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Cost-effective production of stable prodigiosin by *Serratia marcescens* UCP 1549 and application in soap coloring

Produção econômica de prodigiosina estável por *Serratia marcescens* UCP 1549 e aplicação na coloração de sabonete

Producción rentable de prodigiosina estable por *Serratia marcescens* UCP 1549 y aplicación en la coloración de jabón

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Abstract
Prodigiosin is a microbial red pigment, mainly produced by the bacterium *Serratia marcescens*, considered as a promising antimicrobial, immunosuppressive and antiproliferative compound. However, its industrial commercialization is still limited because of the high cost of production mainly due to the use of expensive substrates. Hence, this work aimed to the sustainable production of prodigiosin by *S. marcescens* UCP 1549, using corn bran as alternative and low-cost substrate, and its application in soap coloring. According to the results, both bacterial growth and red pigment production occurred, achieving 7.24 g/L and 1.68 g/L of biomass and pigment yield, respectively. Positive result in the presumptive test indicated red pigment as prodigiosin, which was confirmed by UV-Visible spectrophotometry (maximum absorbance peak at 535 nm), TLC (R*f* 0.9) and the functional groups identified by FTIR spectroscopy. Prodigiosin showed color stability at different pH values and NaCl concentration. The application of pigment in soap coloring was effective, suggesting its promising potential in the cosmetics industry as natural colorant. The results showed the biotechnological potential of *S. marcescens* UCP 1549 in the biotransformation of corn bran into prodigiosin, allowing a more economically and competitively industrial bioprocess.
Keywords: Natural pigment; Corn bran; Cosmetics industry.

Resumo
A prodigiosina é um pigmento microbiano vermelho, produzido principalmente pela bactéria *Serratia marcescens*, considerado um promissor composto antimicrobiano, imunossupressor e antiproliferativo. Contudo, sua comercialização industrial ainda é limitada devido ao alto custo de produção, causado principalmente pelo uso de

substratos caros. Assim, este trabalho teve como objetivo a produção sustentável de prodigiosina por *S. marcescens* UCP 1549, utilizando farelo de milho como substrato alternativo e de baixo custo, e sua aplicação na coloração de sabonete. De acordo com os resultados, ocorreu crescimento bacteriano e produção de pigmento vermelho, atingindo 7,24 g/L de biomassa e 1,68 g/L de rendimento de pigmento, respectivamente. O resultado positivo no teste presuntivo indicou o pigmento vermelho como prodigiosina, o que foi confirmado por espectrofotometria UV-Visível (pico de absorbância máxima em 535 nm), TLC (R*f* 0,9) e os grupos funcionais identificados por espectroscopia FTIR. A prodigiosina apresentou estabilidade na cor em diferentes valores de pH e concentração de NaCl. A aplicação do pigmento na coloração do sabonete foi eficiente, sugerindo seu potencial promissor como corante natural na indústria cosmética. Os resultados mostraram o potencial biotecnológico de *S. marcescens* UCP 1549 na biotransformação do farelo de milho em prodigiosina, permitindo um bioprocesso industrial mais econômico e competitivo.
Palavras-chave: Pigmento natural; Farelo de milho; Indústria cosmética.

Resumen
La prodigiosina es un pigmento microbiano rojo, producido principalmente por la bacteria *Serratia marcescens*, considerado un promisorio compuesto antimicrobiano, inmunosupresor y antiproliferativo. Sin embargo, su comercialización industrial aún es limitada debido al alto costo de producción, causado principalmente por el uso de sustratos costosos. Así, este trabajo tuvo como objetivo la producción sustentable de prodigiosina por *S. marcescens* UCP 1549, utilizando salvado de maíz como sustrato alternativo y de bajo costo, y su aplicación en la coloración de jabón. De acuerdo con los resultados, se produjo tanto el crecimiento bacteriano como la producción de pigmento rojo, alcanzando 7,24 g/L de biomasa y 1,68 g/L de rendimiento de pigmento, respectivamente. El resultado positivo en la prueba presuntiva indicó que el pigmento rojo era prodigiosina, lo que fue confirmado por espectrofotometría UV-Visible (pico máximo de absorbancia a 535 nm), TLC (R*f* 0,9) y los grupos funcionales identificados por espectroscopia FTIR. La prodigiosina mostró estabilidad de color a diferentes valores de pH y concentración de NaCl. La aplicación del pigmento en la coloración de jabón fue eficiente, sugiriendo su promisorio potencial como colorante natural en la industria cosmética. Los resultados mostraron el potencial biotecnológico de *S. marcescens* UCP 1549 en la biotransformación de salvado de maíz en prodigiosina, permitiendo un bioproceso industrial más económico y competitivo.
Palabras clave: Pigmento natural; Salvado de maíz; Industria cosmética.

