Bioresources and Bioprocessing

Modeling bikaverin production by *Fusarium oxysporum* CCT7620 in shake flask cultures

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Abstract

Bikaverin is a fungal red pigment that presents antimicrobial and antitumor activities. Therefore, this substance could be used as an alternative additive in the food and pharmaceutical industries. The aim of this work was to use response surface methodology to optimize the fermentation conditions and maximize the production of bikaverin in shake flasks. The variables investigated were agitation speed (71–289 rpm), temperature (21–35 °C), and substrate (rice) concentration in the culture medium (16.4–83.6 g/L). The agitation speed had a positive effect on red pigment production, while substrate concentration and temperature had the opposite effect. Maximum bikaverin production was predicted to occur using 289 rpm, 24.3 °C, and 16.4 g/L rice concentration. Experimental validation using 289 rpm, 28 °C, and 20 g/L rice concentration was 6.2% higher than predicted by the model. The present investigation was important for defining the best conditions for the production of bikaverin.

Keywords: *Fusarium oxysporum*, Bikaverin, Fungal pigment, Submerged cultures, Red pigment

Introduction

Natural pigments have been increasingly used in the pharmaceutical, textile, and food industries, due to their environmental friendly nature and the fact that they are considered less toxic than their chemical counterparts, resulting in higher market appeal (Ogbonna 2016). Some synthetic dyes are possible carcinogens, highly toxic, and can induce allergic dermatitis, skin irritation, and mutation in humans (Srivastava et al. 2004; Sinha et al. 2012). They can contaminate the environment, leading to negative ecotoxicological effects and bioaccumulation in wildlife (Saha et al. 2010). Therefore, there is increasing preference for natural colorants, particularly in food products, where they can act as functional ingredients (Dufossé et al. 2005; Yolmeh et al. 2014).

Microorganisms are considered a promising source of natural pigments, mainly because they can potentially be produced uninterruptedly, under controlled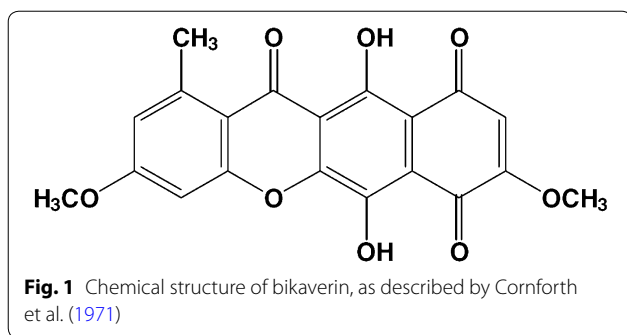 and optimizable conditions. Therefore, the biotechnological production of pigments has attracted the interest of industry. Some examples are already commercially available, such as β-carotene from microalgae, phycocyanins from *Spirulina*, and *Monascus* pigments (Dufossé et al. 2005; Kongruang 2011).

Polyketides are a family of chemicals that are formed by condensation of successive acetate units, as a result of the action of multifunctional polyketide synthase (PKS I) enzymes (Smith and Tsai 2007). The biological activities of these compounds mean that they have significant pharmaceutical importance (Hertweck 2009; Lale and Gadre 2016). Bikaverin is a red polyketide produced by fungi of the *Fusarium* genus, such as *F. verticillioides, F. lycopersici*, and *F. oxysporum* (Balan et al. 1970; Linnemannstöns et al. 2002). The structure of bikaverin (6,11-dihydroxy-3,8-dimethoxy-1-methyl-benzo-xanthein-7,10,12-trion, $C_{20}H_{14}O_8$, molecular mass of 382.068 Da) (Fig. 1) was determined by its chemical synthesis followed by X-ray crystallography (Cornforth et al. 1971).

Feed experiments with acetate isotopes [1,2-13C] demonstrated that bikaverin could be completely biosynthesized by the condensation of nine acetate units in a single polyketide chain, which was then folded and

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Santos *et al. Bioresour. Bioprocess.*   (2020) 7:13

Page 2 of 8

Fig. 1 Chemical structure of bikaverin, as described by Cornforth et al. (1971)

cross-linked to form the backbone of the molecule (McInnes et al. 1976). Further studies revealed that the *bik1* gene, responsible for the synthesis of bikaverin, has five adjacent genes that participate in this process: *bik2*, *bik3*, *bik4*, *bik5*, and *bik6* (Wiemann et al. 2009). The transcription of these genes is affected by pH, nitrogen concentration (Wiemann et al. 2009), and carbon source. Studies found that the highest production of bikaverin was achieved in acid medium, under nitrogen limitation, with sucrose being a better carbon source than glucose (Brewer and Arsenault 1973; Limón et al. 2010).

