Research, Society and Development, v. 11, n. 7, e24711729841, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i7.29841

Red pigment production by *Amycolatopsis* sp. UFPEDA 3422

Produção de pigmento vermelho por *Amycolatopsis* sp. UFPEDA 3422

Producción de pigmento rojo por *Amycolatopsis* sp. UFPEDA 3422

Received: 05/02/2022 | Reviewed: 05/11/2022 | Accept: 05/17/2022 | Published: 05/22/2022

Amanda Maria da Silva
ORCID: https://orcid.org/0000-0003-2269-4982
Federal University of Pernambuco, Brazil
E-mail: amanda.msilva4@ufpe.br
Marcela de Araújo Sobral
ORCID: https://orcid.org/0000-0001-7440-3804
Federal University of Pernambuco, Brazil
E-mail: marcela.clementino@ufpe.br
Iranildo José da Cruz Filho
ORCID: https://orcid.org/0000-0002-5466-6567
Federal University of Pernambuco, Brazil
E-mail: iranildoj@gmail.com
Arthur Travassos de Queiroz Xavier
ORCID: https://orcid.org/0000-0002-9023-3232
Federal University of Pernambuco, Brazil
E-mail: arthurtravassos.xavier@gmail.com
Mariza Severina de Lima Silva
ORCID: https://orcid.org/0000-0002-0833-4238
Federal University of Pernambuco, Brazil
E-mail: mariza.slima@ufpe.br
Beate Saegesser Santos
ORCID: https://orcid.org/0000-0002-9909-5462
Federal University of Pernambuco, Brazil
E-mail: beate.saegesser@gmail.com
Alexandre José da Silva Góes
ORCID: https://orcid.org/0000-0002-3202-2074
Federal University of Pernambuco, Brazil
E-mail: alexandre.goes@ufpe.br
Willames de Albuquerque Soares
ORCID: https://orcid.org/0000-0003-3268-7241
Federal University of Pernambuco, Brazil
E-mail: was@poli.br
Janete Magali de Araújo
ORCID: https://orcid.org/0000-0001-8878-4820
Federal University of Pernambuco, Brazil
E-mail: janetemagali@yahoo.com.br
Gláucia Manoella de Souza Lima-Gomes
ORCID: https://orcid.org/0000-0002-1941-8912
Federal University of Pernambuco, Brazil
E-mail: glaucia.mslima@ufpe.br

Abstract
Red biopigments produced by actinobacteria represent an important substitute in the food industry to synthetic pigments, which can cause harmful effects on human health and the environment. In this study, pigment production by actinobacteria strain UFPEDA 3422, identified by polyphasic taxonomy, was investigated. The cultivation parameters related to growth and pigment production were assayed in solid culture media and submerged fermentation. The red cell pigment was extracted with ethyl acetate and analyzed in a UV-Vis spectrophotometer with maximum production of the pigment (34.9 $AU_{540\ nm}$) in Hickey-Tresner (HT) medium for 48 hours at 37 ºC. The maximum biomass concentration (33.4 g/L) was obtained after 96 hours. Polyphasic taxonomy confirms 97.4% similarity of strain UFPEDA 3422 with the *Amycolatopsis*, a rare genus able to produce pigments on different substrates. In addition, the microorganism has tolerance to temperature variations, pH fluctuations and concentrations of 5% NaCl. The determination of cultivation and pigment production parameters show the potential of *Amycolatopsis* sp. as an alternative source of natural pigments.
Keywords: Biopigment; Carbon source; Polyphasic taxonomy.

Resumo

Biopigmentos vermelhos produzidos por actinobactérias representam um importante substituto na indústria alimentícia aos pigmentos sintéticos, que podem causar efeitos nocivos à saúde humana e ao meio ambiente. Neste estudo, foi investigada a produção de pigmentos pela cepa de actinobacteria UFPEDA 3422, identificada por taxonomia polifásica. Os parâmetros de cultivo relacionados ao crescimento e a produção de pigmentos foram avaliados em meio de cultura sólido e fermentação submersa. O pigmento celular vermelho foi extraído com acetato de etila e analisado utilizando um espectrofotômetro UV-Vis com máxima produção de pigmento (34.9 $AU_{540\ nm}$) no meio Hickey-Tresner (HT) por 48 horas a 37 °C. A concentração máxima de biomassa (33.4 g/L) foi obtida após 96 horas. A taxonomia polifásica confirmou 97.4% de similaridade da cepa UFPEDA 3422 com *Amycolatopsis*, um gênero raro capaz de produzir pigmentos em diferentes substratos. Além disso, o micro-organismo apresenta tolerância a variações de temperatura, oscilações de pH e concentrações de 5% de NaCl. A determinação dos parâmetros de cultivo do microrganismo e produção do pigmento demonstram o potencial de *Amycolatopsis* sp. como uma fonte alternativa de pigmentos naturais.
Palavras-chave: Biopigmento; Fonte de carbono; Taxonomia polifásica.

Resumen

Biopigmentos rojos producidos por las actinobacterias representan un importante sustituto en la industria alimentaria a los pigmentos sintéticos que pueden causar efectos nocivos para la salud humana y el medio ambiente. En este estudio se investigó la producción de pigmentos por la cepa de actinobacteria UFPEDA 3422, identificada por taxonomía polifásica. Los parámetros de cultivo relacionados con el crecimiento y la producción de pigmentos se evaluaron en medio de cultivo sólido y en fermentación sumergida. El pigmento celular rojos se extrajo con acetato de etilo y se analizó con un espectrofotómetro UV-vis al máximo rendimiento del pigmento (34,9 $AU_{540\ nm}$) en medio Hickey-Tresner (HT) durante 48 horas a 37 °C. La máxima concentración de biomasa (33,4 g/L) se obtuvo después 96 horas. La taxonomía polifásica confirmó un 97,4% de similitud de la cepa UFPEDA 3422 con *Amycolatopsis*, un género raro capaz de producir pigmentos en diferentes substratos. Además, el microorganismo es tolerante a variaciones de temperatura, fluctuaciones de pH y concentraciones de 5% NaCl. La determinación de los parámetros de cultivo del microorganismo y la producción de pigmentos demostraron el potencial de *Amycolatopsis* sp. como fuente alternativa de pigmentos naturales.
Palabras clave: Bio Pigmentación; Fuente de carbono; Taxonomía polifásica.

