PRODUCTION OF POLYKETIDES COLORANTS BY *Talaromyces amestolkiae* APPLYING DIFFERENT CARBON SOURCES

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ABSTRACT

The objective of this work was to study the production of natural colorants by *Talaromyces amestolkiae* applying glucose, xylose and pectin as carbon source in submerged cultivation on orbital shaker. Initially, the production of natural colorants was carried out varying the amount carbon source in three rates: 10:0; 10:5; 05:5; 0:10 (g. L^{-1}) of glucose:xylose, respectively. Then, the citric pectin was applied in the rates: 4.0; 2.0; 1.0 (g. L^{-1}). The bioprocess was performed in 250 mL Erlenmeyer flasks with 50 mL of culture media containing 15 mycelium discs in orbital shaker at 150rpm/168h/30°C. The production of colorants reached a maximum of 14.51 AU_{500nm} employing 10:05 g. L^{-1} of glucose:xylose, and 7.93 AU_{500nm} applying 1.0 g. L^{-1} of pectin. Therefore, this work showed the effect of citric pectin in colorant production by *T. amestolkiae* and the capacity of the fungi to metabolize both glucose and xylose as carbon source to produce natural colorants. Additionally, the possibility to employ these two carbohydrates as carbon sources together indicates that this microorganism can possibly metabolize hydrolysates of agro-industrial by-products to produce natural colorants.

Keywords: Filamentous Fungi. Biocolorants. Pectin. Bioprocess. Biotechnology

1 INTRODUCTION

Natural colorants, encompass a diverse array of chemically varied molecules containing a chromophore within their molecular framework, which determines their coloration (De Oliveira *et al*., 2022). These organic compounds can be originate from several sources including microbial, animal, or plant origins, and among them, microorganisms, in particular, offer distinct advantages such as the ability for both submerged and semi-solid cultivation, rapid production, metabolic diversity, and year-round availability (Santos-Ebinuma *et al*., 2013). The biotechnological approach to manufacturing natural colorants has gained traction in the food and pharmaceutical industries due to safety concerns associated with synthetic counterparts, particularly in human consumption (Mussagy *et al*., 2021).

Among the plethora of natural bioproducts obtainable from microorganisms, filamentous fungi emerge as producers of numerous bioactive compounds. *Talaromyces amestolkiae*, a filamentous fungus, exhibits versatility in producing various biocompounds such as β-glucosidases and xylanases enzymes contingent upon the carbon source in the culture medium (De Eugênio *et* al., 2017; Barbieri *et al*., 2022), alongside notable synthesis of yellow, orange, and red colorants (De Oliveira *et al*., 2019).

Given the promising findings and acknowledging the biotechnological benefits of *T. amestolkiae* in a biorefinery concept, this study aimed to explore its potential for natural colorant biosynthesis using different carbon sources, with the intent of laying the groundwork for future utilization of agro-industrial by-products as substrates for colorant production.

2 MATERIAL & METHODS

The microorganism used in this work, *T. amestolkiae* DPUA 1275, was kindly provided by the DPUA Cultures Collection, from the Mycology laboratory of the Federal University of Amazonas, DPUA, AM, Brazil. The inoculum was prepared by activation of the microorganism in Petri plates (90 mm x 10 mm) containing 10 mL of Potato-dextrose agar medium supplemented with yeast extract (PDA-YE). To this purpose, a loop of the *T. amestolkiae* from stock culture was inoculated on a PDA-YE plate and incubated at 30 °C for 168 h.

The cultivation was conducted by varying the concentration of carbon sources at three ratios: 10:0; 10:5; 05:5; 0:10 (g. L^{-1}) of glucose:xylose, respectively. Besides, a commercial citric pectin was applied in the rates: 4.0; 2.0; 1.0 (g. L^{-1}) in the culture medium, which also comprised (g. L^{-1}) of monosodium glutamate (25.0), $CaCl_2$ (0.015), $MgSO_4$ (0.012), and $FeSO_4$ (0.010). A culture medium containing only 10 g. L^{-1} of glucose was prepared as a positive control for comparison.

