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Induction of pigment production through media composition, abiotic and biotic factors in two filamentous fungi

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ABSTRACT

In addition to plant-derived, fungal pigments have become an alternative in respect to synthetic ones. Besides *Monascus* sp., several pigment-producing fungi do not have culture conditions well-established yet. In this research, media composition, light wavelength and co-culture were evaluated, results were reported in Absorbance Units per gram of biomass (AU/Bgr). For *Fusarium oxysporum* a C:N ratio above 7 was advantageous, using both complex and defined media; blue LED light increased the AU/Bgr value from 18013 to 344; co-culture did not enhance pigment production. In *Aspergillus chevalieri* a high C:N ratio with glucose as carbon source was ideal. When exposing cultures to light, UV and red light gave the highest pigmentation; moreover, differential UV-VIS spectra in all wavelengths suggested production of additional pigments. Particularly a pigment observed when cultured in green light was also found in co-culture with yeast and there was an improvement of AU/Bgr value of 52549%. This is the first report regarding light effect and co-culture for these fungi, as well as C:N ratio for *A. chevalieri*.

1. Introduction

The use of synthetic or artificial colors is a frequent subject of debate led by safety regulatory entities; this concern has caused the extensive study of artificial colors regarding their effect on health. The European Food and Safety Authority (EFSA) started a general re-evaluation in 2009 after which, by examining the consumption safety thresholds, they decided to lower the acceptable dairy intake (ADI) of the colorants quinoline yellow (E104), sunset yellow (E110) and ponceau 4R (E124) [1–3]; showing the increase in restrictions concerning the use of some dyes. Given this situation, the fundamental problem to be solved is to deliver a new group of natural pigments. The successful marketing of natural pigments from plant source as food coloring reflects the presence and importance of niche markets [4]; however, it must be outlined that the main bottleneck of natural color production from vegetable origin is that obtainment takes longer, and production is subject to environmental conditions.

Filamentous fungi instead, present more advantages since they have a greater ability to be produced independently of field conditions, such is the case of species of *Monascus sp*. that synthesize yellow, orange, purple and red pigments [5]. Some strains of this genera have already been cultured in rice and in defined media of glucose and monosodium glutamate yielding 89,3 and 64 abs/g dry product respectively [6,7]. In addition, nowadays fungi have started to become a reality in the market of natural colors. Beta-carotene from *Blakeslea trispora* is approved by the EFSA and production reaches concentrations of almost 17 g/L; this fungus as well has been cultured employing several carbon sources such as glucose, lactose and vegetable oils [8,9].

Despite this scenario, for other pigment-producing fungal species, studies on the optimization of production are not available yet; added to this, given that most of this type of metabolites are not growth associated, the study of induction by factors that allow increasing their yield becomes necessary.

Among these scarcely studied fungi *Aspergillus chevalieri* (or teleomorphs in *Eurotium* sp.) and *Fusarium oxysporum* are promising strains and reports of pigment production in submerged culture are very limited. There are some works dealing with pigment production in submerged culture for other strains belonging to *Fusarium* sp. such as *Fusarium verticillioides* and *Fusarium moniliforme*. In the case of *A. chevalieri* and related

Abbreviations: AU/Bgr, (absorbance units per gram of biomass).
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species, literature mainly focuses on characterization of compounds in the fractionated extracts obtained when culturing in standard media as PDA or Malt extract-yeast extract-sucrose [10–12] in search of bio-activities [13–17].

Some species of the genera *Fusarium* sp. like *F. verticilloides*, *F. fujikuroi,* and *F. oxysporum* produce a series of pigments among which bikaverin, norbikaverin, and other compounds like nectriafurone and O-demethylanhydrofusarubrin are found [18]. Bikaverin, a pigment produced by *F. oxysporum* under acidic conditions, is considered by some authors as a naphtoquinone [19]. Others consider it a tetracyclic benzoxanthone resulting from the synthesis performed by a multifunctional class I polyketide synthase, followed by subsequent group modifications mediated by mono-oxygenase and a methyltransferase [20,21].

