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Biotechnological potential of *Talaromyces* sp. isolated from soil of Caatinga biome in the production of pigments with antimicrobial activity

Potencial biotecnológico de *Talaromyces* sp. isolados de solo do bioma Caatinga na produção de pigmentos com atividade antimicrobiana

Potencial biotecnológico de *Talaromyces* sp. aislados de suelo del bioma Caatinga en la producción de pigmentos con actividad antimicrobiana

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Abstract
Global demand for natural pigments and dyes has increased much in recent years, driven by a heightened awareness of the toxic effects of various synthetic dyes on pollution of the environment and health human. The pigments and colorants obtained through plants and microbes are the primary source exploited by modern industries. Among the other non-conventional sources, filamentous fungi particularly are known to produce an extraordinary range of colors including several chemical classes of pigments such as melanins, azaphilones, flavins, phenazines, and quinines. The objective of this work was to evaluate the production of pigments produced by Talaromyces spp. and its antimicrobial activity. In this context, strains of the fungi *Talaromyces* spp. were isolated from soil of biome Caatinga and showed production of dark red, red and yellow pigments. Almost all fungi were able to grow and produce soluble pigments in the solid medium containing different carbon sources (sucrose, fructose, glucose, maltose, and starch), as well as in different salinity concentrations (0.5%, 1%, 2%, 4% and 6%), kept at room temperature (28ºC). All the extracted pigments showed antimicrobial activity against to bacteria Gram negative, as well as to yeasts, evidencing the high potential of application in the textile industry to produce antimicrobial fabrics.
Keywords: Screening; Filamentous fungi; Salinity; Pigment; Colorant. Antimicrobial activity.

Resumo
A demanda global por pigmentos e corantes naturais tem aumentado muito nos últimos anos, impulsionada por uma maior conscientização dos efeitos tóxicos de diversos corantes sintéticos na poluição do meio ambiente e na saúde humana. Os pigmentos e corantes obtidos através de plantas e micróbios são a fonte primária explorada pelas indústrias modernas. Entre as outras fontes não convencionais, os fungos filamentosos são particularmente conhecidos por produzir uma extraordinária gama de cores, incluindo várias classes químicas de pigmentos, como melaninas, azafilonas, flavinas, fenazinas e quininas. O objetivo deste trabalho foi avaliar a produção de pigmentos produzidos por Talaromyces spp. e sua atividade antimicrobiana. Nesse contexto, cepas do fungo Talaromyces spp. foram isolados de solo do bioma Caatinga e apresentaram produção de pigmentos vermelho escuro, vermelho e amarelo. Quase todos os fungos foram capazes de crescer e produzir pigmentos solúveis no meio sólido contendo diferentes fontes de carbono (sacarose, frutose, glicose, maltose e amido), bem como em diferentes concentrações de salinidade (0,5%, 1%, 2%, 4 % e 6%), mantidos à temperatura ambiente (28ºC). Todos os pigmentos extraídos apresentaram

atividade antimicrobiana frente a bactérias Gram negativas, bem como a leveduras, evidenciando o alto potencial de aplicação na indústria têxtil para produção de tecidos antimicrobianos.
Palavras-chave: Seleção; Fungos filamentosos; Salinidade; Pigmento; Corante. Atividade antimicrobiana.