1. Introduction

The use of biopigments or natural pigments as dyes and food additives is growing every year. Color plays a fundamental role in the perception of flavor, aroma and texture of food, providing an attractive and pleasant appearance related to the acceptance of products by consumers (Chatragadda & Dufossé, 2021). The food coloring market presents a projection of USD 5.12 billion by 2023 (Rana et al., 2021). In parallel, the market value of natural pigments, such as carotenoids, one of the most used groups, could reach USD 1.53 billion in 2021 (Venil et al., 2020; Rana et al., 2021).

The search for new compounds with coloring capacity is increasing, since synthetic dyes widely used in various industrial sectors (due, among other factors, to chemical stability and low production cost), have been linked in recent decades to health problems such as allergies, high toxicity, oncogenicity and teratogenic properties (Sen et al., 2019). The characteristics presented by these lead to the banning of some synthetic food additives such as Quinoline Yellow, Ponceau SX and Azorubin (Rana et al., 2021). Additionally, many of these pigments depend on raw material from petroleum and are potentially toxic and harmful to the environment (Kumar et al., 2015; Ramesh et al., 2019).

The research and production of biopigments is growing and follows the worldwide demand of industry and consumers for safe and ecologically viable substances, including for the food industry, where the development of new compounds that are non-toxic, safe and compatible with food is a challenge (Sen et al., 2019).

The need to replace synthetic pigments drives the search for alternative sources of biopigments, which are obtained from animals, plants or microorganisms, the last two being the main sources. In addition to the coloring capacity, many of these have beneficial biological functions for humans such as sun protection, antitumor, antimicrobial activity, as antioxidant, antiproliferative, immunosuppressive agents and for the treatment of Diabetes Mellitus, acquiring high commercial value in the nutraceutical, cosmetic, pharmaceutical and textile industries (Kumar et al., 2015; Ramesh et al., 2019; Celedón & Diaz., 2021). Among the microorganisms, bacteria stand out for the production of several bioproducts, including pigments, due to their short

life cycle, ease of scaling, abundance, and ability to produce different colors and shades of pigments (Venil et al., 2020; Celedón & Diaz, 2021).

Structurally diverse, pigments belong to distinct categories based on their chemical composition, natural source of production and presented functions, and can be classified as: carotenoids, astaxanthin, prodigiosin, among others (Sen et al., 2019).

Natural pigments of microbial origin have advantages over the use of synthetic and natural pigments produced by plants, due to their stability, availability unrelated to seasonal variations, cost-effectiveness, high yield and easy obtainment (Chatragadda & Dufossé, 2021). In addition, the use of microbial pigments has been shown to be a safe alternative that meets ecological issues as they are biodegradable (Ramesh et al., 2019; Rana et al., 2021).

Actinobacteria comprise a widely studied phylum that has important potential for the production of bioactive microbial metabolites and pigments (Sharma et al., 2018). Among actinobacteria, the genus *Streptomyces* is one of the most representative, however, rare genera such as *Amycolatopsis* also have biotechnological importance and potential for the production of antimicrobial agents and biopigments, although little reported in the literature (Bauermeister et al., 2019). According to Bergey's Manual of Systematic Bacteriology, these bacteria are aerobic or facultative anaerobic, Gram-positive, catalase-positive, with branched mycelial structures fragmented into rod-shaped elements and have diffusible pigmentation of varying color: white, red and brown (Goodfellow et al., 2012).

In addition, the production of pigments from microorganisms can be enhanced using fermentation techniques (solid or submerged cultivation), chemical modifications, use of agro-industrial residues, genetic modification techniques, among others, in order to make such an enterprise an alternative attractive to the industry (Ramesh et al., 2019). Thus, the production and application of bacterial pigments as natural dyes has been investigated by several researchers, constituting one of the emerging fields of research for demonstrating its potential in biotechnological industrial applications (Vendruscolo et al., 2013; Chatragadda & Dufossé, 2021).

The productivity of metabolites from microorganisms is related to the fermentation process and for this reason it also has several advantages such as lower production cost (when produced on a large scale), high yield, easy extraction and low cost linked to raw material (Rana et al., 2021).

Thus, in the present study, the identification of the microorganism *Amycolatopsis* sp. UFPEDA 3422 and investigated the production of red pigment obtained from it, with the objective of evaluating the best conditions for submerged cultivation as well as obtaining the kinetic parameters of production. In addition to extracting the pigment, obtaining kinetic production parameters allows optimizing a possible production which, in turn, can result in more cost-effectiveness.

2. Methodology

2.1 Microorganism and inoculum preparation

The microorganism *Amycolatopsis* sp. UFPEDA 3422 was obtained from the Microorganisms Collection of the Department of Antibiotics of the Federal University of Pernambuco (UFPE). The strain was reactivated and seeded in ISP-2 medium for five days at 37 ºC for further microscopic analysis and then stored at 4 ºC.

2.2 Physiological characteristics

The morphological, physiological and biochemical characterization of the microorganism was performed according to the methodology recommended by The International Streptomyces Project (ISP) (Shirling & Gottileb, 1996; Goodfellow et al., 2012). The growth characteristics, mycelial substrate, substrate and aerial mycelium coloration as well as soluble pigment formation were investigated in ISP-2, ISP-3, ISP-4, GAA, CAA, HT, ALA, ISP-6 and ISP- 7 media, kept in incubation for 8

days at 30 °C and 37 °C. The colors presented after cultivation were distinguished by the naked eye, being analyzed for growth, coloration of substrate (reversal of plaque) and aerial mycelium and the presence of soluble pigments. The production of melanin was verified by analyzing the growth of the microorganism in ISP-6 and ISP-7 medium as described by Goodfellow et al., (2012).