For *Aspergillus chevalieri* and related species as *A. glaucus, A. cristatus,* and *A. ruber* pigment production has been reported, specifically hydroxi-anthraquinones. Physcion or parietin, asperflavin, questin, erytroglaucin, and glycosilated anthraquinones can be found among these compounds [15]. Additional to these ones, a type of molecules with a benzaldehyde core can be found as well, having also an isporene unit and lateral chains of 7 carbons, among these, compounds like flavoglaucin, auroglaucin, dihydroauroglaucin, and tetrahydroaurogaucin can be found. These compounds present differences in the level of unsaturation of its lateral chain [16].

These two strains are promissory sources of pigments for diverse reasons, one is their biological activity. In respect to bikaverin from *Fusarium oxysporum*, so far, it has not been defined a clear role of the substance in plant pathogenicity [22,23]. In contrast to this, the purpose of biosynthesis of these compound might be related with inhibition of microorganisms in a saprophytic phase since it has already been reported that the compound has antibacterial and anti-parasitic activity. There is as well one work that highlights its potential as an antioxidant in neuronal cell lines [23–25].

Regarding pigments of *Aspergillus chevaleri* and related species, antioxidant activity of benzil derivatives has been well documented as well as the antimicrobial activity of their anthraquinones, and in fact, this fungi can be found in a fermented food in Japan named Katsuobushi [10,26,27].

The second reason of their potential is that they can also be considered as new alternatives to existing natural pigments of yellow and red hue; in terms of its stability, it is known that natural colors like curcumin and betacyanins are unstable [28,29]. So far there are no research articles regarding the stability of the pigments produced by these two fungi; however, it is well-known that anthraquinones are heat [30,31] and light stable [32]. It is also known that yellow hue anthraquinones are produced by *Aspergillus chevalieri* and related species [27,33,34]. There is also ongoing research in the Biotechnology research group concerning stability assays of the red pigment of *F. oxysporum* using castor oil as a vehicle finding almost the same level of heat stability as carmine (antraquinone lake emulsion) and improved light stability (data not included in this study).

The metabolites produced by these two strains are compounds of secondary metabolism, it is well-known that many of the genes clusters coding for secondary metabolites are just expressed under specific conditions and that some standard laboratory maintenance protocols do not trigger these pathways [35]. For this reason is of great importance the study of the effect of nutritional, biotic and abiotic factors [36–40] in this context is necessary to evaluate the effect of variables as carbon to nitrogen ratio, complex or defined media, stimulation through interaction with microorganisms and exposure to different light wavelengths [41]. Therefore, this paper aims to determine culture conditions for improving pigment production in the filamentous fungi *Fusarium oxysporum* and *Aspergillus chevalieri* through nutritional, biotic and abiotic stimuli.

2. Materials and methods

2.1. Strain maintenance and inoculum preparation

Strains of *Fusarium oxysporum* and *Aspergillus chevalieri* were obtained from a collection of the Biotechnology research group and cultured in PDA for 2 weeks at 23 °C + 1 °C.

In each fungus, for preculture, a modification of the section inoculum preparation found in the method M38-A2 of the CLSI was performed [42], 12 fragments of mycelium of 1 cm Φ were macerated sequentially until disintegration of the mycelial circles; 40 ml of saboreaud liquid medium were added to the macerated biomass with an adjusted pH of 5,6 for *F. oxysporum*, and *A. chevalieri*. Each blend was syringe filtered through a holder with a 50 μm mesh; the filtrate was then disposed in 50 ml conic tubes and used to inoculate seed cultures; pre-inoculum was carried in saboreaud medium. The flasks were left at 24 °C ± 1 °C and 100 rpm with a basal light irradiance of 0,715 W/m^2; through preliminary studies, it could be determined that the proper incubation times for each fungus were 36 h for *F. oxysporum* and 120 h for *A. chevalieri*. For all assays performed, 8 ml of mycelial suspension were used as inoculum (10% of total working volume) equivalent to 0,2 g/L dry weight for *F. oxysporum* and 0,26 g/L for *A. chevalieri*.