Resumen
La demanda mundial de pigmentos y tintes naturales ha aumentado mucho en los últimos años, impulsada por una mayor conciencia de los efectos tóxicos de varios tintes sintéticos sobre la contaminación del medio ambiente y la salud humana. Los pigmentos y colorantes obtenidos a través de plantas y microbios son la principal fuente explotada por las indústrias modernas. Entre las otras fuentes no convencionales, se sabe que los hongos filamentosos en particular producen una gama extraordinaria de colores que incluyen várias clases químicas de pigmentos como melaninas, azafilones, flavinas, fenazinas y quininas. El objetivo de este trabajo fue evaluar la producción de pigmentos producidos por Talaromyces spp. y su actividad antimicrobiana. En este contexto, cepas de los hongos *Talaromyces* spp. fueron aisladas del suelo del bioma Caatinga y mostraron producción de pigmentos rojo oscuro, rojo y amarillo. Casi todos los hongos fueron capaces de crecer y producir pigmentos solubles en el medio sólido que contenía diferentes fuentes de carbono (sacarosa, fructosa, glucosa, maltosa y almidón), así como en diferentes concentraciones de salinidad (0,5%, 1%, 2%, 4 % y 6%), conservado a temperatura ambiente (28ºC). Todos los pigmentos extraídos mostraron actividad antimicrobiana frente a bacterias Gram negativas, así como a levaduras, evidenciando el alto potencial de aplicación en la industria textil para producir tejidos antimicrobianos.
Palabras clave: Cribado; Hongos filamentosos; Salinidad; Pigmento; Colorante.

1. Introduction

The history of the pigments described the painters had used natural dyes from variated sources as plants, insects, molluscs and minerals for their paintings. However, the unique and special character of their works were the result of using different mixtures of dyes and mordants, as varnishes and lacquers responsible for cohesion and the possibility of different colors from pigments and may be protection of the layers promoted some important effects.

Color is the first parameter to be noticed about an article by the receiver. It has immediate perceptual and cognitive significance in human experience. It serves as an activating stimulus that enhances visual awareness and responsiveness. Since color perception is the strongest emotional part of the visual process, it has great strength that can be used to express and reinforce visual information to great advantage, being widely used to make a product more attractive (Sánches-Muños et al. al., 2020). As a result, pigments have become an essential part of human daily life and have extensive applications in many areas, such as agriculture, textiles, cosmetics, pharmaceuticals, food, among others (Daud et al., 2021; Paillié-Jiménez et al., 2020; Venil et al., 2020).

Synthetic dyes are often used in different fields such as in the food industry, paper and agricultural industry and science and technology. But, due to adverse toxicological side effects of synthetic pigments used in industries, research is now focused on products from natural resources (Sánches-Muños et al., 2020). Synthetic dyes are non-renewable, non-biodegradable, sometimes carcinogenic and teratogenic. In addition to causing a negative impact on the environment through pollution by toxic waste, presenting a major challenge in disposing of waste by-products in an economical way (Paillié-Jiménez et al., 2020).

According to Research Nester, the pigment market is on the rise with a projection of raising US$66.3 billion by the end of 2024, against US$46.8 billion that the market generated in 2016. Therefore, the organic pigments are projected to provide an absolute $ opportunity worth US$ 2.3 Bn over the forecast period (2022-2032), and the demand for organic dyes based on type is projected to increase around 5.7%through 2031 (Source: FACT.MR, 2022).

Despite the drawbacks, synthetic dyes still have an advantage in terms of large-scale production at an economical price with consistent color quality and numerous color variations outweighing the benefits of natural dyes. To minimizing their negative environmental impacts of pigments, and various aspects of bio-colorant applications produce a competitive pigment, investments are being made in producing natural pigments based on the use of agro-industrial residues as substrates (Morales-Oyervides et al., 2020; Troiano et al., 2022).

Because of their genetic simplicity compared to plants, microorganisms may be a better source for understanding biosynthetic mechanisms and for being engineered to produce high yields of pigments. Despite the origin of the pigmented microorganism, it seems very important to develop protocols using organic industrial waste and agricultural by-products as substrates for pigment production and to find new green strategies for rapid pigment extraction (Pailliè-Jiménez et al., 2020).

The high demand for natural products is driving an exponentially growing market, and the annual growth rate of the dyes market is estimated at ~7% and is expected to reach $7.79 billion by the year 2020 (Dikshit & Tallapragada, 2018).

