IMPROVEMENT OF BIOSURFACTANT PRODUCTION BY THE ENDOPHYTIC FUNGUS *Aspergillus welwitschiae* CG2-16

Angélica R. Soares[1,2], Bárbara N. Batista[1], Juliana G. C. Rodrigues[1,3], Patrícia M. Albuquerque[1,2,3]*

[1] *Grupo de Pesquisa Química Aplicada à Tecnologia, Escola Superior de Tecnologia/Universidade do Estado do Amazonas, Manaus, Brazil*
[2] *Programa de Pós-graduação em Biotecnologia e Recursos Naturais da Amazônia, Escola Superior de Ciências da Saúde/Universidade do Estado do Amazonas, Manaus, Brazil*
[1] *Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Escola Superior de Ciências da Saúde/Universidade do Estado do Amazonas, Manaus, Brazil*
* *palbuquerque@uea.edu.br*

ABSTRACT

Biosurfactants are amphiphilic molecules produced by microorganisms and exploited for different industrial applications, such as pharmaceuticals, cosmetics, food and environmental. Furthermore, due to their chemical structure, these molecules are considered biocompatible and biodegradable. Among fungi, *Aspergillus* sp. have been described as promising biosurfactant producers. Therefore, this study aimed to improve the production of biosurfactants by the endophytic fungi *Aspergillus welwitschiae* isolated from the Amazonian medicinal plant *Fredericia chica* (crajiru). Variables that significantly affected the submerged cultivation were evaluated: pH, cultivation time and temperature. A central composite experimental design was used. The production of fungal biosurfactants was favoured under the conditions of 32°C, pH 12.4 and cultivation time of 7 days. These conditions lead to a 32% reduction in surface tension of the cultivation liquid medium. pH and cultivation time were the most significant factors for biosurfactant production by the endophytic fungus, and should be further investigated in order to reach the bioprocess optimization.

Keywords: Surface tension. Endophytic fungi. Central Composite Design. Cultivation conditions.

1 INTRODUCTION

Biosurfactants are amphiphilic compounds produced by microorganisms. These molecules can reduce the surface tension of liquids and are of great interest for the industry [1]. Biosurfactants could replace synthetic surfactants, which cause environmental damage due to toxicity and low biodegradability [2]. The wide industrial application has led the biosurfactants market to grow significantly, with prospects of reaching US$2.3 billion by 2028 [3].

The main source of industrially produced biosurfactants are bacteria and yeast. However, fungal production has been increasingly reported, and species of *Aspergillus* has been cited as promising sources of these tension-active molecules [4]. Endophytic fungi, found inside plant species, have recently attracted the attention of scientists due to their ability to significantly produce bioactive compounds. Nevertheless, studies on the production of biosurfactant by endophytic fungi are still scarce [5].

Several parameters affect the production of fungal biosurfactant, such as the medium pH, nitrogen and carbon concentration, time and temperature of cultivation, inoculum, among others [4]. Cultivation conditions, therefore, should be optimize. In this sense, this study aimed to improve the production of biosurfactants by the endophytic fungus *Aspergillus welwitschiae*, isolated from the branches of *Fridericia chica*, by evaluating the pH, cultivation time and temperature, on the surface tension reduction.

2 MATERIAL & METHODS

The microorganism used in this study, *Aspergillus welwitschiae* CG2-16 was isolated from the branches of *F. chica* and is deposited in the Central Microbiological Collection (CCM) of the Amazonas State University (UEA). The fungus was reactivated on Sabouraud solid medium containing soybean oil (0.5 g/L) to induce the production of biosurfactants. After the growth of the microorganism, a suspension of 1×10^8 spores/mL was prepared and used as inoculum [6].

Biosurfactant production was carried out in 125 mL Erlenmeyer flasks in a shaker incubator, at 170 rpm. The liquid medium consisted of 2.0 g/L yeast extract, 1.0 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4$ and 3.0 g/L of Na_2HPO_4. Soybean oil (2.0 g/L) was filtered and added after the medium sterilization [5]. To improve the biosurfactant production, the cultivation conditions were varied, according to a Central Composite Design (CCD) (Table 1). The pH, time and temperature of cultivation were evaluated, since these factors showed to be the most significant to the surface tension (ST) reduction, in a previous 2^{5-1} fractional experimental design. The software Statistica v. 10.0 was used to analyze the data obtained in the CCD ($p < 0.05$).

After fungal cultivation, the liquid medium was centrifugated and the supernatant was used for measuring the ST. The reduction of ST, when compared to the non-inoculated cultivation medium, indicates the production of biosurfactants.

Surface tension was measured according to Du Nouy's ring method, using 25 mL of the cell-free supernatant. Each sample was analyzed using a tensiometer (Krűss-K20), in order to calculate the reduction in surface tension over the cultivation time. Ultrapure water was used to calibrate the equipment [7].

3 RESULTS & DISCUSSION

Table 1 presents the surface tension reduction obtained in each experiment of the CCD. The highest ST reduction (32%) was observed in experiments 10 and 14, when the fungal cultivation was carried out for 7 days, at 32 °C, pH 12.4, and for 7 days, at 38.7 °C, pH 9.0, respectively.

Table 1 Surface tension reduction obtained in each experiment of the Central Composite Design, used to improve the biosurfactant production by the endophytic fungus *Aspergillus welwitschiae* CG2-16,under different cultivation conditions.