2.3 Biochemical characterization of the microorganism

2.3.1 Evaluation of the degradation of carbon sources, Tween and gelatine

The assay was performed using modified bennett's agar (Jones, 1949) as a base medium supplemented separately with carbon sources: guanine (0.4%), hypoxanthine (0.4%), starch (0.1%), xylan (0.4%) and L-tyrosine (0.5%). Tween degradation was evaluated using Sierra's medium (Sierra, 1957) adding 1% (v/v) of Tween 20 and 80 (separately).

In the evaluation of gelatine degradation, the Gordon medium (Gordon et al., 1974) was used, added with 0.4% and 1% of gelatin, respectively. For these assays, a 15 µL aliquot of the actinobacteria suspension was added to the surface of the plates. The plates were incubated for 14 days at 37 ºC, with a halo of degradation being observed after the addition of iodine crystals. The experiment was conducted in triplicate.

2.3.2 Metabolic profile of carbon source utilization

The metabolic profile of carbon source utilization and acid production from it was evaluated using sugar solutions: dextrin, D-trehalose, mannitol, D-maltose. Myo-inositol, D-xylose, L-arabinose, L-rhamnose, adonitol, mannose, glucose, saccharose, D-lactose, D-sorbitol and D-cellobiose at a concentration of 1% (w/v), added to the culture medium ISP-9 agar used as a nutritional basis (Shirling & Gottlieb, 1966) plus phenol red indicator at a concentration of 0.025%. The plates were incubated for 10 days at 37 ºC. The reading of the assays was performed according to the growth of the microorganism and the presence or absence of acid production and modification of the pH of the culture medium. The experiment was carried out in triplicate.

2.3.3 Antibiotic susceptibility profile

The antibiotic resistance profile was verified by the disk diffusion technique described by Kirby-Bauer (Bauer et al., 1966) using the Glucose Yeast Extract Agar culture medium. 50 µL of the actinobacteria suspension (in 0.9% saline solution) were seeded and antibiotic discs were added: nalidixic acid (30 µg); vancomycin (30 µg); rifampicin (5 µg); streptomycin (10 µg); gentamicin (10 µg); tobramycin (10 µg) and cefoxitin (30 µg). The results were interpreted according to CLSI (2017). The experiment was carried out in triplicate.

2.4 Evaluation of the influence of temperature on microorganism growth

The influence of temperature on the growth of *Amycolatopsis* sp UFPEDA 3422 was evaluated using the glucose yeast extract agar culture medium as substrate. The microorganism was incubated at 4 ºC, 25 ºC, 37 ºC and 45 ºC for 10 days. The experiment was carried out in triplicate (Tang et al., 2019).

2.5 Evaluation of the influence of pH on microorganism growth

The influence of the pH on the growth of the microorganism was evaluated using a culture medium glucose yeast extract agar with double concentration, added with a buffer solution composed of a KH_2PO_4 -0.2 M solution and a K_2HPO_4 - 0.2 M solution in different proportions to obtain the pH values. The development of the microorganism was evaluated at pH 4.5, 9 and 10. For each 200 ml of buffer solution, 200 ml of culture medium was added. The plates were incubated at 37 ºC for 10 days, then the growth and color of the colonies was observed (Tang et al., 2019).

2.6 Evaluation of the influence of sodium chloride (NaCl) on the growth of the microorganism

The influence of sodium chloride (NaCl) on the growth of *Amycolatopsis* sp UFPEDA 3422 was evaluated using as a basis the culture medium glucose yeast extract agar added with sodium chloride (NaCl) (1%, 3%, 5%,7% and 10%). Cultures were incubated at 37 °C for 10 days. Then, colony growth in the presence of salt was observed (Tang et al., 2019).

2.7 Identification based on 16S rDNA region

DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. Amplification of 16S DNA was performed by polymerase chain reaction using universal primers for eubacteria 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Weisburg et al., 1991). Reaction mixes consisted of 10 to 50 ng DNA, 5 pmol of each primer, 200 mM dNTP, 1.5 mM $MgCl_2$, ×1 buffer, and 1 U Platinum Taq DNA polymerase (Invitrogen Life Technologies) and adjusted to a final volume of 25 µL with water. The following cycling conditions were used: 5 min denaturation at 94 °C; 25 cycles of 1 min at 94 °C, 30s at 52 °C and 2 min at 72 °C; and a final elongation of 10 min at 72 °C. The PCR product was sequenced by Macrogen (Korea), and the resulting sequence was compared to all sequences available in GenBank using BLAST software from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi. nlm.nih.gov/). The sequences were aligned with Clustal software, and the phylogenetic tree was built with Mega 7 using the neighbor joining method. Tree topology was evaluated by bootstrap analysis (1000 resampling). The sequences obtained were deposited in GenBank.

2.8 Pigment production and extraction

Initially, a suspension of the microorganism in 0.9% saline solution was prepared, then 50 µL of this were applied and spread in petri dishes containing ISP-2 medium. Plates were kept under incubation at 37 ℃ for 5 days. From the culture grown on a plate, blocks of agar (8 mm in diameter) were transferred to Erlenmeyer flasks containing ISP-2 broth, kept under agitation at 150 rpm, at 37 °C for 48 hours. Subsequently, 5 ml of the fermented ISP-2 broth were transferred to Erlenmeyer flasks containing 50 ml of the fermentation medium (selected according to the best growth in solid medium) (HT and 400 broth), kept under stirring at 150 rpm, at 37 °C for 96 hours (Lyra et al., 1964). Every 24 hours, three Erlenmeyer flasks were removed and the liquid fraction was separated from the mycelial by centrifugation at 10.000 rpm for 10 min, with the biomass obtained being weighed and reserved. Pigment extraction was performed by adding 3 mL of ethyl acetate to each gram of biomass (1:3 w/v). Samples were filtered and the extracted pigment was stored away from light. The pigment obtained was diluted 1:100 in ethyl acetate and scanned at a length from 200 to 900 nm in a UV light spectrophotometer for quantification, with the concentration expressed in absorbance unit (AU). The experiments were carried out in triplicate.

