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BIOTECHNOLOGICAL POTENTIAL OF *FUSARIUM* STRAINS IN BIOPIGMENT PRODUCTION

POTENCIAL BIOTECNOLÓGICO DE AMOSTRAS DE *FUSARIUM* NA PRODUÇÃO DE BIOPIGMENTOS

POTENCIAL BIOTECNOLÓGICO DE CEPAS DE *FUSARIUM* EN LA PRODUCCIÓN DE BIOPIGMENTOS

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ABSTRACT

Microorganisms are promising sources of biopigments, many of them with antioxidant, antimicrobial, anticancer and antimutagenic properties, and can be applied in food, pharmaceutical, and textile industries, among others. Among them, bicaverine, which is produced by Fusarium species and which has proven antitumor and antimicrobial properties. The objective of this work was to evaluate different strains of Fusarium oxysporum species in the production of this polycaferid and to analyze the influence of variables such as agitation, temperature and pH on the production of biomass, intracellular and extracellular bicaverin. For this, three strains of this fungus were tested: UCP 1624, UCP 1137 and UCP 0119. The results revealed that all species were able to produce pigment in both solid and liquid mediums, but with different yields. Due to obtaining a higher yield of biomass and bicaverine, the species Fusarium oxysporum UCP 1624 was subjected to a complete factorial design 23 that showed that high levels of agitation

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significantly influence all dependent variables: biomass production and extracellular and intracellular production of bicaverine. However, high pH levels showed a negative effect, indicating that low values contribute to the production of pigment and biomass. To confirm the presence of bicaherin, Infrared Spectroscopy (FT-IR) was performed, which demonstrated the presence of quinones, oxygenated polycyclic aromatic hydrocarbons, as well as the pigment bicaverin, which has in its molecule an oxygen interposed between two carbon atoms (C-O-C) in aromatic chains (benzoxanthone).

Keywords: Filamentous Mushroom. Microbial Polyketides. Pigment. Bicaverina.

RESUMO

Os micro-organismos são fontes promissoras de biopigmentos, muitos deles com propriedades antioxidantes, antimicrobianas, anticancerígenas e antimutagênicas, podendo ser aplicados em indústrias alimentícias, farmacêuticas, têxteis, entre outras. Dentre eles, a bicaverina, que é produzida por espécies de Fusarium e que possui comprovadamente propriedades antitumorais e antimicrobianas. O objetivo desse trabalho foi avaliar diferentes linhagens de espécies de *Fusarium oxysporum* na produção desse policetídeo e analisar a influência de variáveis como agitação, temperatura e pH na produção de biomassa, bicaverina intracelular e extracelular. Para isso, três cepas desse fungo foram testadas: UCP 1624, UCP 1137 e UCP 0119. Os resultados revelaram que todas as espécies foram capazes de produzir pigmento tanto em meio sólido, quanto em meio líquido, porém com diferentes rendimentos. Por obter um rendimento maior de biomassa e bicaverina, a espécie Fusarium oxysporum UCP 1624 foi submetida a um planejamento fatorial completo 23 que mostrou que níveis altos de agitação influenciam significativamente sobre todas as variáveis dependentes: produção de biomassa e produção extracelular e intracelular de bicaverina. Entretanto, altos níveis de pH mostrou efeito negativo, indicando que valores baixos contribuem para a produção do pigmento e biomassa. Para confirmação da presença de bicaverina foi feita Espectroscopia de Infravermelho (FT-IR) que demostrou a presença de quinonas, hidrocarbonetos policíclicos aromáticos oxigenados, assim como o pigmento bicaverina que apresenta em sua molécula um oxigênio interposto entre dois átomos de carbonos (C-O-C) em cadeias aromáticas (benzoxantona).

Palavras-chave: Fungo Filamentoso. Policetídeos Microbianos. Pigmento. Bicaverina.