1. Introduction

Worldwide demand for microbial pigments has increased in recent years due to their better biodegradability and compatibility with the environment, as well as less toxicity and allergic reaction, when compared to those of synthetic origin (Venil, et al., 2020; Nawaz, et al., 2021; Jeong, et al., 2022). Therefore, their biotechnological production has risen exponentially, aiming at the enormous potential for application in diverse areas, such as the food, pharmaceutical, cosmetic and textile industries (Sánchez-Muñoz, et al., 2020).

In this context, prodigiosin is a natural red pigment, that can be produced by various bacterial species, standing out *Serratia marcescens* as the main microorganism producing (dos Santos, et al., 2021; Sebastian, et al., 2022). It is a secondary metabolite with tripyrrole structure and chemical formula $C_{20}H_{25}N_{30}$, that exhibits a wide spectrum of biological properties (Araujo, Fukushima, & Campos-Takaki, 2010; Liu, et al., 2021). Prodigiosin is well-known as a promising therapeutic agent due to its antimicrobial, antimalarial, immunosuppressive and antitumor activities (Lapenda, et al., 2015; Yip, et al., 2019). Antioxidant property of this pigment have been also demonstrated, as well as its application as bioactive component in sunscreen formulation (Suryawanshi, et al., 2015; Arivizhivendhan, et al., 2018). In addition, prodigiosin have been applicated as natural colorant of textile materials, paper, candles and also, as biodegradable ink (Venil, et al., 2013; Ren, et al., 2021; Liu, et al., 2022).

Despite of its promising applications, the large-scale production of prodigiosin remains limited due to the high cost of production, mainly caused by the use of expensive conventional substrates. Hence, several researches have been addressed to obtain prodigiosin using industrial by-products and waste, as alternative sources of carbon and nitrogen, being a promising and cost-effective strategy (Elkenawy, et al., 2017; Arivizhivendhan, et al., 2018; Nguyen, et al., 2020; Nguyen, et al., 2022).

Particularly in Brazil, *S. marcescens* UCP 1549 isolated from the semiarid region of Pernambuco state has shown

excellent potential to produce prodigiosin, as demonstrated in the literature (Araujo, Fukushima, & Campos-Takaki, 2010; Lins, et al., 2014; Montero-Rodríguez, et al., 2018). However, the search for novel substrates is justified, in order to increase productivity and reduce costs. In this context, this study aimed to the cost-effective production of prodigiosin by *S. marcescens* UCP 1549 using corn bran as the sole substrate. Also, its application as soap colorant was addressed, since it has been scarcely studied (Ahmad, et al., 2012; Venil, et al., 2013).

2. Methodology

2.1 Microorganisms and preparation of inoculum

The bacterium *S. marcescens* UCP 1549, isolated from semi-arid soil and identified by Araujo, Fukushima, & Campos-Takaki (2010), was kindly provided by the Culture Collection UCP - Catholic University of Pernambuco (Recife-PE, Brazil), registered to the World Federation for Culture Collections (WFCC) under number 927. This strain was maintained in the solid Luria Bertani (LB) medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L) at 5 °C.