Thus, the organics market and the pigment industries represent vast commercial sectors that would soon be dominated by microbial pigments (Novoveská et al., 2019). Compared to plant and animal sources, the production of microbial pigments by fermentation technology is more dynamic and economical, resulting in biodegradable compounds that can have broad industrial applications as dyes (Silva et al., 2019; Venil et al., 2020) Although microbial pigments are not widespread in dye formulations, they represent an important alternative that has the long-term ability to compete with synthetic dyes (Zerin et al., 2020). Successful application of microbial pigments depends on high production yields, reasonable production costs, regulatory approval, pigment characterization and stability to environmental factors such as temperature and light (Morales-Oyervides et al., 2017).

2. Methology

2.1 Microorganism

Different cultures of fungi of the genus Talaromyces strains isolated from soil of the biome Caatinga belong to Culture Collection UCP (Universidade Católica de Pernambuco), located at the Nucleus of Research in Environmental Sciences and Biotechnology-NPCIAMB, Catholic University of Pernambuco, and registered in the World Federation for Culture Collection (WFCC). The strains ware maintained in Sabouraud dextrose Agar medium, incubated at 28ºC, for 10 days and kept at 5º C.

2.2 Screening of fungal pigments

2.2.1 Effect of salt concentrations on the growth and pigment production

The effect of salt on fungal growth was observed by adding salt to Sabouraud Dextrose Agar (SAB) medium. About 0.5%, 1%, 2%, 4% and 6% (w/v) of NaCl were added to the SAB medium. All media were adjusted to pH 6.5. After the 7-day incubation period, the plates were analyzed.

2.2.2 Effect of carbon sources on growth and pigmentation

For screening of the filamentous fungus for pigment production, all Petri dishes were inoculated with 5% (v/v) of the spore suspension, containing 10^6 cells/mL. To verify the effect of carbon sources on the production of pigments, about 2% of different carbohydrates such as sucrose, fructose, glucose, maltose, and starch were added as the only carbon source to the medium instead of dextrose to verify the effect of different carbohydrates on fungal growth and a dextrose control was used for comparison. All media were adjusted to pH 6.5. After 7 days of fungal incubation, the plates were observed, and the influence recorded.

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2.3 Extraction of pigments

After incubation, the intracellular and extracellular pigments contained in the fermented material were extracted with 70% ethanol in an incubator with agitation at 150 rpm for 24h, followed by centrifugation and filtration. After filtration, the material was lyophilized and weighed (General et al., 2014).

2.4 Disc diffusion assay

The antimicrobial activity was developed according to the methodology described by Bauer et al. (1966). The antibacterial activity assays were carried out using the Disc diffusion method (Balachandran et al., 2013) using clinical bacteria *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*., and the yeasts *Candida sphaerica*, *Candida tropicalis*, *Candida glabrata*, *Candida guillermondii*, *Candida lipolytica* as test organisms. The microorganisms test, obtained by a suspension of standardized cells. at an approximate concentration of 10^7 cells/mL, seeded by the “spread-plate” technique using swab for transferred to surface of the medium. About 10 µg of the sample from pigments isolated from *Talaromyces* sp UCP1337, *Talaromyces* sp UCP1349 and *Talaromyces* sp GT022 were impregnated into Whatman filter paper discs and were used to determine antibacterial activity. All the plates were incubated at 37 °C for 24 h. Zones of inhibition were measured after 24 h. The control discs contained ethanol was used as control of solvent. The test was performed in triplicate for all strains.