Independent factors								Response	
Coded/uncoded								Surface Tension (mN/m)	Surface Tension Reduction (%)
Run	Run Type	pH		t (days)		T (°C)			
1	Factorial	-1	7.0	-1	4	-1	28.0	37.4	29
2	Factorial	1	11.0	-1	4	-1	28.0	39.1	30
3	Factorial	-1	7.0	1	10	-1	28.0	62.5	-19
4	Factorial	1	11.0	1	10	-1	28.0	42.1	25
5	Factorial	-1	7.0	-1	4	1	36.0	39.2	25
6	Factorial	1	11.0	-1	4	1	36.0	38.8	31
7	Factorial	-1	7.0	1	10	1	36.0	46.7	11
8	Factorial	1	11.0	1	10	1	36.0	44.7	18
9	Axial	-1.68	5.6	0	7	0	32.0	39.7	13
10	**Axial**	**1.68**	**12.4**	**0**	**7**	**0**	**32.0**	**36**	**32**
11	Axial	0	9.0	-1.68	2	0	32.0	38.3	27
12	Axial	0	9.0	1.68	12	0	32.0	64	-21
13	Axial	0	9.0	0	7	-1.68	25.3	40.2	24
14	**Axial**	**0**	**9.0**	**0**	**7**	**1.68**	**38.7**	**35.6**	**32**
15	Center	0	9.0	0	7	0	32.0	40	24
16	Center	0	9.0	0	7	0	32.0	39.7	24
17	Center	0	9.0	0	7	0	32.0	39.6	25
18	Center	0	9.0	0	7	0	32.0	39.8	24
19	Center	0	9.0	0	7	0	32.0	39.5	25

t = cultivation time. T = cultivation temperature.

A similar result was observed by Silva et al. [5] in the production of biosurfactants by endophytic fungi isolated from *Piper hispidum*. The authors observed that the fungus *Aspergillus niger* PhIII 23L was able to reduce the surface tension in 36%. These results corroborate the potential of endophytic fungi belonging to the genus *Aspergillus* for the synthesis of tension-active molecules.

The effects of the variables and their interactions on surface tension reduction are shown in Figure 1. The variables with the greatest influence on biosurfactant production were pH (p = 0.0041), time (p = 0.0001) and the combination of pH and cultivation time (p = 0.0347), which had estimated effects of -7.09, 3.96 and 2.53, respectively.

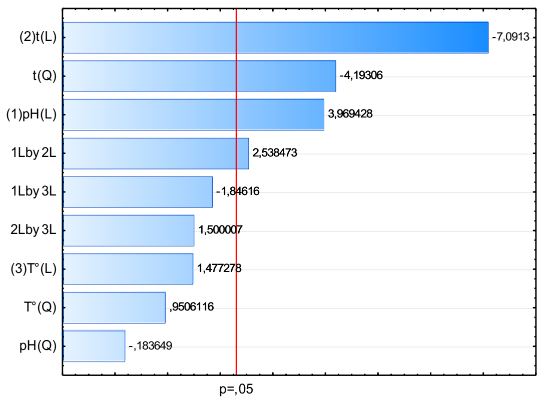

Figure 1 Pareto diagram for the surface tension reduction obtained from the cultivation of the endophytic fungus *Aspergillus welwitschiae* CG2-16 for the production of biosurfactants. 1: pH; 2: cultivation time (t); 3: cultivation temperature (T).

It was observed that lower pH values had a negative influence on surface tension reduction. The pH directly influences the production of biosurfactants and the transport of components across the cell membrane and must be stable to avoid cell lysis [8].

The effect of the interaction between the variables time and pH on the surface tension reduction can be seen in the response surface graph (Figure 2). The combination of these variables showed that the higher the pH combined with a shorter cultivation time, the better the biosurfactant production. Therefore, in order to obtain the best results, it is of the utmost importance to choose these conditions in future steps to obtain the optimization of the bioprocess.

The production of biosurfactants by *Aspergillus* sp. occurs between 30 and 40°C, a range considered ideal for the growth of fungi belonging to this genus. In addition, studies involving the production of biosurfactants by mutagenic *A. niger* showed that the highest production was obtained after 7 days of cultivation at 35 °C. [9], with the optimum time and temperature being similar to those found in the present study. Therefore, the endophytic *A. welwitschiae* proved to be a potential source of biosurfactants without requiring genetic alteration, and further tests involving other factors could be considered in future optimization studies, since the high cost of production is the main disadvantage related to biosurfactants [10].

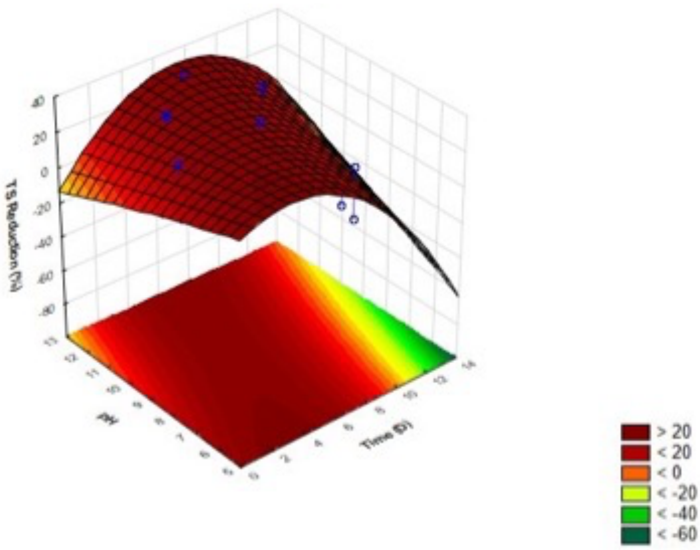

Figure 2 Response surface for the interaction of the variables cultivation time (t) and pH on biosurfactant production using the endophytic fungi Aspergillus *welwitschiae* CG2-16.

4 CONCLUSION

The study determined that the variables pH and cultivation time were significant for obtaining a higher level of biosurfactants from *A. welwitschiae*. The results demonstrate the effectiveness of experimental planning in determining significant factors and improving the fungal biosurfactant production.

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