Due to internal accumulation and excretion of this polyketide, the culture of bikaverin-producing *Fusarium*, such as *F. fujikuroi*, presents a reddish color (Lale and Gadre 2016), which may vary depending on the fungal growth conditions (Linnemannstöns et al. 2002). In addition to its colorant properties, bikaverin has shown antibiotic properties (Deshmukh et al. 2014) and biological activity against the protozoan *Leishmania brasiliensis* (Balan et al. 1970), the oomycete *Phytophtora infestans* (Son et al. 2008), and the nematode *Bursaphelenchus xylophilus* (Kwon et al. 2007). Bikaverin has also been reported to have inhibitory effects against tumor cells, such as MIA Pa Ca-2 (pancreatic carcinoma) (Zhan et al. 2007) and Ehrlich ascites carcinoma (EAC), for which it exhibited ED_{50} of 0.5 µg/mL (Fuska et al. 1975). Recently, bikaverin was tested as an inhibitor of protein kinase CK2. This enzyme is highly expressed and active in several tumor cells, being responsible for suppressing apoptosis and stimulating cell growth. Both cell viability and cell proliferation were drastically reduced after treatment with 10 µM bikaverin for 24 h in MCF7 cells (human breast adenocarcinoma cell line), A427 (human lung carcinoma cell line) and A431 (human epidermoid carcinoma cell line) (Haidar et al. 2019).

Although the bikaverin-producing *Fusarium* species are widespread agricultural phytopathogens, there are no reports of negative effects on human health caused specifically by bikaverin (Limón et al. 2010). In rats, bikaverin at concentrations up to 100 µM showed no genotoxic effect on hepatocyte DNA (Norred et al. 1992). The lack of toxicological data and information about other biological activities of bikaverin motivated our research group to produce this compound for further studies. Considering that bikaverin biosynthesis is affected by different environmental factors such as agitation, aeration, temperature, pH (Giordano and Domenech 1999; Bell et al. 2003; Srivastava et al. 2011), and medium composition, including salt starvation and type of carbon source (Rodríguez-Ortiz et al. 2010), and that the optimal conditions may vary for each microorganism, the aim of the present study was to model the effects of the main parameters on bikaverin production by *F. oxysporum* CCT7620.

Materials and methods

The general experimental steps of this study followed the sequence shown in Fig. 2. In the following sections, a detailed explanation of each procedure may be found.
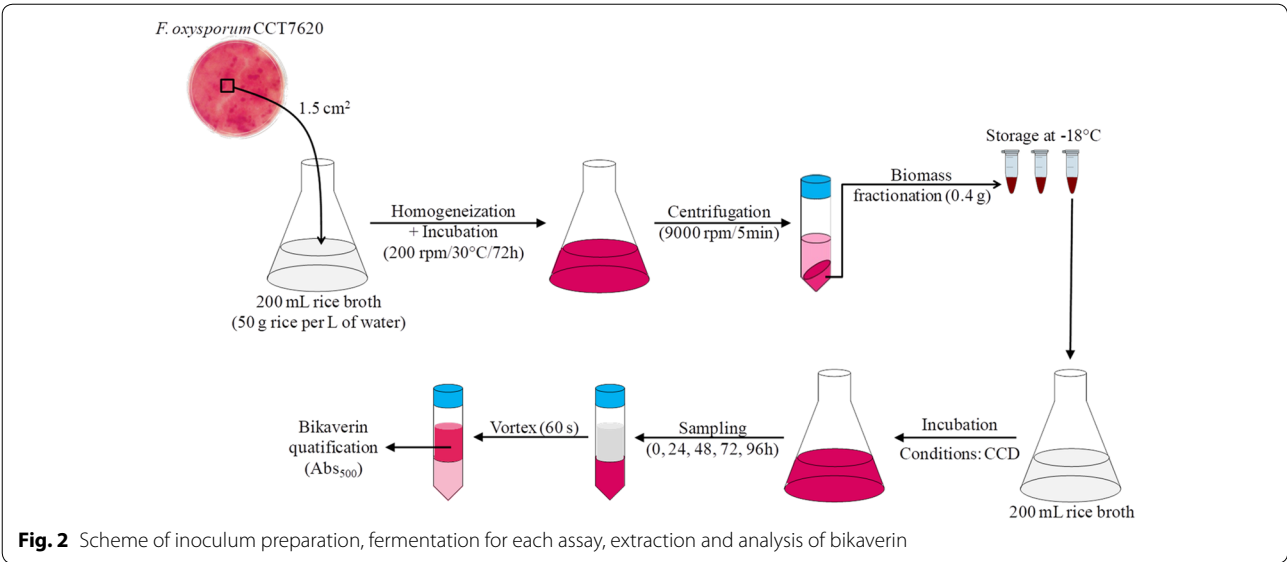
Microorganism and biomass (inoculum) production

Fusarium oxysporum CCT7620, previously isolated from soil, was deposited in the Tropical Cultures Collection of the André Tozello Institute, in Campinas, Brazil (Silva 2013). The microorganism was maintained on potato dextrose agar (PDA) slants at 4 °C, with periodical replication in PDA agar plates.