2.2. Culture media assay

Culture was undertaken with 80 ml of medium. Evaluated media had a base salt composition consisting of (in g/L): $MgSO_4.7H_2O$ - 0,02, KCl - 0,01 KH_2PO_4 - 0,03 and $NaNO_3$ - 0,08 [43]; In addition, a pH of 4,0 was established for *F. oxysporum*; and of 5,5 for *A. chevalieri*; composition of the media is shown in Tables 1 and 2. The incubation time was 15 days for *F. oxysporum* [44,45] and 18 days for *A. chevalieri* [17,26,27,46] with the same incubation conditions used in the inoculum; triplicates were done for all conditions.

2.3. Influence of LED light assay

The fungi were inoculated in 15 flasks for each selected media, incubation conditions were the same as in the culture media assay and five wavelengths were evaluated using light emitting diodes: Blue, green, red, white and UV-A light; a light irradiance of 9 + 0,5 W/m^2 was established for the first four wavelengths and for UV light the value was set at 0,05 + 0,005 W/m^2.

Table 1
C and N content for several media in *F. oxysporum*.

C:N Ratio	Culture Media
C:N 3	M15Y5
C:N 2	M15Y7,5
C:N 1,5	M15Y10
C:N 6	M30Y5
C:N 4	M30Y7,5
C:N 3	M30Y10
C:N 9	M45Y5
C:N 6	M45Y7,5
C:N 4,5	M45Y10
C:N 7,03	BFL 20
C:N 7,03	BFL 30
C:N 7,03	BFL 40

Table 2
C and N content for several media in *A. chevalieri.*

C:N Ratio	Culture Media
C:N 10	G10Y1
C:N 3,3	G10Y3
C:N 2	G10Y5
C:N 20	G20Y1
C:N 6,6	G20Y3
C:N 4	G20Y5
C:N 30	G30Y1
C:N 10	G30Y3
C:N 6	G30Y5
C:N 7,03	BFL 20
C:N 7,03	BFL 30
C:N 7,03	BFL 40

M: Maltodextrin Y: Yeast extract: BFL: Barley Flour.
G: Glucose Y: Yeast extract.

2.4. Co-culture assay

2.4.1. Bacteria and yeast seed culture

Strains of *P. fluorescens* and *K. marxianus* were maintained in Pseudomonas P Agar and Malt extract-YPD Agar correspondingly. For seed culture, these were grown in the same media without agar. Incubation conditions were 130 rpm and 30 °C for *P. fluorescens* and 150 rpm and 37 °C for *K. marxianus*.

2.4.2. Co-culture

The fungi were inoculated in 24 flasks for each selected media, incubation conditions were the same as in the culture media assay; each half was then used for co-culture with the soil dwelling bacteria *P. fluorescens* or the yeast *K. marxianus*; days 6^{th}, 7^{th} and 8^{th} were established for the start of co-incubation in *F. oxysporum*; in the case of *A. chevalieri* it was performed on the 3^{rd}, 4^{th} and 5^{th} day. Final optical density of 0,05 was established for each microorganism (0,0125 g/L dry weight for bacteria and 0,175 g/L for yeast).

2.5. Biomass quantification

The content in the flasks was filtrated using cellulose filter paper and wet biomass was freeze-dried for 3 days at −50 °C and 20 mTorr (EYELA-FDU-1110·2110 freeze dryer).

2.6. Pigment extraction

Dry biomass was processed in an IKA® - A 11 basic analytical mill; and later pulverized in a mortar, to obtain a particle size of 0,3 mm. 100 mg of biomass and 250 mg of 0,5 mm glass beads were weighed into 1,5 ml PFA (Perfluoroalkoxy-alkane) vials. Finally, 0,7 ml of dichloromethane was added and extraction took place in a digital disruptor-genie (Scientific Industries ™) at 3000 rpm for 8 min. After a cycle of extraction, tubes were centrifuged at 13000 rpm, and colored supernatant collected; the process in the disruptor was repeated three times with clean solvent.

