OPTIMIZATION OF CERULENIN PRODUCTION BY *Sarocladium oryzae* BRM 59907

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ABSTRACT

Cerulenin, a secondary metabolite produced by *Sarocladium oryzae*, draws attention due to its broad-spectrum antimicrobial activity. Its production commonly utilizes glycerol and glucose as carbon sources, presenting challenges in efficiency and extraction. This study aimed to optimize cerulenin production by *S. oryzae* BRM 59907 via submerged fermentation. Plackett-Burman design was used to identify key factors influencing production, leading to optimization by Doehlert design through response surface methodology. Temperature and concentration of Mg^{2+} have a negative impact on cerulenin production while iron supplementation increases the concentration of the biomolecule. After optimization a robust model was constructed, resulting in an over 50% increase in cerulenin production compared to the initial condition, achieved with reduced quantities of medium components.

Keywords: Experimental design. Cerulenin. Secondary metabolite. Doehlert. Antimicrobial agent.

1 INTRODUCTION

Cerulenin is a secondary metabolite produced by *Sarocladium oryzae*, known for its broad-spectrum antimicrobial activity. This natural product is capable of irreversibly inhibiting the β-ketoacyl-ACP synthase domain present in the fatty acid synthase (FAS) enzyme. Inactivation of this domain impairs the biosynthesis of fatty acids and the formation of the cell membrane in fungi and bacteria, although other pathways may also be affected, such as ergosterol, melanin and polyketide biosynthesis. It is a low-toxicity[1] and biodegradable compound whose biological action requires low concentrations (0.1-100 mg/L).

The most common production culture medium for obtaining this biomolecule typically employs glycerol and glucose as carbon sources. The presence of glycerol, besides reducing the medium cost, allows the utilization of a co-product from the biodiesel chain, contributing to a more sustainable and green bioprocess. However, one of the main challenges in this bioprocess lies in the low efficiency of its production and extraction from the culture medium. This study aimed to optimize production of cerulenin by *Sarocladium oryzae* BRM 59907 in submerged fermentation based on the standard medium reported in the literature[2].

2 MATERIAL & METHODS

In order to assist in the selection of parameters to be studied, *Sarocladium oryzae* BRM 59907 was cultivated in the standard medium supplemented with salts. The inoculum was propagated in a shaken flask at 28 ºC and 150 rpm for 72 h, in a medium containing 3% glycerol, 1% glucose, 0.5% peptone, 0.3% NaCl, and 1.75 ppm Mg^{2+}. Cerulenin production at 28 ºC and 150 rpm was monitored at 24, 48, 72, 96, and 120 h by aseptically transferring 1 mL of the inoculum to 500 mL flasks with 99 mL of medium containing 3% glycerol, 1% glucose, 0.5% peptone, 0.3% NaCl, 1.75 ppm Mg^{2+}, 9 ppm Ca^{2+}, and 1.3 ppm K^{+} at pH 7. Cerulenin concentration was estimated through a bioassay with *Saccharomyces cerevisiae*, and cellular biomass was determined using the dry mass method. Glycerol and glucose consumption throughout the cultivation were quantified by High-Performance Liquid Chromatography using an Agilent Infinity 1260 HPLC system (Agilent Corp., USA) equipped with an Aminex HPX-87H column (BioRad – 300 mm x 7.8 mm) and a refractive index detector (RID) at 45 ºC, with mobile phase 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min.

Subsequently the most relevant factors for cerulenin production were identified using a Plackett-Burman design at 72 and 96 h of cultivation. Based on previous experiments, ten independent variables were analyzed at two levels (glycerol, glucose, peptone, NaCl, $FeCl_3$, $CaCl_2$, $MgSO_4.7H_2O$ and KCl initial concentrations, temperature and initial pH), totaling 15 experiments with the triplicate of the central point. With the most important factors, optimization of bioprocess conditions using response surface methodology was performed with the Doehlert design at 72 and 96 h. The cerulenin production predicted by regression models was validated by a kinetic study under the best obtained condition. The model quality was evaluated by ANOVA with 95% confidence interval.

3 RESULTS & DISCUSSION

The supplementation of culture media with calcium, magnesium and potassium yields (0.648 ± 0.033) g of dry cell mass in 96 h, approximately three times higher than that reported in the previous work for this strain[2]. There was no diauxic growth of this strain in the proposed culture medium. The glucose concentration, which experienced its greatest reduction at 24 h, varied little throughout the remainder of the cultivation, reaching 0.6% by the end of the bioprocess. Glycerol was progressively metabolized starting from 24 h (2.75%) until reaching a final concentration of 0.5% at 120 h (fig. 1a). The concentration of cerulenin remained around 50 mg/L from 48 h onwards, a value considered lower than the production typically reported in the literature[3,4], with $Y_{P/X}$ of (9.64 ± 0.94) mg/g (fig. 1b). These results reinforce the need for an optimization of this bioprocess.