A piece of agar (1.5 cm^2) containing *F. oxysporum* CCT7620 grown for 72 h in PDA medium, at 30 °C, was transferred to a conical flask containing 200 mL of rice medium, followed by homogenization using an Ultra-Turrax (16,000 rpm). The rice medium consisted of 50 g of rice flour per liter of water, and the rice flour was obtained by milling commercial white rice (polished) in a knife mill. The flasks were incubated for 72 h, at 30 °C and 200 rpm, after which the biomass (inoculum) was recovered by centrifugation at 9000 rpm for 5 min. The supernatant was discarded, the biomass was fractionated (0.4 g wet weight portions), and the material was stored in a freezer (at − 18 °C) for subsequent use as inoculum.

Effect of pH on bikaverin production

Various pH conditions were tested to determine whether pH control would be necessary, considering the effect of this parameter on bikaverin production. Given that an acidic medium is known to induce bikaverin production (Wiemann et al. 2009), the pH of the rice medium (50 g of rice flour per liter of water) was adjusted to different values (2.5, 3.0, 3.5, 4.0, and 4.5), using 0.05 mol L^{-1} citrate buffer, before being inoculated with the biomass produced as described above ("Microorganism and biomass (inoculum) production" section). A buffer-free medium (initial pH of 6.7) was used as the control. The cultures were incubated at 28 °C and 289 rpm (the supposed

Santos *et al. Bioresour. Bioprocess.* (2020) 7:13

Page 3 of 8

Fig. 2 Scheme of inoculum preparation, fermentation for each assay, extraction and analysis of bikaverin

optimal condition), using a rotary shaker. Aliquots of 3 mL were collected at different times (0, 24, 48, 72, and 96 h), to monitor the pigment production kinetics. The samples were extracted and analyzed as described in "Extraction and analysis of the pigment produced" section. The importance of pH for bikaverin biosynthesis was evaluated statistically using one-way ANOVA followed by the Tukey HSD test ($\alpha = 0.05$).

Kinetics of pigment production under different conditions

The experimental design for bikaverin pigment production in liquid culture considered three independent variables (Table 1), employing a 2^3 central composite design (CCD) with 6 axial points and 3 replicates at the center point, resulting in 17 different assays (Table 2). The time course of pigment production (measurements at 0, 24, 48, 72, and 96 h) was determined for each of these different culture conditions, using the extraction and analytical procedure described in "Extraction and analysis of the pigment produced" section.

Extraction and analysis of the pigment produced

Samples (whole fermented broth) were transferred to Falcon tubes and extracted with ethyl acetate (3 mL of solvent for each 3 mL of sample), using a vortex for 60 s, followed by centrifugation at 3600 rpm for 5 min. The recovered organic layer was analyzed using a Beckman Du 640 spectrophotometer, recording the absorbance values at 500 nm. Bikaverin was quantified using a calibration curve ($Abs_{500} = 0.003553$ [bikaverin, mg/L] $-$ 0.029447, $R^2 = 0.9946$), as described in Additional file 1: Fig. S1.

Statistical analysis

Response surface methodology (RSM) using the CCD was applied to model the production of red pigment by *F. oxysporum* CCT7620 in liquid culture medium, according to Eq. 1:

$$Y = \beta_0 + \beta_1 T_1 + \beta_2 A_2 + \beta_3 [S]_3 + \beta_{11} T_1^2 + \beta_{22} A_2^2 + \beta_{33} [S]_3^2 + \beta_{12} T_1 A_2 + \beta_{13} T_1 [S]_3 + \beta_{23} A_2 [S]_3$$

(1)

where Y is the bikaverin concentration (mg/L), T, A, and $[S]$ are the coded values of temperature, agitation speed, and rice concentration, respectively, and β_n are the estimated regression coefficients associated with

Table 1 Parameters and levels used in the central composite design (CCD)

Independent variables	Symbol	Coded levels				
		− 1.68	− 1	0	+ 1	+ 1.68
Temperature (°C)	T	21	24	28	32	35
Agitation speed (rpm)	A	71	115	180	245	289
Rice concentration (g/L)	[S]	16.4	30	50	70	83.6

Santos *et al. Bioresour. Bioprocess.* (2020) 7:13

Page 4 of 8

Table 2 Bikaverin production (mg/L) by *F. oxyporum* CCT7620 for each experimental condition defined by the central composite rotational design