2.9 FTIR Analysis

For FTIR analysis, the pigment was diluted in KBr salt. Then, the FTIR spectra were recorded in the frequency range from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} according to the methodology described by El-Naggar and El-Ewasy, 2017 (with some modifications). Functional groups and types of vibrations were evaluated based on peak values and compared with pigments produced by other microorganisms.

2.10 Evaluation of kinetic parameters of production

The kinetic parameters related to maximum biomass productivity and maximum pigment production per unit of time were determined as described by Bühler et al., (2015).

The maximum cell productivity (biomass) was determined according to the equation (Eq. 1):

$$P_{cells} = (X_{MÁXt}) - (Xt_0) / t - t_0 \quad (1)$$

Being P_{cells}: maximum growth productivity (cell formation) at the instant of time $(t - t_0)$ $(g.h^{-1})$;

$X_{MÁXt}$: maximum biomass formation at time t;

Xt_0: biomass formation at time t_0; t: time to reach the maximum biomass value;

t_0: initial time of cultivation.

The maximum pigment production was determined using equation 2, corresponding to the ratio of the difference between the maximum optical density unit obtained ($ODU_{MÁX}$) and the optical density unit obtained at time zero ($ODUt_0$) by the variation of time, being final time (t) and start time (t_0). (Eq. 2):

$$P_M = (ODU_{MÁXt}) - (ODUt_0) / t - t_0 \quad (2)$$

2.11 Statistical analysis

The results were submitted to Tukey's test, with a significance level of 5%, using the PAST 3.25 software, the graphs were constructed using Microsoft Excel®.

3. Results and Discussion

3.1 Description of *Amycolatopsis* sp. UFPEDA 3422 biochemical and physiological characteristics

The microorganism *Amycolatopsis* sp. UFPEDA 3422 is an aerobic, Gram-positive actinobacterium and its morphology presents branched mycelial structures that fragment into a rod shape. It has the ability to degrade starch, hypoxanthine, xylan, L-tyrosine, tween 20 and 80, gelatin 0.4% and 1%; uses Dextrin, D-trehalose, D-maltose, Adonitol and Mannose as a carbon source with acid production. In addition, it has an intermediate sensitivity profile to vancomycin (30 μg), sensitivity to Streptomycin (10 μg), resistance to Gentamicin (10 μg), Tobramycin (TOB 10), Nalidixic Acid (30 μg), Rifampicin (5 μg) and Cefoxitin (CFO 30).

The microorganism showed good growth in almost all culture medium evaluated (except in GAA and CAA medium), with variations in the development of aerial and substrate mycelium, exhibiting different tonalities in their colonies according to the substrate used (ISP-2, ISP-3, ISP-4, GAA, CAA, modified HT and ALA). The best growth of the microorganism was observed in ISP-2 and HT medium, the nutritional sources available in these substrates (glucose and yeast extract) were responsible for these changes. The aerial mycelium presented coloration reddish pink and pink to whitish salmon in HT medium at 37 ºC and 30 ºC respectively, and opaque pink (37 ºC) and opaque pink orange (30 ºC) in ISP-2 medium. The substrate mycelium of the microorganism showed a reddish pink (37 ºC) and an intense reddish pink (30 ºC) color in the HT culture medium, and in the ISP-2 medium a guava color was observed at both temperatures (Table 1).

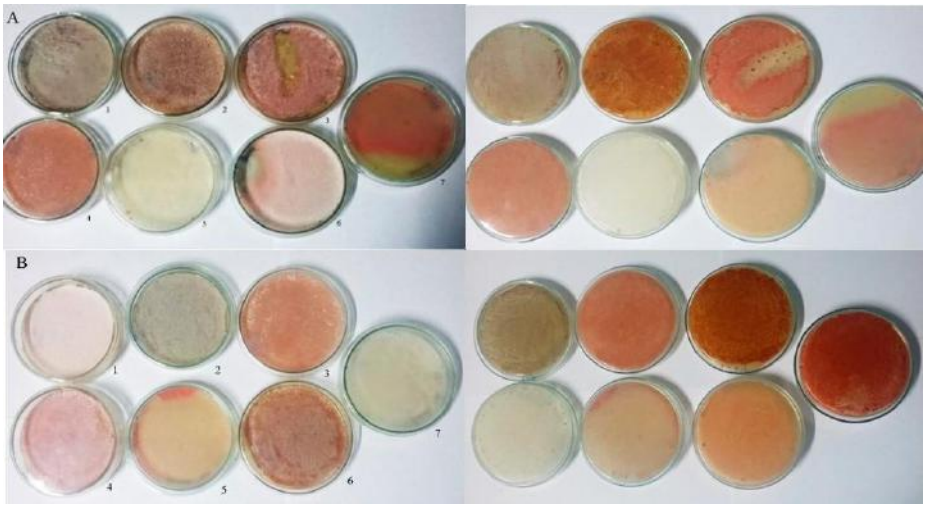
Table 1. Macromorphological characteristics of *Amycolatopsis* sp. UFPEDA 3422 with growth in ISP-2, ISP-3, ISP-4, GAA, CAA, modified HT and ALA culture medium grown at 30 ºC and 37 ºC.