Stored culture of *S. marcescens* was transferred first to LB medium and incubated for 18 h at 28ºC. Then, two colonies were transferred to 50 ml of LB broth and incubated at 28ºC and 150 rpm in an orbital shaker. Once the optical density at 600 nm reached 0.8-1.0, this culture was used as inoculum (Montero-Rodríguez, et al., 2018).

2.2 Agro-industrial substrate

In this study, corn bran was used as low-cost substrate, previously obtained in a local market in city of Recife (Pernambuco, Brazil). It was sieved using Tyler series sieves - mesh 16 and 32- to obtain a fine and homogeneous powder, in order to facilitate dissolution in the culture medium.

2.3 Prodigiosin production

Fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of saline solution (KH_2PO_4 3 g/L, K_2HPO_4 7 g/L, $MgSO_4$.$7H_2O$ 0.2 g/L, $(NH_4)_2SO_4$ 1 g/L) and 1 % corn bran, with pH adjusted to 7. The flaks were sterilized in an autoclave at 121°C for 15 min, inoculated with 5 % of the pre-inoculum of *S. marcescens* grown in medium LB and then incubated at 31ºC and 100 rpm in an orbital shaker for 72 h.

2.4 Determination of biomass yield

After 72 h of cultivation, the fermentation was centrifuged at 10000 *g* for 15 min. The supernatant was discarded and the pelletized cells were washed twice with distilled water by centrifugation at 10000 *g* for 20 min. Then the biomass was frozen, lyophilized and quantified as g/L.

2.5 Extraction and quantification of red pigment

The produced red pigment was extracted from biomass using the solvent system chloroform:methanol, in the proportions 2:1, 1:1 and 1:2 (*v/v*), evaporated and quantified by gravimetry (Araujo, Fukushima, & Campos-Takaki, 2010; Montero-Rodríguez, et al., 2018).

2.6 Purification and identification of red pigment

Preliminary identification of the crude red pigment was performed after its solubilization in 95% ethanol. The colored solution was divided into two portions: one portion was acidified with a drop of concentrated HCl and the other was alkalized

with a drop of concentrated NaOH solution (Gerber & Lechevalier, 1976). The red or pink color in acidic condition and the yellow or brown color in alkaline condition confirmed a positive presumptive test for prodigiosin (Sathishkumar & Aparana, 2014; Mansi & Shah, 2015).

Then, the red pigment was dissolved in 3 ml of methanol and subjected to purification by column exclusion chromatography (column 22 x 1 cm) filled with Sephadex LH-20 activated at 800 °C for 1 h, as an absorbent. The elution was carried out by the solvent system chloroform: methanol (1:1, *v/v*) and then modified to chloroform: methanol: acetone (4:2:3, *v/v*), aiming maximum removal of impurities (Lins, et al., 2014). The red fraction was collected and analyzed by UV-Vis spectrophotometry, and the absorbance range in the range 400-700 nm was determined. Prodigiosin production was confirmed by the presence of a maximum absorbance peak at 535 nm (Araujo, Fukushima, & Campos-Takaki, 2010).

In addition, the red fraction was subjected to thin layer chromatography (TLC), using aluminum plates coated with silica gel, which after application of the sample was placed in a glass vat containing the chloroform: methanol solvent system: (9:1, *v/v*) (Araujo, Fukushima, & Campos-Takaki, 2010; Priya, et al., 2013). The retention factor (R*f*) was calculated according to the formula R*f*: distance travelled by the compound/ distance travelled by the solvent front and then, it was compared to the standard prodigiosin R*f* referred in the literature (Lins, et al., 2014).