Run	Temperature (°C)		Agitation (rpm)		Rice flour (g/L)		Bikaverin production (mg/L)				
	Coded	Real	Coded	Real	Coded	Real	0 h	24 h	48 h	72 h	96 h
1	−1	24	−1	115	−1	30	0	0	0	44.53	75.45
2	+1	32	−1	115	−1	30	0	0	0	100.34	132.74
3	−1	24	+1	245	−1	30	0	0	0	127.90	260.15
4	+1	32	+1	245	−1	30	0	0	0	0	140.43
5	−1	21	−1	115	+1	70	0	0	0	37.86	0
6	+1	32	−1	115	+1	70	0	0	0	38.11	0
7	−1	24	+1	245	+1	70	0	0	0	53.24	111.02
8	+1	32	+1	245	+1	70	0	0	0	0	103.14
9	−1.68	21	0	180	0	50	0	0	0	42.33	138.36
10	+1.68	35	0	180	0	50	0	0	0	32.77	0
11	0	28	−1.68	71	0	50	0	0	0	0	0
12	0	28	+1.68	289	0	50	0	0	0	269.89	293.36
13	0	28	0	180	−1.68	16.3	0	0	94.53	247.20	269.90
14	0	28	0	180	+1.68	83.6	0	0	0	0	46.32
15	0	28	0	180	0	50	0	0	32.29	103.67	139.12
16	0	28	0	180	0	50	0	0	61.61	133.03	158.13
17	0	28	0	180	0	50	0	0	56.92	121.98	171.23

temperature (1), agitation speed (2), or rice (substrate) concentration (3), and their combinations.

The data were treated using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, USA). Only the statistically significant parameters ($\alpha < 0.05$) were used to construct the model (Eq. 1). After analysis of variance (ANOVA), response surfaces were constructed from the fitted models.

Results and discussion
Effect of pH control on bikaverin production
It is known that the metabolic pathway responsible for bikaverin production is affected by pH (Bell et al. 2003; Li and Mira De Orduña 2010), and that acidic pH values tend to increase its production. The *bik* genes responsible for bikaverin biosynthesis are only expressed in acidic medium (Wiemann et al. 2009), suggesting that low pH values are necessary for its synthesis (Medentsev and Akimenko 1998; Wiemann et al. 2009). However, it was unclear if pH control would be beneficial for the present process.

Therefore, instead of including pH as an independent variable in the CCD, an experiment was performed comparing cultures with pH control (with different acidic pH values obtained using citrate buffer) and without pH control. As shown in Table 3, there were no significant differences in bikaverin production (at 72 and 96 h) when the pH was controlled in the range

Table 3 Influence of culture pH on bikaverin production

pH	Mean concentration (mg/L)	
	72 h	96 h
2.5	185.1**±30.5	234.5*±26.8
3.0	177.6**±29.8	241.4*±52.86
3.5	192.3**±26.5	229.5*±21.5
4.0	196.5**±13.5	237.7*±10.3
4.5	27.0***±21.9	24.5**±18.0
Control	352.6*±30.4	302.5*±38.6

* Means followed by different asterisks in the same column differ from each other, according to the Tukey test at 5% significance ($p < 0.05$)

2.5–4.0. On the other hand, growth without pH control resulted in significantly higher concentrations of bikaverin after 72 h of fermentation, while control of the pH at 4.5 resulted in significantly lower bikaverin production, compared to the other conditions tested.

Previous studies by our group, using *F. oxysporum* CCT7620 cultivated in an airlift bioreactor operating with no pH control, indicated that the pH decreased continuously from the initial value (~ 6.0), reaching a value close to 3.5 after 48 h, which then remained stable during the following 48 h. In this case, maximal pigment production occurred between 48 and 72 h (Silva 2013). Similar results were reported for cultivation of *F. decemcellulare* to produce naphthoquinone (Medentsev

et al. 2005). In another study, the maximum production of bikaverin by *F. fujikuroi* occurred when the initial pH of 4.5 decreased to 3.7 at the end of the fermentation, suggesting that the pH decrease during growth favored the metabolic pathway for formation of this pigment (Giordano et al. 1999). These results suggested that the natural acidification of the medium was sufficient to induce bikaverin production. Therefore, this experiment was important, since it could be concluded that the CCD should not include pH as an independent variable and that pH control would not be indicated to maximize bikaverin production.

Modeling of bikaverin pigment production

Based on the experimental design, 17 assays were carried out under different conditions (Table 2). Regardless of the condition tested, maximal red pigment production occurred at 96 h of cultivation. Therefore, this fermentation time was selected for construction of the model, which only considered reasonably significant parameters (p values close to 0.1 or lower). Consequently, the model included the linear coefficients corresponding to temperature, agitation speed, and rice concentration, the quadratic coefficient for temperature, and the interaction of temperature and agitation speed (Additional file 1: Table S1), resulting in the model shown in Eq. 2:

$$[\text{Bikaverin, mg/L}] = 146.11 - 22.19\,T - 32.55\,T^2 + 65.90\,A - 56.43[S] - 23.11\,T.A \quad (2)$$