The bioprocess was conducted in 250 mL Erlenmeyer type flasks containing 50 mL of culture medium inoculated with 15 mycelium discs (8.0 mm in diameter) and incubated on an orbital shaker at 150 rpm/168 h/30 °C. Subsequently, the fermented broth was vacuum-filtered using Whatman n°1 paper filters. The filtrate was analyzed to quantify the produced colorants, while the filtrate residue was utilized to measure fungal biomass on a dry mass basis. The colorants production was quantified indirectly by the measurement at 500 nm and expressed in Units of absorbance (UA_{500nm}).

3 RESULTS & DISCUSSION

In the production of fungal colorants, different carbon sources have been applied with the aim of understanding the behavior and metabolism of these compounds, which act not only as a carbon source but also as energy in these processes (Morales-Oyervides *et al.*, 2020). It is important to highlight that each microorganism presents different conditions of metabolism and assimilation of carbon and nitrogen sources. Therefore, the application of glucose, xylose and pectin was made in order to understand the interference of these compounds in the production of the red colorant and to increase the knowledge about the ability of the microorganism *T. amestolkiae* to metabolize such compounds. **Figure 1** presents the data of red colorant production in the assays carried out using glucose, xylose, and commercial pectin.

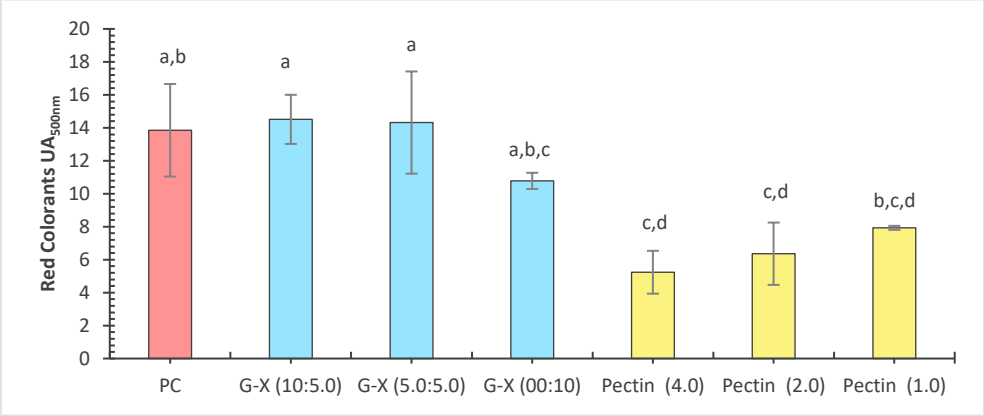

Figure 1 - Production of red colorant by *T. amestolkiae* using commercial glucose, xylose and pectin in the culture medium conducted at 30°C; 150 rpm on orbital shaker for 168h.

*PC corresponds to Positive control of the culture medium supplemented with the following composition (g.L-1): MSG - Monosodium glutamate (25.0), G - glucose (10.0), CaCl (0.015), MgSO4 (0.012), FeSO4 (0.010). X – means Xylose. All Pectin assays were carried out containing GMS, G and salts at the same concentrations as the standard medium. The values described are the means of the triplicates and the error bar ± indicates the standard deviation. Statistical differences between contents were evaluated using the Tukey test (Minitab 19.1 software) with a significance level of 0.05. Means identified with different letters (a, b, c, d) are significantly different.

According to the Figure 1, it is possible to observe that red colorants were produced in all assays, being between (UA_{500nm}): 10.78 -14.51 for conditions with Glucose:Xylose, and 5.24 – 7.93 for medium containing pectin. In tests performed with glucose:xylose in different proportions, a production of red colorants similar to the positive control could be observed. However, in a culture medium composed only of xylose, the production was shown to be lower than in culture medium containing both monosaccharides. These results corroborate with data acquired in the literature, since xylose is a monosaccharide that can be consumed and used as an energy source for colorant production by other microorganisms (Morales-Oyervides *et al.*, 2020). In these works, the authors report that the presence of xylose in the cultivation medium increases productivity, as in the case of red colorants produced by *Penicillium spp* (Sopandi *et* al., 2012; Chintapenta *et al*., 2014) and/or is the source of carbon preferred or most consumed, such as in the production of red colorants by *P. purpurogenum* (Patil *et al*., 2015). Regarding the results obtained in the presence of commercial pectin, despite the gradual interference in production, *T. amestolkiae* was able to biosynthesize colorants, with production being statistically similar in tests with the lowest concentrations of pectin in relation to the control. However, it is worth noting that the presence of pectin, even in the lowest condition (1.0 g. L^{-1}), reduced approximately 40% of the production of red colorants. In the **Table 1**, it is possible to observe the data related to de colorant production, like pH, Carbon source consumption and Biomass.