2.6.1. Estimation of produced pigments

Extracts were filtered with a 0,45 μm PTFE syringe filter unit, (SLCR025 EMD-Millipore) and the volume of filtered extract was recorded before making dilutions or stocking undiluted extracts in glass vials (to discard bias of solvent volatility) The estimation of pigment production in terms of absorbance units was calculated according to formula 1.

Formula 1. Absorbance Units

$$AU = \frac{[Abs * V_{fl}]}{V_r}$$

Where:

AU: Absorbance Units

V_{fl}: Filtered Volume

V_r: Read Volume in spectrophotometer

With this information, the yield of Absorbance units per biomass (AU/Bgr) was obtained.

The different extracts were taken to a quartz microplate to be read at 520 nm and 395 nm, for *Fusarium oxysporum* and *Aspergillus chevalieri* respectively in the culture media experiment. For the experiments of LED light and the co-culture effect, scans of the extracts in the spectrophotometer were done in order to take into consideration the presence of other pigments whose production have been stimulated under these diverse conditions.

2.7. Statistical analysis

Logarithm transformation was applied to the data of C:N ratio in *Fusarium oxysporum* and media type in *Aspergillus chevalieri,* this allowed the analysis of data by one way-ANOVA. Untransformed data for LED light effect was analyzed in the same manner; and finally the co-culture effect by two way-ANOVA; only in *Fusarium oxysporum*; which was not possible for *Aspergillus chevalieri* since several pigments occurred merely in specific conditions. Significant differences were considered for those sets of data with p-value < 0,05.

3. Results

3.1. Media composition and C:N ratio

Through literature revision and the spectrophotometric scan of the dichloromethane crude extract of *F. oxysporum* obtained from cell disruption, it can be inferred that the red pigment produced is bikaverin, with a λ_{max} of 520 nm [44,47].

The effect of media composition as well as the C:N ratio could be determined (Fig. 1); there is satisfactory stimulation of pigment production if there is a high C:N ratio and the concentration of the nitrogen source is ≤ 5 g/L. Since the p-value for media type is > 0,05, there is no statistical difference between chemically defined media and complex media; nevertheless, p-value is < 0,05 for the factor C:N ratio (Table 3).

In the present research, it is possible to prove that the complex media BFL 40 with a C:N ratio of 7,03 that gave the highest biomass concentration did not give the highest AU/Bgr value. In contrast, the best result of pigment production was achieved in the M45Y5 media with a C:N ratio of 9 but with lower biomass (7,65 g/L- Fig. 1), showing that bikaverin production is not necessarily proportional to biomass production. This media was selected for further assays.

Concerning *Aspergillus chevalieri*, spectrophotometric scan and literature give some insight in the production of a yellow pigment,

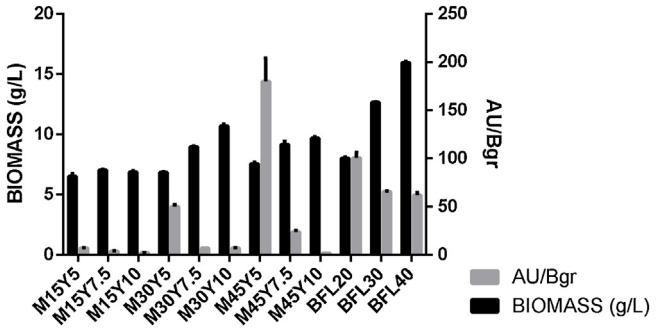

Fig. 1. Biomass and Pigment production in MY and BFL media for *F. oxysporum.*

Table 3
p-value for different treatments.

FACTOR	p – value	
	F. oxysporum	*A. chevalieri*
C:N ratio	3,439 e-14*	2,2 e-16
Media Type	0,912	1,267 e-4
LED Light	4,217 e-13*	n/a[a]
Microorganism	1,607 e-06*	n/a[a]
Co-culture Day	0,07056	n/a[a]

[a] Not applicable.

probably tetrahydroauroglucin a benzyl derivative which λ_{max} is of 396 nm [10].