Culture medium	ISP-2	ISP-3	ISP-4	GAA	CAA	HT	ALA
Growth:							
37 °C	Great	Good	Good	Scarce	Scarce	Great	Good
30 °C	Great	Good	Good	Scarce	Scarce	Great	Good
Mycelium Development:							
Aerial							
37 °C	Present	Present	Present	Scarce	Scarce	Present	Scarce
Substrate							
37 °C	Present	Present	Present	Present	Present	Present	Present
Aerial							
30 °C	Present	Sparce	Present	Scarce	Scarce	Present	Scarce
Substrate							
30 °C	Present	Present	Present	Present	Present	Present	Present
Mycelium color:							
Aerial 37 °C	Opaque pink	Guava	Whitish pink	Opaque white to beige	Opaque beige to pink	Reddish pink	Grayish white cotton
Substrate 37 °C	Reddish yellow	Guava	Reddish beige	Grayish white	Whitish pink to gray	Reddish pink	Gray
Aerial 30 °C	Orange opaque pink	Opaque pink	Whitish pink	White to beige	Pink	Pink to whitish Salmon	Grayish white cotton
Substrate 30 °C	Brown	Guava	Whitish pink to cream	Gray to white	Grayish pink	Intense reddish pink	Gray

Source: Authors.

The morphology and development of mycelia presented by the microorganism at 30 ºC and 37 ºC did not differ significantly (Figure 1). The coloration presented in the HT culture medium was the most intense the growth of the microorganism, being its composition favorable to the production of pigments. Thus, this substrate was selected for submerged fermentation with medium 400 (tested in previous trials).

Figure 1. Macromorphology of *Amycolatopsis* sp. UFPEDA 3422 in ALA culture media; 2 ISP-2; HT; ISP-3; GAA; ISP-4 and CAA. (A) Growth at 30°C: 1 – ALA; 2 – ISP-2; 3 – HT; 4- ISP-3; 5-GAA; 6 – ISP-4 and 7 – CAA. (B) Growth at 37 ºC: 1 – ALA; 2 – ISP-3; 3- ISP-2; 4- GAA; 5- CAA; 6 – ISP-4 and 7 – HT.



Source: Authors.

Amycolatopsis sp. UFPEDA 3422 also produces melanoid pigments, as observed in culture in ISP-6 and ISP-7 medium, this characteristic configures an important possibility of its application as a photoprotective agent, since melanin has been reported to have a strong self-protective action, including UV radiation blocking, free radical adsorption, toxic iron chelation, elimination of phenolic compounds and environmental stress, being popularly used in pharmacology and cosmetic development, with high potential as a bioactive compound (Celedón & Diaz, 2021).

The ease of growth of the microorganism in different culture medium is a positive factor for its use as a pigment producer, since for the industrial production of pigments it is desirable that the microorganism has properties such as acceptability of a wide variety of substrates, varied sources of carbon and nitrogen, tolerate temperature and pH oscillations, in addition to presenting high production yield, and the possibility of optimization of cultural conditions is one of the great advantages associated with pigment production by these organisms (Kumar et al., 2015; Keekan etal., 2020).

The results presented reveal high pigment production capacity by *Amycolatopsis* sp. UFPEDA 3422, related to the source of nutrients used during cultivation, which may indicate ways to increase the production of pigments using the sources mentioned as substrate.

3.2 Influence of temperature and pH on microorganism growth

The microorganism grows at temperatures of 25 ºC, 37 ºC and 45 ºC, showing well-developed aerial mycelium and substrate mycelium with a red color at 37 ºC, however it does not develop at 4 ºC. The possibility of growth at high temperatures demonstrates the microorganism's easy adaptation to oscillations in growing conditions, showing that thermal plasticity is a good condition for optimizing the growth process.

Growth at 45 ºC also enables the obtainment of heat shock metabolites (HSM) which are metabolites encoded by "dormant" genes that are reactivated when subjected to high temperatures (Saito et al., 2020). This new category of metabolites opens up a new field of study for the extraction and production of substances from microorganisms. The optimum pH for growth of the microorganism was 4.5 with well-developed aerial mycelia being observed, with colony growth whose back was reddish-orange and the back was white to light pink. Good growth was also evidenced at pH 9.5, although with less development of mycelia, the colonies presented verse in tonality reddish-brown and the reverse with pink coloration. This result differs from that reported by Manikkam et al., (2015) who, in a study with *Streptomyces* sp, found that pH values between 7 and 11 provided better microorganism growth and greater pigment production, while at pH 5 this development was harmed, with no growth at all.

For some microorganisms, pH acts as a metabolic activation factor so that changes that cause oscillations in this parameter produce metabolic stress and can favor the production of metabolites, including pigments. The organism's tolerance to pH variations provides the possibility of optimizing the fermentation process, as well as helping to recognize the microorganism's cultivation characteristics. Actinobacteria that have the ability to grow at low pH are considered acidophilic and can be divided into two groups: neutrotolerant acidophilic (optimal growth between pH 5.0 and 5.5) and strict acidophilic (which shows optimal growth at pH 4.5). Microorganisms isolated from soil in arid regions usually have a low pH tolerance profile. *Amycolatopsis* sp. was isolated from the rhizospheric soil of the Caatinga, and thus presents resistance to environmental stresses. The adversity of the Caatinga biome is a source of great biodiversity still unexplored and secondary metabolites (eg. biopigments) produced by prospected microorganisms in this region demonstrate great biotechnological and medical potential (Rodrigues et al., 2018).

3.3 Influence of NaCl concentration on microorganism growth

Amycolatopsis sp. UFPEDA 3422 shows good growth in the presence of 1% and 3% of sodium chloride (NaCl).

Concentrations of 5% and 7% of NaCl harm the growth of the microorganism, with no development of aerial mycelium, only substrate mycelium with colors ranging from cream to colorless. At a concentration of 10% of the salt the microorganism does not develop. At the concentration of 1% of NaCl, the colonies have a red color and the concentration of 3% of the salt resulted in colonies with an intense red wine color. This result corroborates what was observed by Manikkam et al., (2015) where NaCl concentrations below 7.5 influenced the growth and production of pigments by *Streptomyces* sp. D25. Abraham and Chauhan (2018), identified an isolate of *Streptomyces* sp. JAR6, which grows in the presence of 5% NaCl and tolerates the presence of up to 10% salt. This behavior may be associated with the hyperosmotic effect in bacteria, where osmotic shock may have led to the suppression of microorganism growth in high salt contractions. Subramanian and Gurunathan (2020), observed that Cell biomass was affected with 10% NaCl (w/v), however, the pigment production of microorganisms improved under the effect of salt.