3. Results and Discussion

3.1 Morphological characteristics of isolated fungi

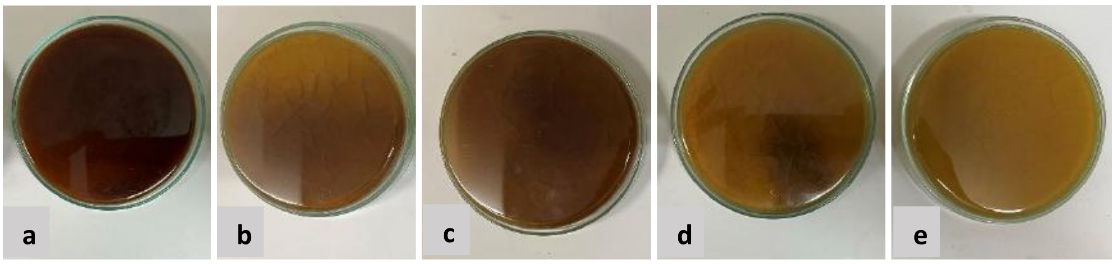
The colonies of the genus *Talaromyces* spp. were characterized by a gray-green color with white edges. The conidiophores were branched and the metulae and phialides extended from these branches. Conidia were globose (round) to subglobose (somewhat rounded).

3.2 Growth and pigment production by *Talaromyces* spp. strains

3.2.1 Influence of different NaCl concentrations

The results showed the influence of NaCl concentrations on the growth and the pigments production of the strains *Talaromyces* UCP 1337, *Talaromyces* UCP 1349 and *Talaromyces* Ga022, (Figures 1, 2 and 3), respectively.

Figure 1. Influence of different NaCl concentrations [(a) 0,5%, (b) 1%, (c) 2%, (d) 4% e (e) 6%] on the growth and pigment production and was solubilized into the media by the growth of the filamentous fungus *Talaromyces* sp. UCP 1337.

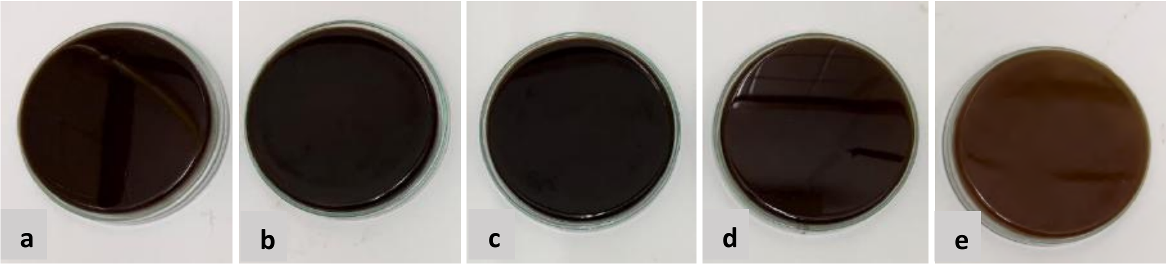


Source: Authors (2022).

The filamentous fungus *Talaromyces* UCP 1337 showed excellent growth on different concentrations of salinity up to (Figure 1). The growth of the fungal strains *Talaromyces* UCP 1349 and *Talaromyces* Ga022 developed at all tested salinity concentrations NaCl can apply stress to the fungus, so it can be verified that the studied strains of the genus *Talaromyces* are resistant to high salinity concentrations, showing that the tested fungi are able to grow well in saline conditions.

The strain *Talaromyces* UCP 1337 only obtained visible growth of pigment in the condition of absence of salinity and using the carbon source glucose (Figure 4a), under these conditions the fungus produced an intense red pigment after 8 days of cultivation. Venkatachalam et al. (2019) also reached its optimal condition in medium with no salinity using the fungus *Talaromyces albobiverticillius* 30548 for pigment production.

Figure 2. Influence of different NaCl concentrations [(a) 0,5%, (b) 1%, (c) 2%, (d) 4% e (e) 6%] on the growth and pigment productions and was solubilized into of the filamentous fungus *Talaromyces* UCP 1349.