Table 4 presents the ANOVA results for this model (Eq. 2). The ratio between the mean square of regression and the mean square of the residuals (MS_R/MS_r), or F_{cal}, was 18.3, which was close to six times higher than the critical value at 95% significance ($F_{tab} = 3.20$). The ratio between the mean square of the lack of fit and the mean square of the pure error (MS_{LF}/MS_{PE}) was 6.3, which was three times lower than the critical value at 95% significance ($F_{tab} = 19.38$). The coefficient of determination (R^2) was satisfactory (0.893) and the p value for the regression

was < 0.0001. Therefore, this model was considered suitable for the prediction of bikaverin pigment production after 96 h of cultivation of *F. oxysporum* CCT7620 in rice-based medium. The graphical representation of this model (Eq. 2) could then be constructed (Fig. 3).

The analysis of Fig. 3a–c indicated that the agitation speed had a positive effect on bikaverin production. This
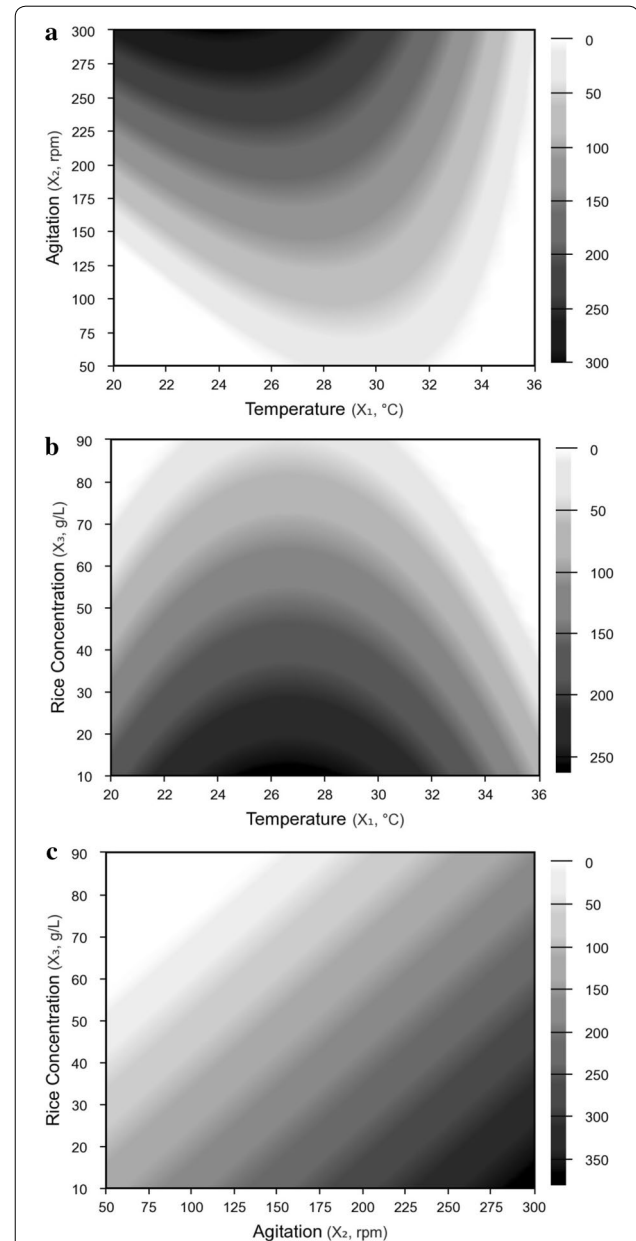

Fig. 3 Interactive effect of **a** temperature and agitation speed; **b** temperature and rice concentration; and **c** agitation speed and rice concentration on bikaverin pigment production (mg/L). Rice concentration, agitation speed, and temperature were kept at 50 g/L, 180 rpm, and 28 °C in Fig. 3a–c, respectively

Table 4 Analysis of variance (ANOVA) for the experimental results of the central composite design used for bikaverin production by *F. oxysporum* CCT7620

	SS (x 10³)	df	MS (x 10³)	*F* value	*p* value
Regression	127.581	5	25.516	18.3	< 0.0001
Residual	15.323	11	1.393		
Pure error	0.521	2	0.260		
Total SS	142.904	16			
$R^2 = 0.893$				$F_{0.95\,(5,11)} = 3.20$	

SS Sum of squares, *df* degrees of freedom, *MS* mean square

Santos *et al.* Bioresour. Bioprocess.   (2020) 7:13

Page 6 of 8

was expected, since agitation increases mass transfer and gas exchange, resulting in increased growth and metabolite production (Ahmad et al. 1994). It is known that some enzymatic reactions depend on the concentration of oxygen, such as pre-bikaverin C6 and C7 hydroxylation by monoxygenase (Wiemann et al. 2009). Elsewhere, decreased biomass and bikaverin production were observed when a low air/medium ratio was employed for the cultivation of *F. fujikuroi* (Giordano and Domenech 1999).