In an osmotic stress scenario, when the salt content inside and outside the cell is out of balance, the bacteria activates mechanisms to maintain turgor and hydration, triggering responses at the genomic (gene transcription) and protein levels. The activation of these mechanisms can, therefore, influence the production of secondary metabolites, such as pigments in pigmented microorganisms.

Information on NaCl tolerance provides data on the physiology of the microorganism and the production of metabolites by them, since salt stress is one of the factors that most affect colony development and that the production of metabolites is generally related to a response to environmental stresses (Subramanian & Gurunathan, 2020).

The importance of NaCl concentration, therefore, can be seen in preserving the characteristic color of the strain. That is, if the concentration is adequate, the color of the colony will be favored and, consequently, the extracted pigment will have the desired characteristic color, thus the salt concentration may represent a positive factor for the production of pigments by the micro-organism.

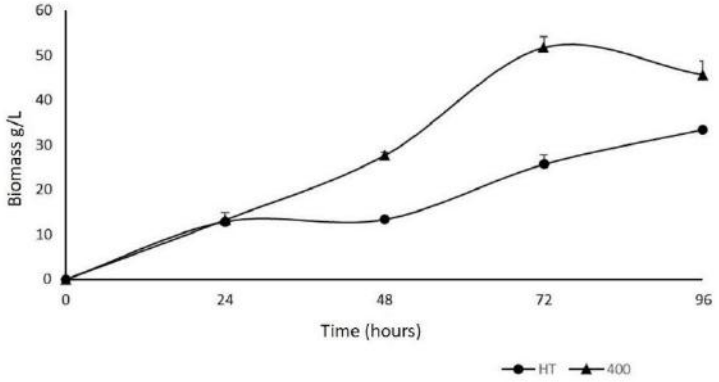
3.4 Identification based on 16S rDNA region

The 941 bp partial sequence was sent to GenBank and assigned an accession number of OK649762. The result of the phylogenetic analysis of the 16S rRNA gene of *Amycolatopsis* sp. UFPEDA 3422 demonstrates a 97.4% similarity with other *Amycolatopsis* present in the database (NCBI). The application of taxonomy and genomic screening techniques has shown that these actinobacteria are significant sources of metabolites of interest, being observed in some exemplary stress-related genes and the ability to adapt to extreme environmental conditions (Sayed et al., 2019).

3.5 Fermentation Assay, pigment extraction and evaluation of kinetic parameters

The highest biomass production was evidenced in the exponential growth phase of the microorganism, 51.6 g/L after 72 hours in 400 culture medium, corresponding to a maximum cell production of 0.035 $g.h^{-1}$ and 33.4 g/L after 96 hours in HT medium, corresponding to 0.017 $g.h^{-1}$ (Figure 2). Cellular biomass from fermentation in HT medium has a wine-red color while biomass produced in 400 medium has a pinkish tonality. The pigment produced by *Amycolatopsis* sp. is obtained from the discoloration of its cells, so the quantification of the biomass and the identification of the color produced by it are important data for the evaluation of the concentration of pigments produced.

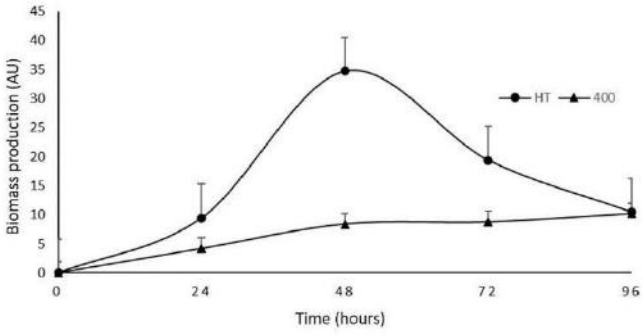
Figure 2. Biomass production by *Amycolatopsis* sp. UFPEDA 3422 in HT culture media and 400 in submerged fermentation at 150 rpm, 37 ºC. Demonstrating maximum biomass production peak obtained in 400 media after 72 hours of fermentation and in HT media after 96 hours of fermentation.



Source: Authors.

The production of pigments by the microorganism was significantly higher in the HT culture medium. The extracted pigment showed maximum absorbance peak in the region of 540 nm approximately with shoulder at 470 nm in the visible UV spectrophotometer. The maximum production of pigments evidenced in these peaks was equivalent to 34.9 $AU_{540\ nm}$ (0.72 $ODU.h^{-1}$) and 45.9 $AU_{470\ nm}$ (0.95 $ODU.h^{-1}$) in 48h, 37 ºC in the medium HT. On the other hand, in medium 400, 8.4 $AU_{540\ nm}$ (0.17 $ODU.h^{-1}$) and 13.1 $AU_{470\ nm}$ (0.27 $ODU.h^{-1}$) were obtained under the same culture conditions (Figure 3). Absorbances between 380-490 nm correspond to yellow to orange coloration, while absorption between 490-595 corresponds to red coloration (Heo et al., 2018), thus, the pigment produced by the *Amycolatopsis* sp. isolate can be described as red. Red natural dyes are part of the group of the most used in the food industry and are often associated with classes of carotenoids, anthocyanins and betacyanins, which have a wide range of representatives (Sen et al., 2019). The red pigment has many biotechnology applications, and changes in formulation or composition of culture medium can provide, in addition to greater stability, increased production yield and variety of shades, making it even more attractive to the food industry (Keekan et al., 2020). Red biodyes are especially important since restrictions on the use of synthetic dyes have affected the color palette used in this market.