The purified red pigment was submitted to Fourier transform infrared (FT-IR) spectroscopic analysis on the Shimadzu equipment, IR-TRACER 100, using an attenuated total reflection (ATR) accessory consisting of a mixed "diamond/ZnSe" crystal. The peaks obtained were compared with the literature to confirm the presence of prodigiosin

2.7 Pigment stability

Color stability of the prodigiosin produced by *S. marcescens* was investigated following the methodology proposed by Perumal, et al. (2009) and Velmurugan, et al. (2011), with modifications. Briefly, glass test tubes containing 10 ml of the ethanol extract of prodigiosin were adjusted to pH 2, 4, 6, 8, 10, 12 and 14, homogenized for 10 min and the absorbance measured using a UV–visible spectrophotometer. Another set of tubes containing 10 ml of extract were amended with 0.1, 0.2, 0.5, 1 and 5% (*v/v*) salt solution (NaCl) and kept at rest for 1 h to determine absorbance. Relative color stability (%) was calculated according to Equation 1.

$$\%S = (A_1 \times 100)/ A_0 \quad (1)$$

where A_0 is pigment absorbance before treatment and A_1 is absorbance after treatment. Absorbance of the pigment was measured by spectrophotometry at 535 nm.

2.8 Application of prodigiosin in soap coloring

The potential application in soap coloring of the pigment produced by *S. marcescens* was investigated. For this, 40 g of white soap were fractionated and melted over a low fire with 50 ml of distilled water in a container. When fully melted, 0.3 g of the pigment diluted in water was added for 3 min. Then, the mixture was placed in a mold and allowed to cool to room temperature until it solidified (Meenakshi et al., 2018). The test was done in triplicate.

3. Results and Discussion

3.1 Production of biomass and pigment

Several synthetic media have been widely used for the growth of prodigiosin-producing microorganisms (Bhagwat & Padalia, 2018; Yip, et al., 2019). However, the high cost of conventional substrates justifies the need for the formulation of cost-effective production medium that allows the bacterial growth and the biosynthesis of this useful pigment. In this sense, low-cost substrates such as brown sugar, cassava wastewater and ram horn peptone have been previously used (Araujo, Fukushima, & Campos-Takaki, 2010; Aruldass, et al., 2014; Kurbanoglu, et al., 2015).

In this study, the use of corn bran reached a biomass yield of 7.24 g/L, which was higher than that obtained by the same strain in medium containing corn steep liquor (3.47 g/L) and cassava wastewater (3.65 g/L) (Araujo, Fukushima, & Campos-Takaki, 2010). In addition, the pigment yield was 1.68 g/L, a result similar or superior to that previously obtained by *S. marcescens* strains in different agro-industrial based medium (Table 1).

Table 1: Comparison of prodigiosin production from *S. marcescens* UCP 1549 of this study and previous studies using agro-industrial substrates.

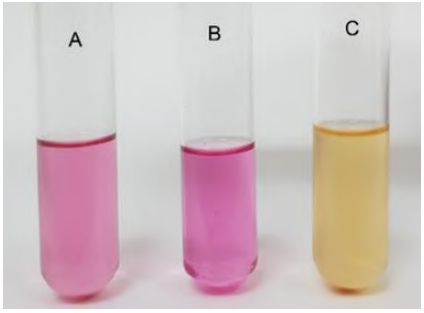
Strain	Agro-industrial substrate	Prodigiosin yield (g/L)	Reference
Serratia marcescens	Sesame oil	0.767	Giri, et al. (2004)
	Coconut seed	1.940	
	Coconut oil	1.420	
S. marcescens TKU011	Peanut powder	1.168	Wang, et al. (2012)
	Squid pen powder	0.978	
S. marcescens UTM1	Brown sugar	0.237	Aruldass, et al. (2014)
S. marcescens MO-1	Ram horn peptone	0.278	Kurbanoglu, et al. (2015)
S. marcescens	Kitchen waste	0.870	Xia, Veony & Yang et al. (2018)
S. marcescens UCP 1549	Corn bran	1.680	Present study

Source: Authors.

3.2 Identification of pigment

Presumptive test for prodigiosin was carried out for red pigment produced by *S. marcescens* UCP 1549 (Figure 1). An intense pink color was detected when the pigment was subjected to acidic pH, while a yellow color was observed in alkaline conditions, indicating a positive result for prodigiosin production (Rajasekharan, et al., 2014; Mansi & Shah, 2015; Faraag et al., 2017; Rakh, et al., 2017).