The major difference in respect to *Fusarium oxysporum* was that, in this case, a complex media composed of barley flour did not give a high pigment content (Fig. 2); this can also be supported by both p-values which are lower than <0,05 (Table 3). Behavior regarding high C:N ratio remains in defined media with the best results presented in ratios of 10, 20 and 30 respectively. The highest AU/Bgr value was achieved with G20Y1 media; nevertheless, biomass concentration was not above 2,58 g/L; this media was selected for further assays.

3.2. Wavelength effect

Taking into consideration that there was no proportional relation between biomass concentration and pigment production in culture media assay, wavelength assay was executed as another approach not only to stimulate pigment biosynthesis and accumulation but also biomass production.

Through our experimentation, we found that pigment biosynthesis was improved in *F. oxysporum* when using blue and green light in respect to the AU/Bgr obtained in the media M45Y5 with standard light irradiance (Fig. 3). In our case, production was improved in a 191,2%, along with a significant statistical difference (Table 3). Biomass concentration in blue and green light was also higher (8,8 and 9,72 g/L respectively).

For *A. chevalieri,* a positive effect of light was encountered; in this case, resulting in the production of more than one pigment. This inference was established after performing the spectrophotometric scan of the dichloromethane mycelial extract as well as the changes in the hue obtained in this crude extract (Supplementary File, Fig. A and B), which allowed finding a wide peak different from the initial one obtained in the culture media assay and in the case of blue light the presence of two peaks.

Red and UV light managed to improve the results obtained in terms of AU/Bgr value (Table 4), where the value increased more than two times with red light and more than three times with UV light. Biomass content increased in blue, green and white light respectively. The fact that there is no significant increase of AU/Bgr

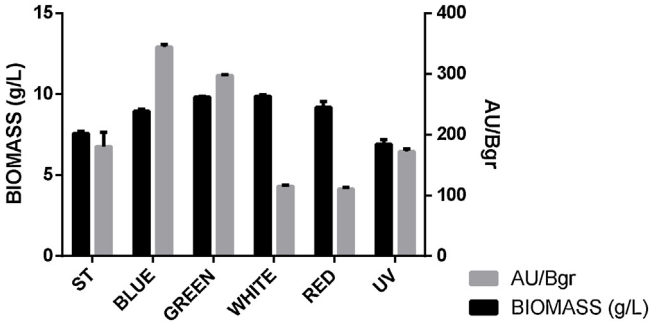

Fig. 3. Biomass and Pigment production in different LED light for *F. oxysporum.*

Table 4
Biomass and Pigment production in different LED light for *A. chevalieri.*

λ_{max}	LED	BIOMASS	AU/Bgr
360 nm	Blue	9,95 + 0,92	2750,68 + 451,30
455 nm	Blue	9,95 + 0,92	2343,57 + 113,05
435 nm	Green	7,77 + 0,96	2526,71 + 341,63
	White	9,69 + 0,94	1746,83 + 108,03
430 nm	Red	2,85 + 0,15	7306,59 + 80,46
	UV	3,02 + 0,03	7976,46 + 438,23

value in blue light might be related with a higher molecule diversity since the spectra for this condition gave the widest peaks (Supplementary File, Fig. A)

3.3. Co-culture effect

Co-culture with *P.fluorescens* had no effect on pigment production by *F. oxysporum*, on the contrary, it considerably diminished production when comparing AU/Bgr value with the one obtained in the culture media with standard light conditions. Regarding the assay with yeast, it was noticed that pigment production did not decrease drastically (Fig. 4), AU/Bgr for day 8 was almost the same as the one obtained in media with standard light conditions. This demonstrates that the type of microorganism used in co-culture has a negative statistical effect (Table 3).

For *A. chevalieri* presence of a pigment with λ_{max} of 365 nm was observed, there was also an increment of a pigment with λ_{max} of 435 nm in comparison to green light where it was initially found. Elicitation occurred when co-cultured with *P.fluorescens* at day 5 and for *K. marxianus* at day 4 and 5 (Fig. 5); this demonstrates that for this fungi microorganism type as well as starting time of co-incubation influence concentration and differential production of pigments.