In contrast, the substrate (rice) concentration presented the opposite effect (Fig. 3b, c). This was also expected, since nutrient limitation is known to induce pigment production in several microorganisms (Stahmann et al. 2001; Lamers et al. 2008; Akilandeswari and Pradeep 2016). In the case of bikaverin, its synthesis is repressed by high nitrogen concentrations (Linnemannstöns et al. 2002). After depletion of the assimilable nitrogen, the pigment is excreted into the culture medium during the stationary phase (Avalos et al. 1999). Similarly, other researchers found that bikaverin production started when nitrogen was depleted and continued until sufficient carbon substrate was available (Giordano et al. 1999). It has reported that bikaverin production by *F. fujikuroi* continued even under carbon source limitation (Chávez-Parga et al. 2005). Therefore, this might explain why rice medium poor in nitrogen (approximately 1.0%, Additional file 1: Table S2), especially at lower rice concentrations, resulted in increased bikaverin production.

The effect of temperature was nonlinear, with maximum bikaverin production close to 25 °C (Fig. 3a, b). The temperature is an important parameter in fermentations, since it influences the metabolic activity of fungi, consequently affecting their growth and pigment production. Temperatures between 27 and 29 °C were reported as optimal for the biosynthesis of fungal pigments in liquid culture media (Dufossé et al. 2005). For *Fusarium oxysporum*, the optimal growth temperature was 28 °C (Gupta et al. 2010). The present results showed that at 250 rpm and substrate concentration of 20 g/L, maximal bikaverin production (317.6 mg/L) occurred at 25.1 °C, although variation of temperature in the range 21.1–29.1 °C led to less than 10% variation of pigment production ($\geq$ 285 mg/L), indicating that the bioprocess was quite robust, in terms of temperature requirement. However, no pigment production was observed at 35 °C, since this temperature is not ideal for fungal growth and constitutes a limiting factor for the synthesis of naphthoquinones, such as bikaverin (Medentsev and Akimenko 1998).

Therefore, according to the proposed model (Eq. 2), the conditions for maximal bikaverin production (380.2 mg/L) would be cultivation for 96 h at 24.3 °C and 289 rpm, with 16.4 g/L of rice in the medium. A validation experiment was performed, in triplicate, under the conditions usually employed for other bioprocesses in our laboratory (28 °C, 250 rpm, and rice concentration of 20 g/L), to evaluate the reliability of the model. This resulted in a bikaverin concentration of 320.5 $\pm$ 30 mg/L after 96 h, which was 6.2% higher than the predicted value for these conditions (301.7 mg/L). This indicated that the model was able to satisfactorily predict bikaverin production. Furthermore, production equivalent to 84% of the predicted maximum was obtained under these conditions, which were therefore considered most suitable for bikaverin production. These parameter values were similar to the ideal conditions for *Fusarium* fermentation reported in previous studies. For example, the optimum production of *F. moniliforme* pigment was achieved using 2% potato dextrose broth, 2% glucose, 1% peptone, and 0.5% methionine, at pH 5.5, with 8 days of incubation at 28 °C (Pradeep and Pradeep 2013). The highest production of gibberellin (preceding bikaverin) by *F. fujikuroi* in a bioreactor was achieved using 30 °C, C:N ratio of 36.8, and pH of 5.0 (Escamilla-Silva et al. 2001).

Conclusion

Response surface methodology was useful for modeling the effects of temperature, agitation speed, and rice concentration on bikaverin production by *F. oxysporum*, enabling identification of the optimal conditions. The ideal conditions for this process consisted of growing *F. oxysporum* CCT7620 in a rice medium (20 g rice flour per liter of water), with incubation for 96 h at 28 °C and 250 rpm, without pH control. This resulted in bikaverin production of 320.5 mg/L. It should be noted that the experimental bikaverin concentration could be underestimated, because the pigment extraction process was not exhaustive and the calibration curve did not consider the matrix effect. The present study represents an important step toward further investigations of this molecule. Current work by our group aims to define the best conditions for extraction of this compound from the culture broth, as well as for purification of the extract.

Supplementary information

> **Additional file 1: Fig. S1.** Calibration curve for bikaverin. **Table S1.** Significant regression coefficients for bikaverin production after 96 h of fermentation. **Table S2.** Centesimal composition of the culture medium centrifuged at 9000 rpm for 5 min, before inoculation.