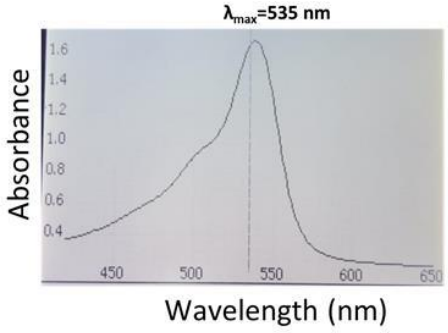
Figure 1: Presumptive test for prodigiosin of the red pigment produced by *S. marcescens* UCP 1549: Ethanol extract of the pigment without alteration (control) (A), in acidic (B) and alkaline (C) conditions.



Source: Authors.

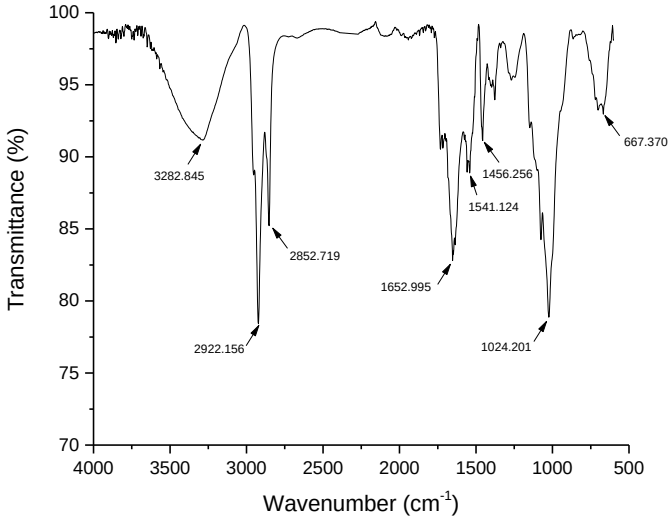
In addition, the spectrophotometric analysis showed the peak of maximum absorbance at 535 nm (Figure 2), while the TLC revealed an R*f* = 0.9, corresponding to prodigiosin (Araujo, Fukushima, & Campos-Takaki, 2010; Gondil et al., 2017; Mandal, et al., 2021). The main absorption bands identified in the FTIR spectra (Figure 3) of the red pigment are summarized in Table 2, and they are in accordance to the prodigiosin peaks reported in previous studies (Khanam & Chandra, 2018; Zhao, et al., 2020).

Figure 2: Absorbance spectrum of the red pigment produced by *S. marcescens* UCP 1549.



Source: Authors.

Figure 3. Infrared spectrum of the red pigment produced by *Serratia marcescens* UCP 1549 in medium containing corn bran.



Source: Authors

Table 2: Comparison of the absorption bands identified in the FTIR spectrum of the red pigment produced by *S. marcescens* UCP 1549 with functional groups of prodigiosin reported in previous studies.

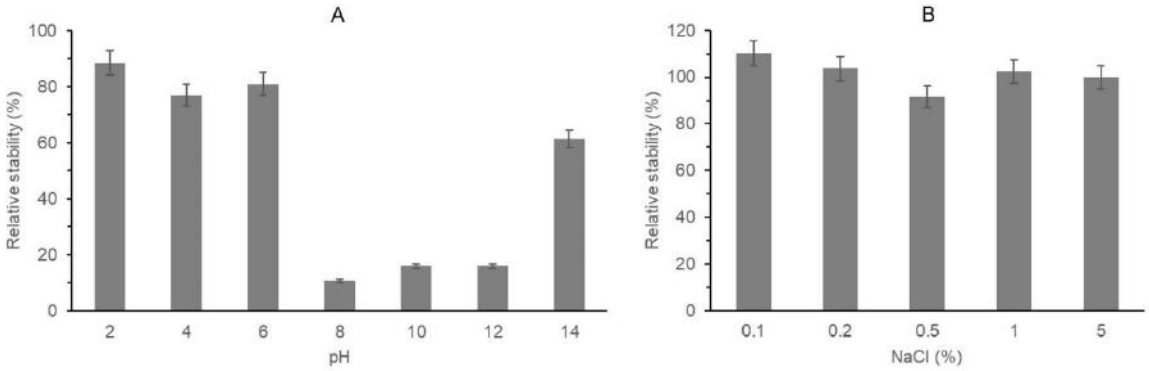
Absorption bands identified in the FTIR spectrum [cm^{-1}]	Functional groups	Band assignment in prodigiosin nature	Reference
3282.845	N-H stretch	heterocyclic amines	Khanam & Chandra (2018), Hernández-Velasco, et al. (2020)
2922.156	C-H stretch	methylene	Manas, et al. (2020)
2852.719	C-H stretch	methylene	Sumathi, et al. (2014), Manas, et al. (2020)
1652.995	C=C stretch	aliphatic amine	Sumathi, et al. (2014), Hernández-Velasco, et al. (2020)
1541.124	C=C stretch	pyrrole ring structure	Ahmad, et al. (2014)
1456.256	C–H bend	methyl groups	Hernández-Velasco, et al. (2020), Zhao, et al. (2020)
1024.201	C-N bend	amines	Zhao, et al. (2020)