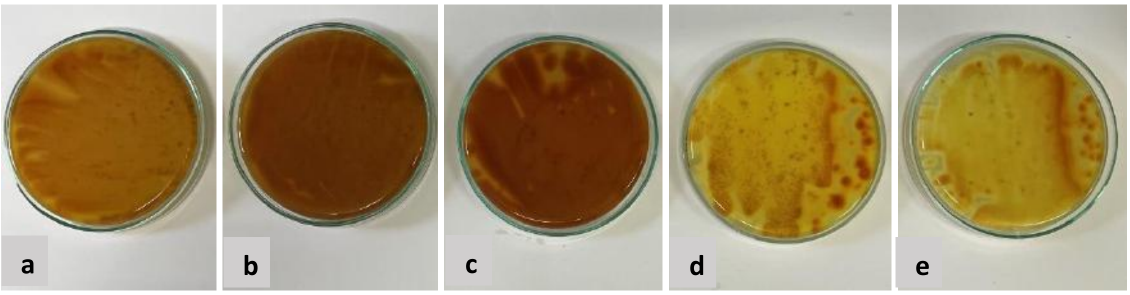


Source: Authors (2022).

The pigment produced by *Talaromyces* UCP1349 showed a red color and was visibly produced at all salinity concentrations (Figures 2 a-e) tested, reaching its peak at 2% NaCl concentration (Figure 2c), showing more intense coloring than in the control medium (Figure 5a). The strain was shown to synthesize the secondary metabolite under saline stress conditions. The effects of salinity on pigment production by various strains of *Penicillium* were studied by Huang et al. (2011). In their study, it was reported that NaCl effectively promoted growth in 91.5% of its 47 strains and antimicrobial activity in 14.5%. A strain of *Penicillium sp* obtained 4.4- and 4.9-times higher yields in treatments with 3% and 6% NaCl, respectively.

This result was similar from that found by Chadni et al. (2017) for *Talaromyces verruculosus* that obtained a growth inhibition at all tested salinity concentrations, showing a growth inhibition rate of 75% at 6% NaCl concentration.

Figure 3. Influence of different NaCl concentrations [(a) 0,5%, (b) 1%, (c) 2%, (d) 4% e (e) 6%] on the growth and pigment productions and was solubilized into of the filamentous fungus *Talaromyces* Ga0022.



Source: Authors (2022).

Salinity is a crucial factor that influences the growth and production of secondary metabolites (Venkatachalam et al., 2019), corroborating what was obtained by the *Talaromyces* Ga0022 strain, which at high salinity concentrations (4% and 6%) produced coloring pigments yellow (Figures 3d and 3e) and at low concentrations (Figures 3a, 3b and 3c) and in the absence (Figure 6a) it produced red pigment.

Table 1. Comparison of pigments produced by *Talaromyces* spp. Strains growth in different concentrations of salinity.

MICROORGANISM	NaCl CONCENTRATIONS/PIGMENT					
	Absence	**0,5%**	**1%**	**2%**	**4%**	**6%**
***Talaromyces* sp. 1337**	Dark Red	Red Brown	Reddish yellow	Reddish yellow	Reddish yellow	Reddish yellow
***Talaromyces* sp. 1349**	Red Brown	Dark Red	Dark Red	Dark Red	Red	Red
***Talaromyces* sp. Ga0022**	Red Intense	Red Intense	Yellow	Yellow	Yellow	Yellow

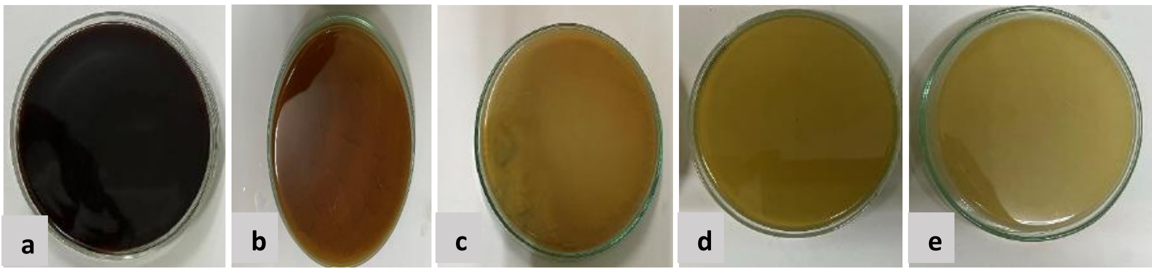
Source: Authors (2022).