There were higher AU/Bgr values for experiments performed with *K. marxianus*; it is possible then that *P. fluorescens* can produce

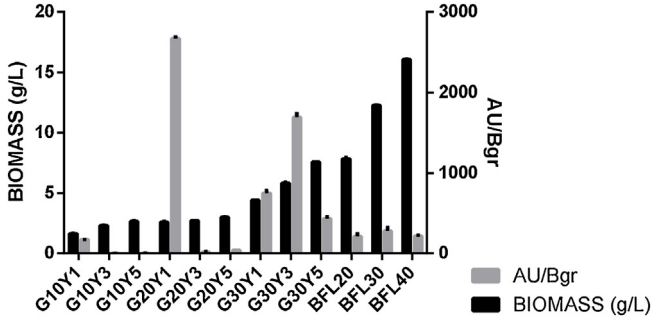

Fig. 2. Biomass and Pigment production in GY and BFL media for *A. chevalieri.*

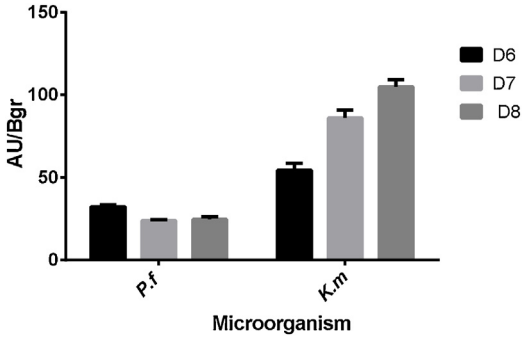

Fig. 4. Pigment production in co-culture for *F. oxysporum* with bacteria or yeast.

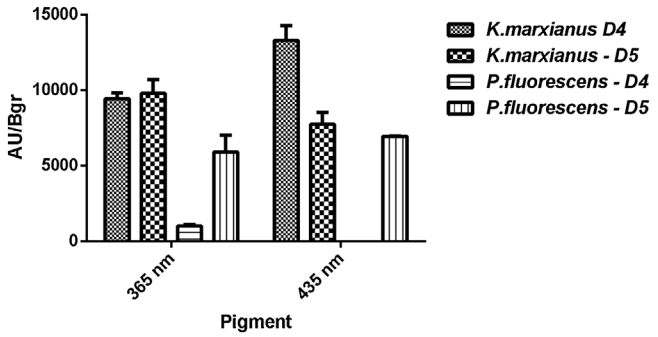

Fig. 5. Pigment production in co-culture for *A. chevalieri* with bacteria or yeast.

compounds with a slight activity against *A. chevalieri* as well. In the case of co-culture with yeast, the competition mechanism performed by *A. chevalieri* might be directed preferably to avoid yeast from consuming carbon source and other nutrients. This implies that metabolism in *A. chevalieri* has a turnover with preference for metabolite production intended to stop yeast growth; with biomass reduced to less than 2,60 g/L (data not shown), which can be supported by the fact that in the experiment with green light, a lower AU/Bgr was obtained but with higher biomass content.

4. Discussion

4.1. Media composition and C:N ratio

In general, for all fungi, it was observed that a C:N ratio equal or above 7 induced pigment production; however, this occurred only with defined simple media for *A. chevalieri* while in *F. oxysporum* this took place for both defined and complex media. Regarding the effect of the nitrogen source in pigment production, some research shows the preference for organic nitrogen sources in biosynthesis (except for glutamine) over nitrates and ammonium salts [48]; especially if these are as complex as yeast extract; in our case the presence of sodium nitrate did not have a negative effect given its low concentration.

Other authors have performed as well assays evaluating culture media composition for extracellular red pigment production in other species of *Fusarium* sp. as *F. fujikuroi*, in which it has been found that high proportions of carbon to nitrogen and that organic nitrogen sources are beneficial for pigment accumulation (Measurement at 500 nm) [49]; another example is the decrease in bikaverin biosynthesis for an over-producing mutant of *F.fujikuroi* with rising concentration of soy bean meal [50], showing as well that biomass and pigment production are not directly correlated. Also, other works with *F. moniliforme* show that the highest production takes place in media with high carbon and low nitrogen concentration such as potato dextrose and malt extract broth. (Measurement at 500 nm) with an AU of 1,5/ml [51,52].