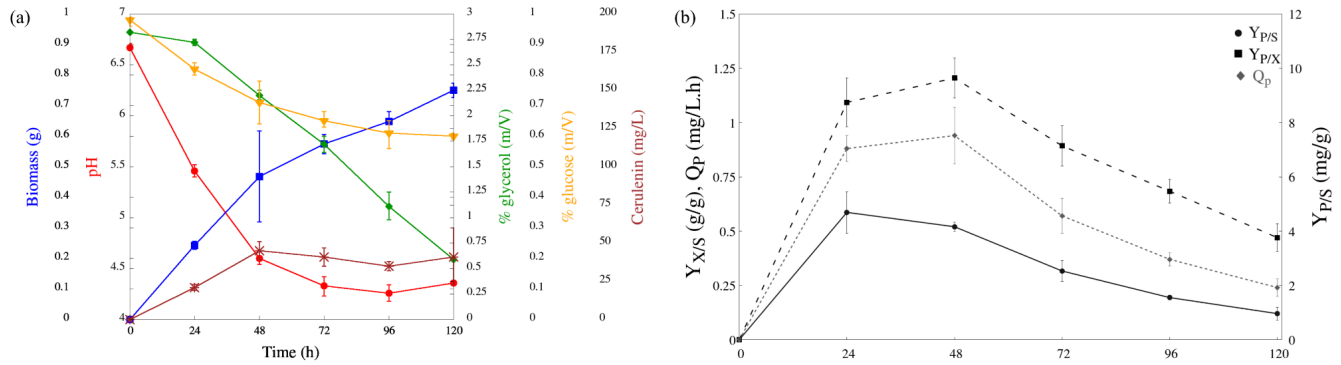

Figure 1 (a) Growth, carbon sources consumption, pH change and cerulenin production kinetics by *Sarocladium oryzae* BRM 59907 in the salt supplemented media. (b) Kinetic parameters of the bioprocess. Q_p – volumetric productivity; $Y_{P/X}$ – product/biomass yield coefficient; $Y_{P/S}$ – product/substrate yield coefficient.

The Plackett-Burman designs showed that the concentration of $FeCl_3$ in culture media was found to be significantly affecting cerulenin yield in a positive manner (α = 0.05) for experiments at 72 and 96 h. On the other hand, both magnesium concentration (96 h) and temperature (72 and 96 h) decreased the production of the secondary metabolite when these factors are used at the high level. Thus, these factors were selected for the modeling stage. The remaining variables were kept at their low level, except for the initial pH, which was adjusted to 6.5 for operational reasons.

The results of the Doehlert design at 72 h showed that the optimal region for antibiotic production lies between 26-28°C and 10-15 ppm Fe(III), resulting in concentrations around 110 mg/L of cerulenin (Fig. 2a). At 96 h, the response surface generated in the absence of magnesium supplementation showed an optimal region between 3.33-10 ppm Fe(III) and 28-30°C, but had lower production compared to that obtained at 72 h (Fig. 2b). Thus, the new production condition can be defined from experiments at 72 h with a medium containing 2% glycerol, 0.5% glucose, 0.25% peptone, 0.0025% NaCl, 0.0029% $FeCl_3$ and initial pH of 6.5 at 28°C and 150 rpm. The selected model was well adjusted to data and no lack of fit was detected (Tab. 1).