Table 1 above shows the pigments produced by the *Talaromyces sp*. 1337, *Talaromyces sp*. 1349 and *Talaromyces sp.* Ga0022 under different salinity concentrations using the adapted Sabouraud Dextrose Agar medium (20g/L Glucose, 10g/L peptone and 20g/L Agar).

3.2.2 Influence of different carbon sources

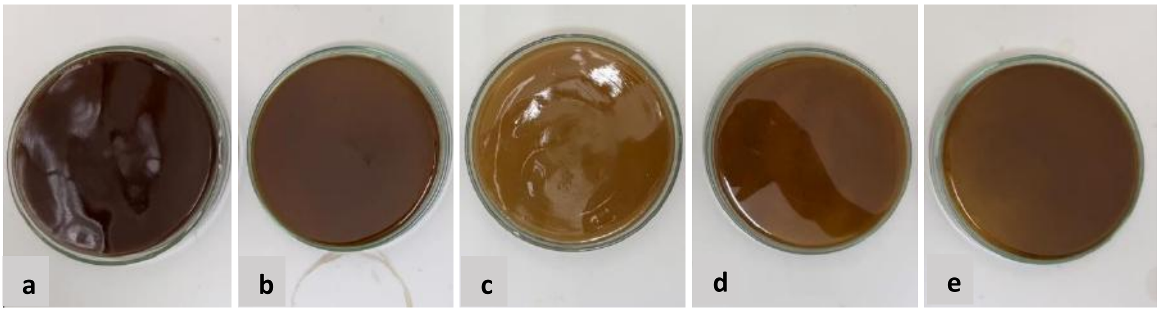
As for the carbon sources tested, analyzing the production of pigments, the strains *Talaromyces* UCP 1337 (Figure 4), *Talaromyces* UCP 1349 (Figure 5) and *Talaromyces* Ga0022 (Figure 6) obtained their best condition using glucose as a carbon source.

Figure 4. Influence of different carbon sources as glucose (a), fructose (b), starch (c), maltose (d) and sucrose (f) on the growth and pigment was solubilized into the media by *Talaromyces* sp. UCP 1337.



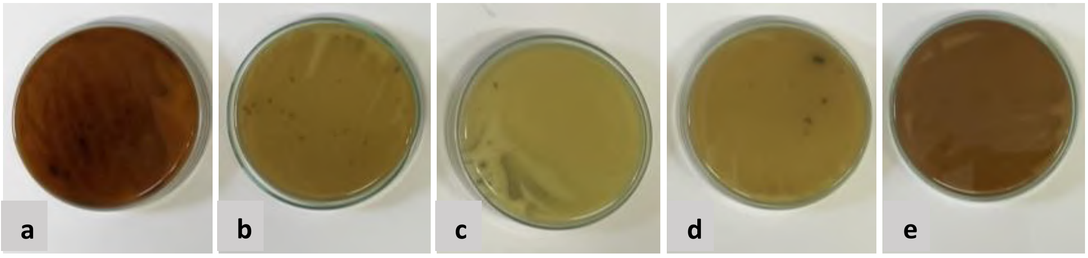
Source: Authors (2022).

Figure 5. Influence of different carbon sources as glucose (a), fructose (b), starch (c), maltose (d) and sucrose (f) on the growth and pigment was solubilized into the media by *Talaromyces* sp. UCP 1349.



Source: Authors (2022).

Figure 6. Influence of different carbon sources as glucose (a), fructose (b), starch (c), maltose (d) and sucrose (f) on the growth and pigment was solubilized into the media by *Talaromyces* sp. Ga022.



Source: Authors (2022).

The *Talaromyces* UCP 1337 strain only showed visible pigment production using glucose as a carbon source (Figure4a); while the fungus *Talaromyces* Ga0022 clearly showed no pigment production using the carbon sources: fructose (Figure 5b), starch (Figure 5c), maltose (Figure 5d) and sucrose (Figure 5e); and the *Talaromyces* UCP 1349 strain produced little or no pigment using the carbon sources: fructose (Figure 6b), starch (Figure 6c), maltose (Figure 6d) and sucrose (Figure 6e). The results obtained corroborate with Nimnoi et al. (2011).