For many secondary metabolites of the genera *Aspergillus* sp. down-regulation of some pathways for secondary metabolism occurs when glucose is used [53]; but not in the case of this research. In the future it would be important to determine why in this specific case production took place with the use of glucose instead of using a complex media with diverse polysaccharides; however, results concerning C:N in other species of *Aspergillus* sp. are coherent with those obtained in the present research; Casas-López and collaborators found the highest production of lovastatin from *A. terreus* in a medium with a C:N ratio of 41,3; nevertheless, this was achieved with a disaccharide [54].

4.2. Wavelength effect

Regarding light effect, increase in production was observed in *F. oxysporum* mainly with blue light; whereas for *A. chevalieri* increase was noted with red and UV light, showing that there are several photoreceptors and specific transcription factors involved and that these vary with fungal species and metabolic route. In general, this outcome is concordant with the work of other authors in which for five filamentous fungi tested, satisfactory yields were obtained with red and blue light; also with data of *Monascus* sp. that presents the highest absorbance units for the intracellular extract when exposed to red light [36]

When comparing the results obtained by the latter author, specifically for *Fusarium verticilliodes* with the ones obtained in this work for *F. oxysporum* there is difference since it can be seen that the highest intracellular yield is achieved with red light instead of blue light [55]. It is feasible to suggest that in the cited research work other red pigments different from bikaverin were taken into consideration. (Since the specific wavelength at which the extract was read is not specified), or that response to light can significantly vary among species of the same genera.

The results seen in *F. oxysporum* with different wavelengths support the idea that bikaverin biosynthesis is light-regulated. It is important to outline that various research have found that production of this pigment in related species, such as *F. fujikuroi,* was improved in mutants of the DASH-cryphtochrome, white collar complex as well as velvet complex when compared to the wild type under low nitrogen conditions [56–58]. Such findings can indicate, that, blue light induction can be regulated by the photoreceptor VIVID [59]. For the case of green light, opsin-related proteins *OpsA* and *CarO* might be related in stimulation. Regarding white, red and UV light some hypothesis can arise; one, that the photoreceptor for the red wavelength is not involved in bikaverin biosynthesis; concerning white light, since it is the mixture of all wavelengths, possibly does not activate a specific photoreceptor, and secondly UV light might have had an effect in metabolism which can be reflected in lower biomass and pigment production.

In the case of *Aspergillus chevalieri* UV and red light yielded the highest AU/Bgr numbers; a scan of the extracts showed that a molecule absorbing at a λ_{max} of 430 nm (in $CHCl_3$) can correspond to parietin which λ_{max} reported in methanol is 433 nm [60,61] and 431 nm in ethanol [62]. The enhancement of pigmentation with red and UV wavelength can shed light on the involvement of photoreceptors in the biosynthesis of this compound; phyto-chrome (red light); and photolyase *CryA* (UVA light). In this context, it is necessary to highlight that UV light might have induced some stress since it has already been reported that rise in parietin production has occurred in *Xanthoria parietina* with increasing exposure to UV light. The main pigment could have served in a feasible manner as a photo-protector or as an antioxidant in *A. chevalieri*. In *Blakeslea trispora* oxidative stress caused an increase in carotenoid content, therefore it is reasonable to propose that other fungal pigments serve this function [63–66].

Nonetheless, it is important to mention that blue light, in addition, has two absorption maxima with λ_{max} of 360 nm and 455 nm respectively, this second one shows a wider peak with a noticeable decrease in absorption mostly evident above 530 nm. This suggests the presence of several orange-red pigments within the extract that have already been reported for the teleomorph *Eurotium chevalieri* as well as some close species as *E. repens*, *E. rubrum, E. cristatum,* and the anamorph *A. glaucus;* some of these are erythroglaucin with λ_{max} of 465 nm, 493 nm, 510 nm, 523 nm in ethanol [67,68], catenarin with a λ_{max} of 463 nm in ethanol [27]. There are not enough reports that expose pigments with λ_{max} near to 360 nm, there is only research that presents coloured compounds as Neoechinulin B or TMC-120 derivative present in

Eurotium sp. [69]. Overall, the results obtained are in line with others exposed for other fungal isolates such as the case of some marine fungi, in which production of secondary metabolites is given in a wavelength specific manner with satisfactory results obtained using red and blue light [70].