RESUMEN

Los microorganismos son fuentes prometedoras de biopigmentos, muchos de ellos con propiedades antioxidantes, antimicrobianas, anticancerígenas y antimutágenas, y pueden aplicarse en las industrias alimentaria, farmacéutica y textil, entre otras. Entre ellas, la bicaverina, que es producida por especies de Fusarium y que tiene propiedades antitumorales y antimicrobianas probadas. El objetivo de este trabajo fue evaluar diferentes cepas de especies de Fusarium oxysporum en la producción de este policaferido y analizar la influencia de variables como la agitación, la temperatura y el pH en la producción de biomasa, bicaverina intracelular y extracelular. Para ello, se analizaron tres cepas de este hongo: UCP 1624, UCP 1137 y UCP 0119. Los resultados revelaron que todas las especies podían producir pigmento tanto en medios sólidos como líquidos, pero con rendimientos diferentes. Debido a la obtención de un mayor rendimiento de biomasa y bicaverina, la especie Fusarium oxysporum UCP 1624 fue sometida a un diseño factorial completo 23 que mostró que altos niveles de agitación influyen significativamente en todas las variables dependientes: la producción de biomasa y la producción extracelular e intracelular de bicaverina. Sin embargo, los niveles altos de pH mostraron un efecto negativo, indicando que valores bajos contribuyen a la producción de pigmento y biomasa. Para confirmar la presencia de bicaherina, se realizó una espectroscopía infrarroja (FT-IR), que demostró la presencia de quinones, hidrocarburos aromáticos policíclicos oxigenados, así como el pigmento bicaverina, que en su molécula contiene un oxígeno interpuesto entre dos átomos de carbono (C-O-C) en cadenas aromáticas (benzoxantonos).

Palabras clave: Hongo Filamentoso. Policetidos Microbianos. Pigmento. Bicaverina.

INTRODUCTION

Color plays a key role in the acceptance of a product in various industrial segments, as it makes it more attractive to the customer (Venil et al., 2020; Wrolstad & Culver, 2012) and dyes and pigments are the substances that give color to these products (Lopes & Ligabue-Braun, 2021).

The increase in industrial demand in modern times has led to the development of rapid formulations and the application of synthetic dyes in high quantities in various segments, regardless of their risks to public health and the environment.

The search for natural dyes is mainly due to problems with artificial dyes, which are capable of causing allergies, in addition to some classes and their by-products having a carcinogenic and mutagenic character, which interfere with the biological processes of water bodies, reflecting high risks to public health (Narsing Rao et al., 2017).

The widespread public awareness and advances in biotechnology have driven the search for pigments of natural origin that emerges as an alternative to these synthetic substances (Narsing Rao et al., 2017).

The production of natural pigments by different groups of microorganisms such as fungi, bacteria, algae, and lichens can be exploited to obtain a wide variety of pigments with different properties such as antimicrobial, anticancer, and antimutagenic, with applicability in the textile, cosmetics, food, and pharmaceutical industries (Hernández et al., 2019; Narsing Rao et al., 2017; Pailliè-Jiménez et al., 2020).

Interest in natural pigments is on the rise, according to a report by "BUSINESS WIRE". The global natural color market is expected to grow by about 11% in the period from 2018 to

2024, generating a revenue of approximately $5 billion. The report suggests that this demand is directly linked to growing evidence of the harmful effects of synthetic pigments, as well as the protection of the environment (Research and Markets ltd, 2019).

Thus, natural pigments, or biopigments, are produced from natural sources, mainly plants and microorganisms. Compared to pigments from plants, pigments obtained by microbial route prove to be promising alternative sources, enabling production in limited spaces, with easy and rapid growth, independent of seasonal conditions and can be produced from renewable and low-cost substrates (Narsing Rao et al., 2017; Sethi et al., 2016).

THEORETICAL FRAMEWORK

The use of pigments has accompanied humanity since cave paintings (Abel et al., 2023; Barnett et al., 2006). Over time, the discovery of natural pigments broadened the color spectrum, but introduced toxic substances and industrially intensive processes, raising environmental and health concerns that today underpin the search for more sustainable alternatives (Barnett et al., 2006; Jose et al., 2019).

In this scenario, microbial pigments – produced by bacteria, algae and fungi – emerge as a strategic alternative, as they combine a wide chromatic range, potential low cost and relevant biological activities (antioxidant, antimicrobial, antitumor, among others), with applications already reported in food, cosmetics, pharmaceuticals and textiles (Hernández et al., 2019; Narsing Rao et al., 2017; Ogbonna, 2016).

The genus *Fusarium* belongs to a broad and complex group of filamentous fungi of the class *Sordariomycetes* that produce a large amount of colored secondary metabolites, stand out for the production of naphthoquinones and anthraquinones with antimicrobial and antitumor activities, as well as pigments applicable to the dyeing of wool, cotton, leather and even coloring in food and polymeric matrix (Lebeau et al., 2019; Nagia & EL-Mohamedy, 2007; Palacio-Barrera et al., 2019).