Source: Authors.

3.3 Relative stability of prodigiosin

The potential application of natural pigments in various industrial fields depends on the stability against variables or extreme conditions. In this sense, prodigiosin produced by *S. marcescens* UCP 1549 was subjected to several physical-chemical treatments and the results are shown in Figure 4.

Figure 4: Color stability of prodigiosin produced by *Serratia marcescens* UCP 1549 at different values of pH (A) and salinity (B).



Source: Authors.

As evidenced, prodigiosin showed excellent color stability when subjected to different values of salinity (Figure 4B). However, in the case of pH, it showed less stability in alkaline conditions (pH 8-12), where there was a color change from pink to yellow, and consequently, a decrease in absorbance at 535 nm (Figure 4A). These results corroborated that obtained previously in the presumptive test, where in basic pH there was also a color change of the ethanol extract of the red pigment (Figure 1), confirming the presence of prodigiosin. Yuan, et al. (2005) obtained similar results when they verified the stability of prodigiosin produced by *Pseudomonas* sp. after pH 2 and 5, while the pigment showed instability in alkaline conditions.

The stability of pigments of natural origin such as the prodigiosin produced by *S. marcescens* UCP 1549, confirm its potential for application as an alternative to synthetic dyes, in several industrial processes, where they are generally subjected to adverse conditions, such as high temperature and acidic pH.

3.4 Application of prodigiosin in soap coloring

There is a growing interest in the use of natural dyes in various industrial areas due to the prohibition on the use of some synthetic dyes with demonstrated carcinogenicity of the precursor or product, as well as the toxic effect of their industrial residues on ecosystems. The lower toxicity and allergic reactions of natural pigments make them more compatible with human use, due to the reduction of exposure to harmful chemicals (Venil, Dufossé & Devi, 2020; Nawaz, et al., 2021).

In this study, prodigiosin produced by *S. marcescens* UCP 1549 in low-cost medium containing corn bran was applied in soap coloring, as shown in Figure 5. The effectiveness of the coloring demonstrates the potential application of this pigment as a natural dye in the cosmetic industry. Previously, Rodríguez (2017) demonstrated the effectiveness of prodigiosin produced by this strain, in textile dyeing and candle coloring.

Figure 5: Application of prodigiosin produced by *Serratia marcescens* UCP 1549 in soap coloring.

Source: Authors.

4. Conclusion

In this study, *Serratia marcescens* UCP 1549 demonstrated its ability to use corn bran as the sole carbon source for the cost-effective production of prodigiosin. The pigment demonstrated color stability at different values of pH and NaCl concentrations, as well as effectiveness in soap coloring, confirming its potential application in the cosmetic industry. However, some analyzes will soon be carried out in order to confirm the antimicrobial properties as well as the low toxicity of soaps colored with prodigiosin, adding value to the cosmetic and personal hygiene products industry.

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