4.3. Co-culture effect

Through co-culture only pigment production was improved in *A. chevalieri* employing bacteria and yeast as well, nevertheless displaying differences in AU/Bgr. For *F. oxysporum*, although there were differences between bacteria and yeast, no enhancement in AU/Bgr was observed regarding production in standard media; this proves that this approach at an interaction level for *F. oxysporum* is species specific. Possible explanations are: The increase in pH caused by the addition of bacteria culture to the flasks caused a down-regulation of the bikaverin pathway, since production is favoured under acidic conditions [47]; alternatively, that the C:N ratio could have been altered as a result of a faster consumption rate of the carbon source characteristic of bacterial growth kinetics compared to the fungi. Diminished production can also be a consequence of the antifungal molecules synthesized by *P. fluorescens*; with activity against this fungi [71].

The above does not imply that this fungus cannot present a response to other microorganisms since it has been reported in another species of the genera, as *F. tricinctum,* a different profile of metabolites when co-cultured with *B. subtilis* [72].

Molecules observed in *A. chevalieri* have λ_{max} of 365 nm and 435 nm; identity for the first compound (365 nm) cannot be secured; nevertheless, it is feasible that neoechinulin B or TMC 120 derivative 2 [69] are present in the extract. The second λ_{max} might correspond to emodin, also found when stimulated with green light, this last compound is an anthraquinone with well-known activity against some gram-positive bacteria [27,73–75] and has shown antifungal effects to yeast like *C. albicans*, and *C. neoformans* [76]. Reports of negative effects are limited in gram negative bacteria, and until now, only described in *Haemophilus parasuis* [77]. These findings are reasonable considering that antimicrobial activity has been reported for some metabolites of *Eurotium* sp. [17,26] and its anamorphs (*Aspergillus* sp.); therefore, accumulation of compounds in this research might be destined to later secretion in order to inhibit competing microorganisms; however, this must be confirmed with further experimentation evaluating pure emodin from wasted media of *A. chevalieri*. Other experiments in progress not included in this work with biomass extracts of this fungi have shown an IC50 (Inhibitory concentration) of 17,5 ppm against *Staphylococcus aureus* (Unpublished data).

In the present work, yeast stimulates higher accumulation of pigment in *A. chevalieri*; other research in which pigment of a strain of *Penicillium* sp. could only be yielded through co-cultivation with *Candida tropicalis* exemplifies another positive outcome [78].

In other species such as *A. fumigatus* meroterpenoid production was induced when co-cultured with *S. rapamycinicus,* showing slight activity against this bacteria [79]; in contrast accumulation of metabolites for *A. terreus* did not occur when co-cultured with two *Streptomyces* sp. [80]. This confirms that stimulation can be species-specific. Equally, increment in synthetized desipeptides has been found in teleomorphs such as *Emericella* sp. [81] which displayed moderate activity against *S. aureus.*

5. Conclusions

C:N ratios equal to or higher than 7 stimulate high pigment production in *F. oxysporum*, while for *A. chevalieri* ideal C:N ratios are among 10–20.

Blue and green light favour increment in bikaverin production in *F. oxysporum* while several pigments can be produced in *A. chevaileri* when stimulated with different light wavelengths.

Induction of pigment production by means of co-culture with bacteria and yeast is species-specific, it was not observed for *F. oxysporum*, but it could be found in *A. chevalieri*.

This work allows considering other approaches for the biotechnological production of pigments besides the study of culture media, further work can be performed to optimize the value of light irradiance or photoperiod instead of continuous exposure. Also, for *F. oxysporum* and other fungi, if required, evaluation of several strains of bacteria or yeast in a culture collection (preferably biosafety I) can be performed in search of the specific interaction that enhances pigment biosynthesis.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2019.e00308.

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