3.3 Antimicrobial activity

The red and yellow pigments of the *Talaromyces* Ga022, and the red pigments of the *Talaromyces* strains UCP 1337 and UCP 1349 were extracted with the solvent 70% ethanol due to their low cost and for being less harmful to the environment compared to solvents used for the extraction of secondary metabolites (Kantifedaki et al., 2018).

The red pigments produced by the *Talaromyces* strains UCP 1337 and UCP 1349 showed action against *Pseudomonas aeruginosa* and the strain UCP 1349 also showed action against *Candida lipolytica* (Table 2). Velmurugan et al. (2009) obtained an inhibition halo of only 1 mm with pigment produced by *Monascus purpureus* and 0.6 mm with pigment produced by *Penicillium purpurogenum* against Pseudomonas aeruginosa, while the pigments produced in this study by the strains of *Talaromyces* UCP 1337 and UCP 1349 obtained an inhibition halo of 15 mm and 17 mm, respectively, demonstrating better antimicrobial activity (Table 2).

Table 2. Antimicrobial activity of the pigments produced by *Talaromyces* spp. strains against to bacteria and yeasts.

Microoganism	HALO/ mm								
	Pigment	*Escherichia coli* UCP0175	*Pseudomonas aeruginosa* UCP1559	*Bacillus subtilis*	*Candida sphaerica* UCP0095	*Candida tropicalis* UCP0996	*Candida lipolytica* UCP0988	*Candida glabrata* UCP1002	*Candida guillermondii* UCP1592
Talaromyces sp. UCP1337	RED	-	15	-	-	-	-	-	-
Talaromyces sp. UCP1349	RED	-	17	10	-	-	12	-	10
Talaromyces sp. Ga022	RED	10	-	15	10	-	10	10	15
	YELLOW	9	10	-	-	-	12	-	-

Resistente (-). Source: Authors (2022).

While the red pigment produced by *Talaromyces* Ga022 showed action against *C. sphaerica, E. coli, C. lipolytica, C. glabrata, C. guillermondii and B. subtilis* and the yellow pigment produced by the same strain at high salinity concentrations showed action against *E. coli, C. lipolytica, P. aeruginosa and C. guillermondii*. Vendruscolo et al. (2014) also obtained fungal pigment with antimicrobial activity against foodborne bacteria: *E. coli* (Table 2).

Due to the antimicrobial activity developed by the extracted pigments, they have a high potential to be used in the manufacture of antimicrobial fabrics, such as the pigment produced by Bisht et al. (2020).

The pigments are considered as secondary metabolites of *Talaromyces* sp. strains and are described as esters, including macrolides, linear polyesters, aromatic lactones, coumarins, phthalides and they show various biological activities, including antibacterial (Song et al. 2022). The antimicrobial activity detected in *Talaromyces* sp. strains isolated from Caatinga soil showing zone of inhibition to bacteria and yeasts are shown in Table 2. The results indicated high potential of the pigments isolated and these compounds showed potential inhibitory effects against *Pseudomonas aeruginosa*, *Bacillus subtilis* and all *Candida* species, except to *Candida tropicalis*.

4. Conclusion

Filamentous fungi were able to produce promising pigments with antimicrobial activity and suggest higher potential for application in the textile industry to produce antimicrobial fabrics. The best yields of the red biopigments produced by *Talaromyces sp*. UCP 1337, red produced by *Talaromyces sp*. UCP 1349, red by the fungus *Talaromyces* Ga0022 and yellow by the fungus *Talaromyces* Ga0022 were in the media formulated from the absence of salinity and glucose; 2% NaCl and glucose; absence of salinity and glucose and 6% NaCl and glucose, respectively.

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