Figure 3. Pigment production by *Amycolatopsis* sp. UFPEDA 3422 in HT and 400 culture media in submerged fermentation at 150 rpm, 37 ºC, at a wavelength of 540 nm. Demonstrating maximum pigment production peak in HT media after 48 hours of fermentation.



Source: Authors.

The production of biomass and secondary metabolites by actinobacteria is influenced by the type, concentration of nitrogen source, carbon source and mineral composition of the culture medium, where carbon sources such as glucose, glycerol

and saccharose are rapidly used for the synthesis of cellular material (Abraham & Chauhan 2018). Furthermore, culture medium that have greater amounts of soluble components tend to favor cell growth, however this growth can modify the viscosity of the medium (Zhou et al., 2018). Thus, the soluble starch present in the 400 medium may have favored the development of the microorganism's biomass (20g soluble starch, 10g glucose, 3g meat extract, 3g peptone, 5g extract of yeast and 3g of $CaCO_3$), when compared to the modified HT medium (1 g of yeast extract, 1 g of meat extract, 10 g of dextrose, 2 g of tryptone, $CaCl_2 7H_2O$). Koim-Puchowska et al., (2021) also the observed the influence of the carbon source on the development of microorganism biomass, observing better cell mass development in the cultivation carried out with soluble starch.

Red pigment production by *Amycolatopsis* sp. UFPEDA 3422, however, was positively influenced by the presence of glucose as a carbon source, with greater cell pigmentation being observed in the biomass produced in the HT medium, a result that corroborates what was presented by Manikkam et al., (2015) in evaluating the effects of the components of culture medium on pigment production by *Streptomyces* sp. D25.

However, studies have shown that although the concentration of glucose is commonly related to a significant increase in cell biomass, it also leads to an increase in the production of red pigments because it is more easily assimilated, but when in high concentrations it has a negative effect on the production of antimicrobial metabolites (Keekan et al., 2020).

Thus, the differences observed between the production of biomass and pigments in HT and 400 medium can be correlated to the different nutritional sources used in each substrate, as these are limiting factors to the growth of the microorganism as well as aeration, movement of microelements, between others. Variations in the composition of the culture medium also imply changes in oxygen availability. Dissolved oxygen in the culture is limited by the specific absorption rate by the cells and is influenced by factors such as viscosity, surface tension, solute concentration and microorganism morphology (Zhou et al., 2018) and, therefore, the productivity of metabolites closely related to the fermentation process, including the nutrients involved and their cultivation conditions with a reduction in the production of microbial metabolites, including pigments and in the production of biomass when the agitation rate is reduced (Venil et al., 2020).

The presence of a smaller amount of biomass associated with a high production of pigments can be explained by the difference in cell structure during fermentation in each medium, that is, the fact that the microorganism has developed more easily in HT medium, forming well-developed cells with larger size, but in few units. On the other hand, the cell mass observed in the 400 medium showed less mycelial development, but a greater number of units, which increases its weight value. In this way, the production of pigments can be controlled by the limitation of the substrate, and the composition of pigments depends on the available nutrients, such as a source of nitrogen and the use of specific strains (Keekan et al., 2020).

The production of pigments by microorganisms isolated from the soil, such as actinobacteria, can confer an evolutionary advantage, since these organisms are constantly exposed to environmental and nutritional stress (Abraham & Chauhan, 2018). Some of these pigments also provide protection against oxidative damage (Rana et al., 2021). The use of natural pigments with antioxidant properties in food materials adds value to them, as they also increase their nutritional value and provide greater acceptability.

Vendruscolo et al., (2013) in a similar work, with research and production of pigments extracted from the biomass of *Monascus ruber* (a fungus widely used for the production of natural pigments) observed the formation of peaks in the region of 470 nm corresponding to an orange coloration and another peak of 510 nm corresponding to a red coloration. Parmar and Singh (2018) characterized a pigment produced by *Streptomyces*, whose absorbance peak was 277.44 nm. Bühler et al., (2013), in an experiment with *Monascus ruber*, using a bioreactor, achieved maximum red pigment production of 8.28 $AU_{510\,nm}$ after 60 hours of cultivation and 7.26 $AU_{470\,nm}$ of orange pigment after 72 hours of cultivation. Heo et al., (2018) in an investigation of the production of pigments by fungal strains obtained maximum production of red pigments equivalent to approximately 3.5 AU. Bühler et al., (2015) in evaluating the influence of light on pigment production by *Monascus ruber* achieved maximum red

pigment production of 8.32 AU and biomass of 8.82 $g.L^{-1}$. Terán Hilares et al., (2018) in a study of the production of red pigments by *Monascus ruber* from sugarcane bagasse hydrolyzate obtained a maximum production of 18.71 $AU_{490\ nm}$, with glucose being metabolized. The differences in wavelengths observed may be related to the structure of the constituent molecules of the pigment.