Among these metabolites is bicaverin, a reddish polyketid pigment from *Fusarium*, which combines bioactive properties (antimicrobial and antitumor) with partially elucidated biosynthesis, but still requires optimization of cultivation conditions and sustainable strategies to increase yield (Limón et al., 2010; Zhao et al., 2020).

Thus, the present work aims to evaluate different strains of Fusarium oxysporum species

(UCP 1624, UCP 1137 and UCP 0119) in the production of biopigment and to analyze the influence of variables such as agitation, temperature and pH in the production of biomass, intracellular and extracellular bicaverin.

METHODOLOGY

Microorganisms

Three samples of fungi of the species *Fusarium oxysporum* belonging to the UCP Culture Collection (Universidade Católica de Pernambuco), located at the Center for Research in Environmental Sciences and Biotechnology of the University, registered in the World Federation for Culture Collection-WFCC, were used, namely: *Fusarium oxysporum* UCP 1624, *Fusarium oxysporum* UCP 1137 and *Fusarium oxysporum* UCP 0119.

Radial Growth Assessment

Initially, the samples of *Fusarium oxysporum* were analyzed according to their ability to produce pigment, according to the modified method of (Sethi et al., 2016). The samples of *F. oxysporum* were cultured in Petri dishes containing the Sabouraud Dextrose Agar (SAB) medium for 7 days, at 28º C, until young spores were obtained. The young spores were inoculated in the center of the Petri dishes, 9 cm in diameter, containing the SAB medium, at pH 6.0 and incubated at 28 ± 1°C, under light and dark conditions. Mycelium growth was analyzed by measuring the diameter in millimeters, every 24 hours, until full plate coverage. The experiments were carried out in five replicates. The growth profile will be established by calculation of the Mycelial Growth Rate (MIGR) in mm (Equation 1), according to (Guadarrama-Mendoza et al., 2014).

$$MIGR = \sum \frac{(D - D_a)}{N} \quad (1)$$

Where:

D = Current average diameter of the colony.

D_a = the average diameter of the colony from the previous day

N = Number of days after inoculation.

Screening of Pigment Production in Liquid Medium

The fungal cultures were cultivated in Petri dishes containing SAB at 28º C for 6 days until young spores were obtained. The colonies were drilled with a sterile 12 mm diameter well cutter that resulted in discs used in submerged fermentation (Pradeep & Pradeep, 2013), modified.

Pigment production by submerged fermentation (FS): Potato dextrose broth (PDB) was used for submerged fermentation. Twenty 12 mm discs grown for 5 days were used as inoculum and transferred to 250 mL Erlenmeyers containing 100 mL of PDB medium and incubated in the absence of light, at 25°C, 200 rpm and pH 4.5, for 7 days.

The metabolic liquid was separated from the biomass by means of filtration in nylon mesh (120 mesh). The pigment extraction from the metabolic liquid was done with ethyl acetate in a 1:1 ratio, homogenized in a manual shaker for 60s and centrifuged at 5000 rpm for 10min for phase separation.

To extract the pigment from the biomass, a process similar to that of the metabolic liquid was used. 5mL of ethyl acetate for 100mg of lyophilized biomass were used. Ethyl acetate and biomass were homogenized in the manual stirrer for 3 minutes. Then they were subjected to centrifugation for 30 min to separate the phases.

The organic phase was submitted to a scan for analysis of the spectrometric peak and subsequent absorbance reading at the wavelength established by the scan. The extract obtained was stored in amber flasks, covered with aluminum foil and in a refrigerator until it was read in a spectrophotometer. The results were presented as optical density at 485 nm (OD485 = Absorbance × dilution factor) at the wavelength of 485nm (Dos Santos et al., 2020).

Complete Factorial Design 2^3

The microorganism selected by submerged fermentation was submitted to a complete factorial design 2^3 to analyze the main effects and interactions between the independent variables: initial pH, temperature and agitation (Table 1) and to define the best cultivation condition with the highest pigment yield, having as response variable the yield of biomass and pigment production, using StatSoft®'s STATISTICA version 6.0 software. In this phase, both the pigment excreted into the medium (extracellular) and the biomass (intracellular) were analyzed.