Santos *et al. Bioresour. Bioprocess.* (2020) 7:13

Page 7 of 8

References
Ahmad MN, Holland CR, Mckay G (1994) Mass transfer studies in batch fermentation: mixing characteristics. J Food Eng 23:145–158. https://doi.org/10.1016/0260-8774(94)90083-3
Akilandeswari P, Pradeep BV (2016) Exploration of industrially important pigments from soil fungi. Appl Microbiol Biotechnol 100:1631–1643. https://doi.org/10.1007/s00253-015-7231-8
Avalos J, Fernández-Martín R, Prado MM et al (1999) Biosíntesis de giberelinas, bikaverina y carotenoides en *Gibberella fujikuroi*. In: López C, Alonso JE (eds) Biotecnología y aplicaciones de microorganismos pigmentados. Universidade da Coruña, Coruña, pp 169–188
Balan J, Fuska J, Kuhr I, Kuhrová V (1970) Bikaverin, an antibiotic from *Gibberella fujikuroi*, effective against *Leishmania brasiliensis*. Folia Microbiol (Praha) 15:479–484. https://doi.org/10.1007/BF02880192
Bell AA, Wheeler MH, Liu J et al (2003) United States department of agriculture—agricultural research service studies on polyketide toxins of *Fusarium oxysporum* f sp *vasinfectum*: potential targets for disease control. Pest Manag Sci 59:736–747. https://doi.org/10.1002/ps.713
Brewer D, Arsenault GP (1973) Production of bikaverin by *Fusarium oxysporum* and its identity with lycopersin. J Antibiot Antibiot 26:778–781. https://doi.org/10.7164/antibiotics.26.778
Chávez-Parga MDC, González-Ortega O, Sánchez-Cornejo G et al (2005) Mathematical description of bikaverin production in a fluidized bed bioreactor. World J Microbiol Biotechnol 21:683–688. https://doi.org/10.1007/s11274-004-3854-0
Cornforth W, Robinson PM, Ireland N (1971) Isolation and characterization of a fungal vacuolation factor (bikaverin). J Chem Soc 16:2786–2788. https://doi.org/10.1039/J39710002786
Deshmukh R, Mathew A, Purohit HJ (2014) Characterization of antibacterial activity of bikaverin from *Fusarium* sp. HKF15. J Biosci Bioeng 117:443–448. https://doi.org/10.1016/j.jbiosc.2013.09.017
Dufossé L, Galaup P, Yaron A et al (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16:389–406. https://doi.org/10.1016/j.tifs.2005.02.006
Escamilla-Silva E, Poggi-Varaldo H, Mayra M et al (2001) Selective production of bikaverin in a fluidized bioreactor with immobilized *Gibberella fujikuroi*. World J Microbiol Biotechnol 17:469–474. https://doi.org/10.1023/A:1011913316988
Fuska J, Proksa B, Fuskova A (1975) New potential cytotoxic and antitumor substances. I. In vitro effect of bikaverin and its derivatives on cells of certain tumors. Neoplasma 22:335–338
Giordano W, Domenech CE (1999) Aeration affects acetate destination in *Gibberella fujikuroi*. FEMS Microbiol Lett 180:111–116. https://doi.org/10.1016/S0378-1097(99)00471-1
Giordano W, Avalos J, Cerdá-Olmedo E, Domenech CE (1999) Nitrogen availability and production of bikaverin and gibberellins in *Gibberella fujikuroi*. FEMS Microbiol Lett 173:389–393. https://doi.org/10.1016/S0378-1097(99)00106-8
Gupta VK, Misra AK, Gaur RK (2010) Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium guajava* L. in India. J Plant Prot Res 50:452–462. https://doi.org/10.2478/v10045-010-0076-3
Haidar S, Aichele D, Birus R et al (2019) In vitro and in silico evaluation of bikaverin as a potent inhibitor of human protein kinase CK2. Molecules 24:1–16
Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chemie Int Ed 48:4688–4716. https://doi.org/10.1002/anie.200806121
Kongruang S (2011) Growth kinetics of biopigment production by Thai isolated *Monascus purpureus* in a stirred tank bioreactor. J Ind Microbiol Biotechnol 38:93–99. https://doi.org/10.1007/s10295-010-0834-2
Kwon HR, Son SW, Han HR et al (2007) Nematicidal activity of bikaverin and fusaric acid isolated from *Fusarium oxysporum* against pine wood nematode, *Bursaphelenchus xylophilus*. Plant Pathol J 23:318–321. https://doi.org/10.5423/PPJ.2007.23.4.318
Lale GJ, Gadre RV (2016) Production of bikaverin by a *Fusarium fujikuroi* mutant in submerged cultures. AMB Express 6:34. https://doi.org/10.1186/s13568-016-0205-0
Lamers PP, Janssen M, De Vos RCH et al (2008) Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications. Trends Biotechnol 26:631–638. https://doi.org/10.1016/j.tibtech.2008.07.002
Li E, Mira De Orduña R (2010) A rapid method for the determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance. Lett Appl Microbiol 50:283–288. https://doi.org/10.1111/j.1472-765X.2009.02789.x
Limón MC, Rodríguez-Ortiz R, Avalos J (2010) Bikaverin production and applications. Appl Microbiol Biotechnol 87:21–29. https://doi.org/10.1007/s00253-010-2551-1
Linnemannstöns P, Schulte J, Del Mar Prado M et al (2002) The polyketide synthase gene pks4 from *Gibberella fujikuroi* encodes a key enzyme in the biosynthesis of the red pigment bikaverin. Fungal Genet Biol 37:134–148. https://doi.org/10.1016/S1087-1845(02)00501-7
McInnes AG, Walter JA, Smith DG (1976) Biosynthesis of bikaverin in *Fusarium oxysporum*. J Antibiot (Tokyo) 29:1050–1057. https://doi.org/10.7164/antibiotics.29.1050
Medentsev AG, Akimenko VK (1998) Naphthoquinone metabolites of the fungi. Phytochemistry 47:935–959. https://doi.org/10.1016/S0031-9422(98)80053-8
Medentsev AG, Arinbasarova AY, Akimenko VK (2005) Biosynthesis of naphthoquinone pigments by fungi of the genus *Fusarium*. Appl Biochem Microbiol 41:573–577. https://doi.org/10.1007/s10438-005-0091-8
Norred W, Plattner R, Vesonder RF et al (1992) Effects of selected secondary metabolites of *Fusarium moniliforme* on unscheduled synthesis of DNA by rat primary hepatocytes. Food Chem Toxicol 30:233–237. https://doi.org/10.1016/0278-6915(92)90038-M
Ogbonna CN (2016) Production of food colourants by filamentous fungi. African J Microbiol Res 10:960–971. https://doi.org/10.5897/AJMR2016.7904
Pradeep FS, Pradeep BV (2013) Optimization of pigment and biomass production from *Fusarium moniliforme* under submerged fermentation conditions. Int J Pharm Pharm Sci 5:526–535

