLC analyses. As well as the separation of other possible molecules from the bikaverin metabolite cycle, observed with purple and light pink coloring.

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