Table 1

Complete factorial design 2^3

Factors	Levels		
	-1	0	+1
Agitation (RPM)	0	100	200
Temperature (°C)	25	28	31
Initial pH	4,5	5,5	6,5

Minimum level (−1); Intermediate level (0); Maximum level (+1).

Fourier Transform Infrared Spectroscopy (FTIR)

The presence of biopigments was characterized by infrared spectroscopy in a spectrometer. Fourier Transform Infrared Spectroscopy provides evidence of the presence of functional groups present in the structure of substances that will be used to identify the chemical composition of the extract. The region of the electromagnetic spectrum of greatest interest for this technique is between 4000 and 400 cm-1.

RESULTS AND DISCUSSIONS

Radial Growth

The *Fusarium* samples showed an estimated mycelium growth velocity in millimeters, every 24 hours, up to the total coverage of the 9 cm plate. The experiments were carried out in replicates of 5 and the mean of these values are described in Table 2, as well as the value of the growth profile (MIGR) established by Equation 1. It was observed that the behavior of *F. oxysporum* UCP 1137 and *F. oxysporum* UCP 1624 showed the influence of light on radial growth, with lower rates, considering greater development of the colonies. However, *F. oxysporum* UCP 0119 showed a very small difference in radial growth rate between light and dark conditions.

Table 2

Radial colony growth in millimeters under light and dark conditions.

	AVERAGE RESULTS (mm)					
TIME (h)	*F.oxysporum* UCP 1137		*F.oxysporum* UCP 0119		*F.oxysporum* UCP 1624	
	WITH LIGHT	**NO LIGHT**	**WITH LIGHT**	**NO LIGHT**	**WITH LIGHT**	**NO LIGHT**
0	12,00	12,00	12,00	12,00	12,00	12.00
24	20,80	20,90	22,20	21,20	18,20	18,80
48	36,50	35,60	38,25	37,25	35,00	35,60
72	44,40	43,60	44,80	45,60	42,40	39,00
96	57,40	57,20	59,00	60,00	57,00	54,60
120	71,40	72,25	73,75	74,30	71,60	69,75
144	87,00	88,60	89,25	89,75	88,80	88,00
MIGR	**12,600**	**12,725**	**12,583**	**12,575**	**12,866**	**13,041**

Production of Pigments in Liquid Medium

The samples of F.oxysporum UCP 1137, F.oxysporum UCP 0119 and F.oxysporum UCP 1624 were submitted to submerged fermentation, in order to select the best sample for pigment production. After the fermentation time, it was possible to qualitatively observe the pigment production by the three species, as shown in Figure 2. Table 3 lists the results of the quantification of the lyophilized biomass and the values of the Optical Density (OD) of the extracts.

Figure 1

Pigment production by species (A) F1137, (B) 0119 and (C)1624 after submerged fermentation

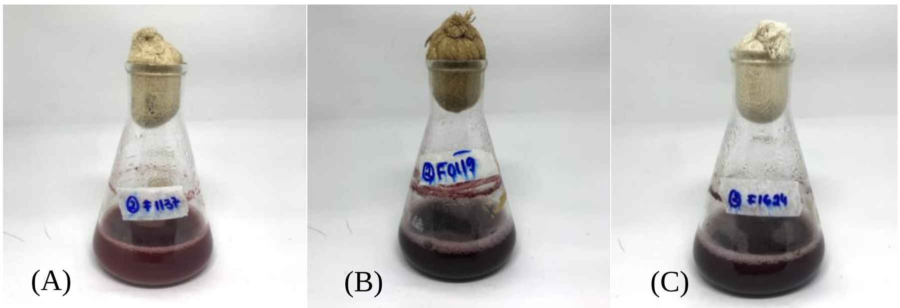


Table 3

Results of Absorbance (DO) measured at peak absorption (485nm) and biomass production (g/L) by each species of Fusarium oxysporum.

	Biomass (g/L)	Absorbance (DO) Extracellular pigment	Absorbance (DO) Intracellular pigment
F1137	3,3	0,097	0,460
F0119	4,4	0,093	0,607
F1624	4,7*	0,427*	0,796*

Table 3 shows that Fusarium oxysporium UCP 1624 was the strain with the highest production of extracellular (0.427) and intracellular (0.796) pigment. As well as the biomass production was achieved by F. oxysporium UCP 1624 (4.7g/L).