Rodríguez-Ortiz R, Mehta BJ, Avalos J, Limón MC (2010) Stimulation of bikaverin production by sucrose and by salt starvation in *Fusarium fujikuroi*. Appl Microbiol Biotechnol 85:1991–2000. https://doi.org/10.1007/s00253-009-2282-3

Saha P, Chowdhury S, Gupta S, Kumar I (2010) Insight into adsorption equilibrium, kinetics and thermodynamics of Malachite Green onto clayey soil of Indian origin. Chem Eng J 165:874–882. https://doi.org/10.1016/j.cej.2010.10.048

Silva WS (2013) Produção de pigmentos fúngico e seu uso no tingimento de tecidos. Dissertation, Federal University of São João Del-Rei

Sinha K, Das Saha P, Datta S (2012) Response surface optimization and artificial neural network modeling of microwave assisted natural dye extraction from pomegranate rind. Ind Crops Prod 37:408–414. https://doi.org/10.1016/j.indcrop.2011.12.032

Smith S, Tsai S-C (2007) The type I fatty acid and polyketide synthases: a tale of two megasynthases. Nat Prod Rep 24:1041. https://doi.org/10.1039/b603600g

Son SW, Kim HY, Choi GJ et al (2008) Bikaverin and fusaric acid from *Fusarium oxysporum* show antioomycete activity against *Phytophthora infestans*. J Appl Microbiol 104:692–698. https://doi.org/10.1111/j.1365-2672.2007.03581.x

Srivastava S, Sinha R, Roy D (2004) Toxicological effects of malachite green. Aquat Toxicol 66:319–329. https://doi.org/10.1016/j.aquatox.2003.09.008

Srivastava P, Srivastava S, Pathak N (2011) Identification of limiting factors for the optimum growth of *Fusarium oxysporum* in liquid medium. Toxicol Int 18:111–116. https://doi.org/10.4103/0971-6580.84262

Stahmann KP, Arst HN, Althöfer H et al (2001) Riboflavin, overproduced during sporulation of *Ashbya gossypii*, protects its hyaline spores against ultraviolet light. Environ Microbiol 3:545–550. https://doi.org/10.1046/j.1462-2920.2001.00225.x

Wiemann P, Willmann A, Straeten M et al (2009) Biosynthesis of the red pigment bikaverin in *Fusarium fujikuroi*: genes, their function and regulation. Mol Microbiol 72:931–946. https://doi.org/10.1111/j.1365-2958.2009.06695.x

Yolmeh M, Habibi Najafi MB, Farhoosh R (2014) Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). Food Chem 155:319–324. https://doi.org/10.1016/j.foodchem.2014.01.059

Zhan J, Burns AM, Liu MX et al (2007) Search for cell motility and angiogenesis inhibitors with potential anticancer activity: beauvericin and other constituents of two endophytic strains of *Fusarium oxysporum*. J Nat Prod 70:227–232. https://doi.org/10.1021/np060394t

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