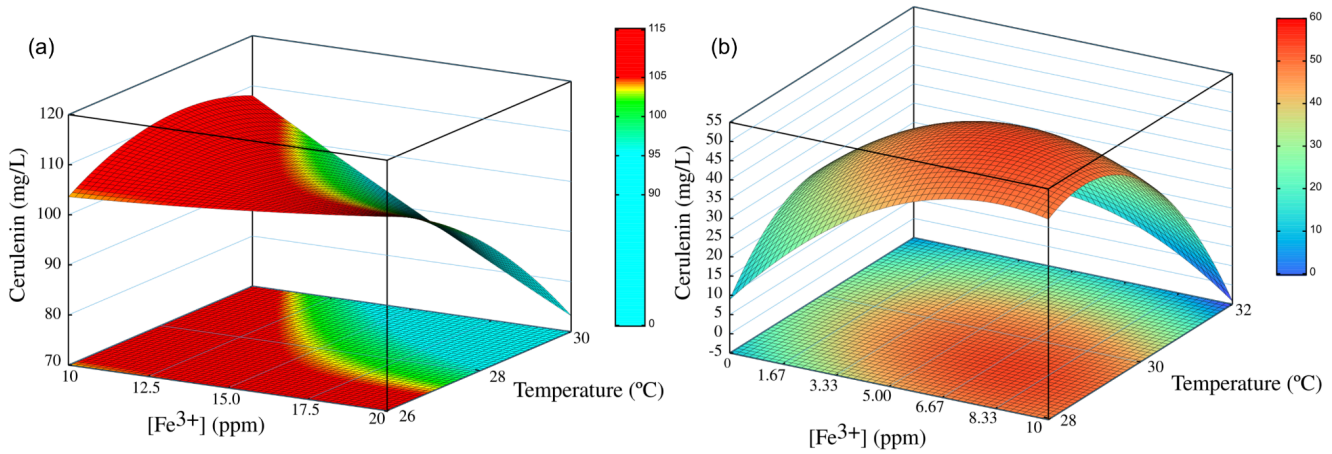

Figure 2 Response surface of cerulenin production generated by a Doehlert design with (a) two variables (temperature and Fe^{3+} concentration) in 72 h and (b) three variables (temperature and concentrations of Fe^{3+} and Mg^{2+}) in 96 h, fixing magnesium concentration at 0 ppm.

Table 1 Analysis of variance (ANOVA) of the multiple linear regression from the Doehlert design - 72 h.

	Degrees of freedom	Sum of squares	Square mean	F value	p-value
Regression	5	510.46	102.09	6.260	0.0499

Residues	4	65.23	16.31	–	–
Lack of fit	1	10.40	10.402	0.569	0.5056
Pure error	3	54,87	18,289	–	–
R^2	0.887		R^2 (adjusted)	0.746	

The production of cerulenin after optimization significantly increased compared to the standard medium supplemented with salts, reaching (105 ± 5) mg/L at 48 h and peaking at 96 h with (121 ± 8) mg/L (Fig. 3a). At 48 h, the product/biomass yield coefficient ($Y_{P/X}$) was (62.18 ± 7.47) mg/g, and the volumetric productivity (Q_P) was (2.26 ± 0.10) mg/L.h (Fig. 3b). Considering these kinetic parameters, it can be inferred that antibiotic production can be conducted for at least 48 hours to achieve good efficiency in the bioprocess. In a partial technical-economic analysis, considering only the expenses related to the culture medium, the production cost of cerulenin is estimated at U$ 32/g.h.

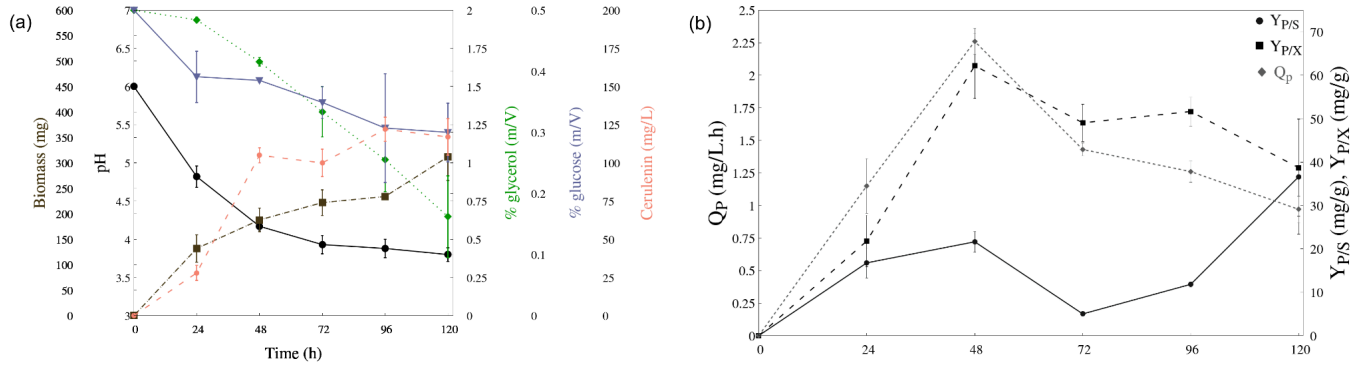

Figure 3 (a) Growth, carbon sources consumption, pH change and cerulenin production kinetics by *Sarocladium oryzae* BRM 59907 in the optimized media. (b) Kinetic parameters of the optimized bioprocess.

4 CONCLUSION

The supplementation of the standard medium with salts, especially Mg^{2+}, significantly impacted the growth of the strain *S. oryzae* BRM 59907 but inhibited cerulenin production. The addition of ferric chloride exerted a positive effect on its biosynthesis and proved to be a suitable source of Fe(III) for the bioprocess. Through experimental designs, an optimal cultivation condition was reached, which increased cerulenin production by over 50% compared to the initial condition, using smaller quantities of medium components, resulting in a halved cost.

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