Table 1 – Carbon source consumption, pH and Biomass of red colorant production by *T. amestolkiae* using commercial glucose, xylose and pectin in the culture medium conducted at 30°C; 150 rpm on orbital shaker for 168h

Carbon source	(g.L^{-1})**	Final pH	Glucose Consumption(%)	Xylose Consumption(%)	Biomass (g.L^{-1})
Positive Control*(Glucose)	(10)	4,95 ± 0,02^a	77,52 ± 13,45^a	-	2,35 ± 0,22^a
Glucose-Xylose	(10:5,0)	4,94 ± 0,01^a	36,09 ± 3,64^b	13,66 ± 1,74^b	3,60 ± 0,82^a
Glucose-Xylose	(5,0:5,0)	4,97 ± 0,02^a	85,78 ± 6,87^a	41,51 ± 2,63^a	3,24 ± 1,25^a
Glucose-Xylose	(00:10)	5,00 ± 0,01^a	-	47,39 ± 4,01^a	3,09 ± 0,14^a
Glucose-Pectin	10,0:4,0	4,85 ± 0,02^b	35,59 ± 0,93^b	-	3,80 ± 0,61^a
Glucose-Pectin	10,0:2,0	4,91 ± 0,11^a	51,60 ± 1,27^b	-	4,47 ± 1,50^a
Glucose-Pectin	10,0:1,0	4,90 ± 0,01^a	56,13 ± 3,90^b	-	3,45 ± 0,66^a

Source: Author data.

*PC (Positive control) corresponds to the culture medium supplemented with the following composition (g.L-1): MSG - Monosodium glutamate (25.0), G - glucose (10.0), CaCl (0.015), MgSO4 (0.012), FeSO4 (0.010). All Pectin assays were carried out containing GMS, G and salts at the same concentrations as the standard medium. The values described are the means of the triplicates and the error bar ± indicates the standard deviation. Statistical differences between contents were evaluated using the Tukey test (Minitab 19.1 software) with a significance level of 0.05. Means identified with different letters (a, b, c, d) are significantly different.

** Concentration corresponding to the sugar inserted in the cultivation medium;

According to the biomass data, it is evident that the microorganism was able to multiply and produce colorants under all conditions, even in the presence of only xylose. There was no statistical difference in relation to biomass in all conditions analyzed. These results, combined with data on consumption of the carbon source (xylose), indicate that the microorganism is capable of metabolizing xylose. The results obtained together with the literature analysis demonstrate that the correlation between the consumption of the carbon source and the production of colorants can vary depending on the proportion and source used. In all experiments that contained glucose and xylose together, the fungus preferentially consumed glucose. Thus, these results demonstrate that xylose does not inhibit the metabolism of *T. amestolkiae*. Furthermore, it is possible to note that in none of the tests with glucose-xylose there was a significant change in pH, which may have contributed to the production of the colorant, considering that the optimum pH for the production of red colorants by *T. amestolkiae* is 5.0 (De Oliveira *et al*., 2019).

Regarding the results obtained in the presence of commercial pectin, it was possible to observe a decrease of around 20% in glucose consumption compared to the standard, even with the pH maintained at 5.0, which is optimal for production. This may indicate that, although pectin does not completely block the production of colorants, its presence in the cultivation medium may affect or partially inhibit their production.

4 CONCLUSION

This study demonstrated the effect of citric pectin in red colorant production by *T. amestolkiae* and the microorganism ability to metabolize both glucose and xylose as carbon sources for natural colorant production. Furthermore, the potential to employ these two carbohydrates simultaneously suggests that this microorganism may metabolize hydrolysates derived from agro-industrial by-products to generate natural colorants. Therefore, this work highlighted the versatility of this microorganism and the application capacity of *T. amestolkiae* in biorefinery and bioprocesses

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ACKNOWLEDGEMENTS

Acknowledgments to FAPESP (Process n°2021/06686-8, 2022/10809-0, 2023/01368-3), CNPq (312463/2021-9); CAPES.