Complete Factorial Design 2^3

In view of the results obtained, the species F1624 was selected for the analysis of fermentation conditions by means of complete factorial design 2^3 having agitation, temperature and pH as independent variables, and response variables were biomass and biopigment production.

A factorial design aims to evaluate the effect of factors under various conditions with a reduced number of experiments. In this study, the experimental design was conducted to select the significant independent factors, in this case, agitation (1), temperature (2) and pH (3) on the dependent variable, in this case, bicaverine, in order to obtain an effective bioprocess. Factor planning is widely used as tools for evaluating factors in pigment production processes (Elkenawy et al., 2017; Gmoser et al., 2018).

Table 4 shows the effects of the independent variables on the production of biomass, intracellular and extracellular bicaverin by Fusarium oxysporum UCP 1624 during 168 hours were investigated according to factor design 2^3 (Table 1).

Table 4

Factorial design matrix 2^3 *to evaluate the effect of the independent variables agitation, temperature and pH on the production of biomass, intracellular and extracellular bicaverin by FusariumoxysporumUCP 1624 for 168 hours.*

Conditions	Agitation	Temperature	pH	Biomass (g/L)	Extracellular bicaverin (mg/L)	Intracellular bicaverin (mg/L)
1	0	25	4,5	2,5	13,92	66,55
2	200	25	4,5	4,9	145,64	133,53
3	0	31	4,5	2,4	13,92	89,63
4	200	31	4,5	4,0	149,30*	258,50*
5	0	25	6,5	2,7	13,07	82,31
6	200	25	6,5	4,1	26,86	134,94
7	0	31	6,5	2,7	13,64	84,28
8	200	31	6,5	4,1	33,06	158,86
9	100	28	5,5	3,3	16,17	101,17
10	100	28	5,5	3,7	16,73	100,32
11	100	28	5,5	2,9	17,86	96,38
12	100	28	5,5	2,8	15,04	95,26

Figure 2 illustrates each individually fermented trial of the plan after the 7 days of fermentation. It is possible to observe that the non-agitated assays practically did not release pigment to the medium (1, 3, 5 and 7), while the agitated assays released pigment making the medium purplish, the assays performed at 200rpm showed stronger coloration (2, 4, 6 and 8) and those at 100rpm presented weaker coloration (9, 10, 11 and 12).

Figure 2

Pigment production by F. oxysporium UCP 1624 cultivated for 168h, according to complete factorial design 23 assays (Table 4) after 7 days of incubation

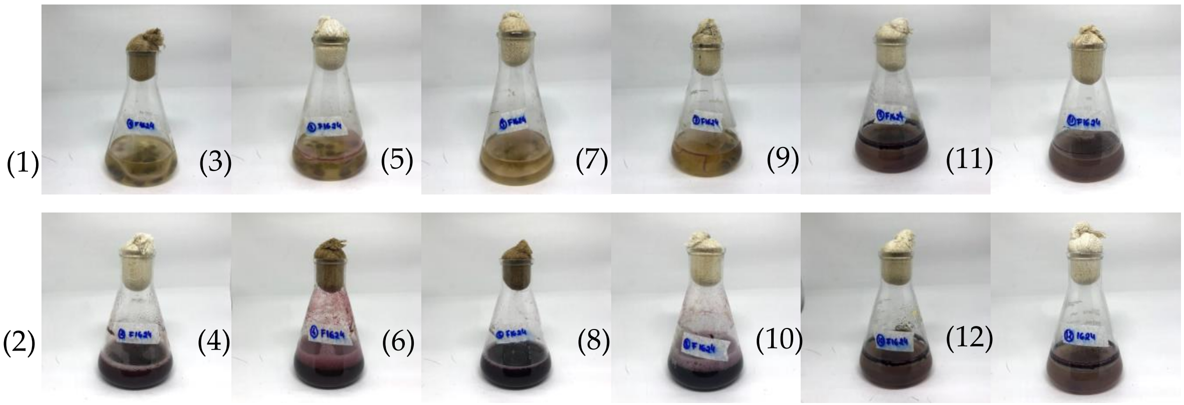
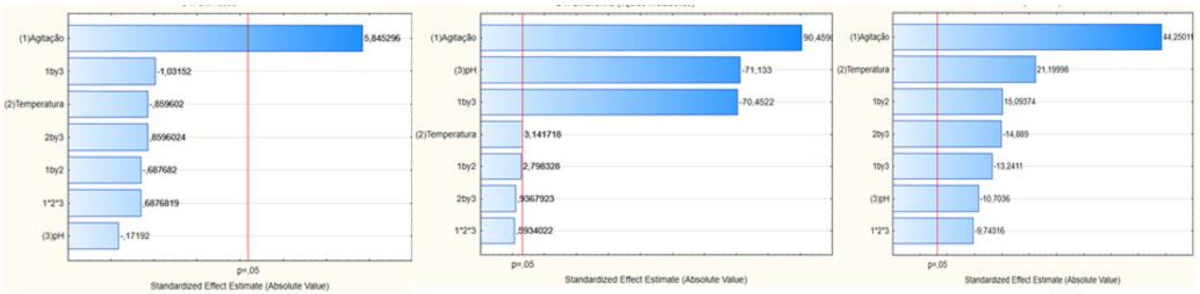


Figure 3 represents the Pareto plots, which show the estimated effects of the factors and their interactions on the response variables (biomass, intracellular and extracellular bicaverin). Pareto charts present a standard limit (p-value limit) that indicates significant effects, at a 95% confidence level.

The Pareto plots (Figure 3) show that high levels of agitation (1) have a significant influence on all dependent variables: biomass production (Figure 3A), extracellular bicaverin production (Figure 3B) and intracellular bicaverin production (Figure 3C). On the other hand, high pH levels (3) showed a negative effect, indicating that low values contribute to the production of pigment and biomass.

Figure 3

Pareto plots of the standardized effects of Agitation (1), Temperature (2) and pH (3) on the production of biomass (A), extracellular bikaverine (B) and intracellular bikaverin (C) by F. oxysporum UCP 1624.



(A) (B) (C)

According to Li & Mira de Orduña (2010) and Dos Santos et al. (2020), the metabolic pathway responsible for the production of bicaverin is affected by pH, in which lower pH values favor its production. In the study by Dos Santos et al. (2020), agitation has a positive effect on bicaverin production. According to Wiemann et al. (2009), some enzymatic activities depend on oxygen concentration, such as pre-bicaverin C6 and hydroxylation of C7 by monooxygenase.

In Figure 3A, the Pareto diagram shows that only agitation (1) favors biomass production. In Figure 3B, the Pareto diagram shows that low pH levels (3) significantly influence the production of extracellular bicaherin. As well as the antagonistic interaction of the independent variables agitation (1) and pH (3) significantly influence the dependent variable.

In the diagram of Figure 3C, all the independent variables were significant on the production of intracellular bicaverin. High levels of the variables agitation (1) and temperature (2) significantly influence the production of intracellular pigment, as well as the synergistic interaction of both variables were significant. However, lower pH levels (3) favor the production of the pigment. All interactions with the variable pH (3): temperature x pH (2x3), agitation x pH (1x3) and agitation x temperature x pH, show significant antagonism.

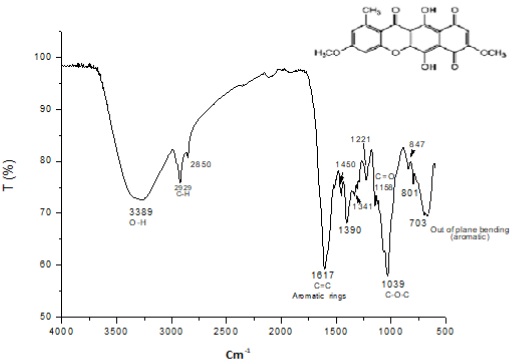
Fourier Transform Infrared Spectroscopy (FTIR)

In FT-IR spectroscopy, variations in the vibrational energy states of molecules are detected, and each frequency is specific to each functional group. And although the infrared spectrum is characteristic of every molecule, certain groups of atoms give rise to bands that occur close to the same frequency, regardless of molecular structure (Campbell et al., 2017; Kačuráková et al., 2002; Wolkers et al., 2004).

The purification of bicaherin from *Fusarium spp*. allowed the determination of its chemical structure through various techniques, including UV and IR spectra, TLC separation, nuclear magnetic resonance, mass spectrum, and X-ray crystallography. These techniques allowed the analysis of the occurrence of two different molecules, called bicaverin and norbicaverine, with a benzoxanthone ring system (as shown in Figure 4) (Limón et al., 2010).

Figure 4

Infrared spectrum of pigmented extract produced by Fusarium oxysporum UCP1624



The IR spectrum (Figure 4) of the pigmented extract of *Fusarium oxysporum* UCP 1624 showed wide OH stretch at 3,389 cm-1. The O–H elongation in the region of 3200–3550 $^{\text{cm-1}}$ corresponds to hydrogen bonding in a 5- or 6-member ring system (Sathyanarayana, 2004).

For aromatic molecules, the C–H group in the warp plane usually arise in the 1000–1300 cm^{-1} region (Socrates, 2004). In the present study, the vibration of C – H bending vibration in the plane is presented at 1221 cm^{-1}. A medium force band is observed at 801 and 847 cm^{-1} that is attributed to flexion outside the C – H plane. Strong force infrared bands between 1150 and 1180 represent stretches of C=O shapes (Socrates, 2004), this vibration is attributed at 1158 cm^{-1} in our extract.

Vibrations of the aromatic rings are proven with the infrared band of medium strength at 1341 cm^{-1}, corresponding to the elongation C=C of the phenyl rings. In this study, the aromatic ring R1 presents stretch vibration at 1465 cm^{-1} in the IR spectrum. The R3 C=C elongation presents vibrations in two absorptions, the medium force band at 1524 cm^{-1} and the strong force band at 1617 cm^{-1}.

According to Qu et al., (2012) xanthone molecules have a "very strong" stretch (ν) between 1400 and 1500 cm^{-1} for the C=C R1 group and inflection in the plane (β) of the O-H group. "Strong" stretches at ~1300 to 1200, ~1617 to 1670 cm^{-1}, ~1675 to 1748 cm^{-1}, ~3065 to 3200 cm^{-1}, ~3600 to 3840 cm^{-1} are attributable to vibrations v C-C, v C=C R3, v C=O, v C-H, V O-H, V O-H, respectively. In the study of Azuma et al. (2013) synthesized (di)benzoxanthones showed vibrational bands at ~3423, 3170, 1606, 1489 cm^{-1}, which correspond to the O-H, C-H, C=C, C=O group and 1267, 1163, 850 cm^{-1} which correspond to the fingerprints confirming the curvatures of C-H and C=O.

Based on the analytical data of the FTIR, the extract has quinones, oxygenated polycyclic aromatic hydrocarbons, as well as the pigment bicaverin, which has in its molecule an oxygen interposed between two carbon atoms (C-O-C) in aromatic chains (benzoxanthone). However, more detailed studies are needed to fully characterize the composition of the extract.

CONCLUSION

The results obtained showed that, although the three strains of *Fusarium oxysporum* evaluated (UCP 1137, UCP 0119 and UCP 1624) were able to produce pigments in solid and liquid media, the *strain F. oxysporum* UCP 1624 showed superior performance, with higher biomass, higher pigment intensity and higher production of bicaverin in the extracellular and intracellular fractions. In addition, the 2^3 factorial design confirmed that high levels of agitation and temperature significantly favor the intracellular production of the pigment, while low pH values increase the biosynthesis of both biomass and bicaverin, reaffirming the determining role of physicochemical conditions in the polyketid metabolism of these fungi.

From a social and technological point of view, the findings of this study contribute significantly to the advancement of sustainable alternatives in the production of natural dyes. Bicaverin, a pigment with recognized antitumor and antimicrobial activity and potential textile application, represents a promising strategy to reduce dependence on synthetic dyes, often associated with environmental impacts and health risks. Thus, the work reinforces the importance of low-cost, scalable and ecologically favorable bioprocesses, strengthening the field of microbial pigment biotechnology and opening paths for greener industrial applications.

Despite the consistent results, the research has some limitations. The cultivation conditions were analyzed on a laboratory scale, and factors such as nutritional composition of the medium, fungus-substrate interaction and use of agro-industrial residues as a carbon source were not investigated. Therefore, it is recommended that future studies include bioprocess scaling and optimization assays using alternative substrates and semi-industrial conditions in addition to an investigation of the molecular and regulatory mechanisms of the polytydic pathway in *Fusarium oxysporum*, contributing to the understanding of secondary metabolism in fungi.

Thus, the research offers evidence that *F. oxysporum* UCP 1624 constitutes a promising biotechnological source for the production of bicaverine, while opening new perspectives for the consolidation of microbial dyes as sustainable alternatives to synthetic pigments.

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