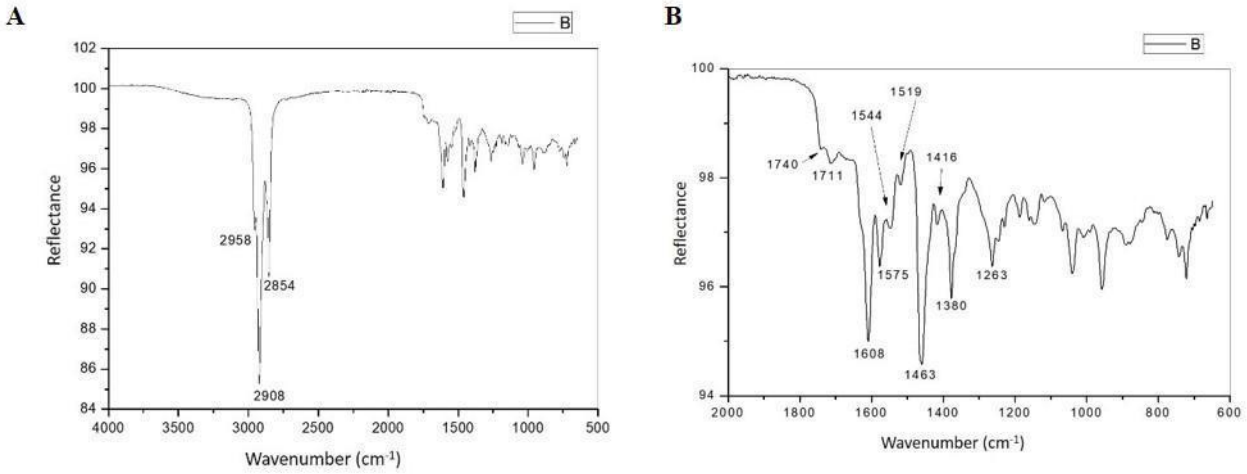
The results obtained contribute to the identification of the HT culture medium as a viable alternative for the production of the red pigment by *Amycolatopsis* sp. UFPEDA 3422, as this allows for a greater production of the pigment. The selection of the best result substrate and the identification of cultivation parameters favor the optimization of production and consequently benefit the obtainment of metabolites on a large scale, making it attractive for commercial use, including in the food industry, in which large amounts of pigments are used (Keekan et al., 2020).

Secondary metabolites of microbial origin have been related to a large number of molecules of industrial interest that are not always easily characterized, but which may have, among other properties, conserved domains typical of enzymes chemically characterized as polyketides, which have biological activities (PKS), non-ribosomal peptides (NRPS), terpenes and other molecules of interest, which indicates a great biotechnological potential (Challis, 2014).

3.6 FTIR Analysis

The infrared spectrum of the raw pigment produced by *Amycolatopsis* sp. was analyzed and the results suggest the following peak assignments: 2958 cm^{-1}, 2908 cm^{-1} characteristic of the presence of alkyl grouping (3000–2800 cm^{-1}), 2950 cm^{-1} characteristic of elongations of the CH bond (El-Naggar & El-Ewasy, 2017), 2854 cm^{-1} corresponding to the C-C bond (Asnani et al., 2016), 1740 cm^{-1} corresponds to the C=O ester bond (Asnani et al., 2016). In addition to these, signals were also presented in the region of 1711 cm^{-1} corresponding to C=O ketone bond (Asnani et al., 2016), 1608 cm^{-1}, 1575 cm^{-1} (1550-1640 region) corresponds to secondary NH bond (Asnani et al., 2016). 1544 cm^{-1} corresponding to the presence of NH in the flexion mode (El-Naggar & El-Ewasy, 2017). Deformation was also observed at 1519 cm^{-1}, 1463 cm^{-1} corresponding to $-CH_2-$ bond (Asnani et al., 2016), 1416 cm^{-1} corresponding to α-CH_2 (aldehyde) or can be due to aliphatic C-H groups (El-Naggar & El-Ewasy, 2017); 1380 cm^{-1} referring to the C-O grouping (Metwally et al., 2017) and 1263 cm^{-1}. Peaks in the region from 1243 to 1305 indicate the existence of C-O anhydrous groupings (El-Naggar & El-Ewasy, 2017). Figure 4 shows the infrared spectra of the pigment. The presence of the peak close to 1750 cm^{-1} may result from the oxidation of lycopene/zeaxanthin or lutein and may result in the formation of carotenoids such as astaxanthin and canthaxanthin (red/orange ketocarotenoids). The existence of the $-CH_2-$ radical was also reported in red pigment produced by *Bacillus subtilis*. This radical is observed in β-carotene, being standard in the region of 1450.68 cm^{-1} (Trivedi et al., 2017). The ketone functional group and the presence of hydroxyl are described as the main groups for the identification of the pigment astaxanthin, in addition the methyl and alkene groups may be related to the esters in this pigment (Elumalai et al., 2014). The methyl group found in the peak of 1385 cm^{-1} would be responsible for the umbrella shape in the aliphatic chain and aromatic rings in one of the forms presented by this type of pigment (astaxanthin). This information provides important data about the pigment, such as the main functional groups present in its structure, but it is not enough for the total elucidation of the molecule.

Figure 4. Infrared spectrum of the pigment (raw extract) produced by *Amycolatopsis* sp. spectrum (A) and detailed peaks (B).



Source: Authors.

4. Conclusion

Amycolatopsis has a great potential for the production of natural pigments to replace synthetic pigments. Although little is reported in the literature, the microorganism offers new possibilities for obtaining pigments of industrial interest and has attractive characteristics for the food coloring, textile, cosmetic and pharmaceutical market, such as easy obtainment, higher yield and production that can be cheaper. The cultivation facilities presented by the microorganism, such as tolerance to physicochemical variations and growth on varied substrates, allow its easy manipulation and large-scale production. Considering that red biopigments are attractive to the industry due to its wide range of applications in the market and its ecological appeal, the results obtained in the production of pigments by *Amycolatopsis* sp UFPEDA 3422 place it as a potential source for obtaining these compounds. The isolate produces large amounts of pigment compared to those obtained using other microorganisms including fungi, algae and other widely studied vegetables, according to data provided in the literature.

Thus, *Amycolatopsis* sp UFPEDA 3422 represents a promising source for obtaining biopigments. However, complementary studies regarding toxicity and other parameters established by the legislation of the market of interest are necessary, aiming at its suitability and consumer safety. In addition, studies to optimize the production process can favor the obtaining of these biopigments, increasing their yield and lowering their cost.

Acknowledgments

The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Abraham, J., & Chauhan, R. (2018). Profiling of red pigment produced by *Streptomyces* sp. JAR6 and its bioactivity. *3 Biotech*, 8(1), 1-9. https://doi.org/10.1007/s13205-017-1044-7.

Asnani, A., Ryandini, D., & Suwandri. (2016). Screening of Marine Actinomycetes from Segara Anakan for Natural Pigment and Hydrolytic Activities. *IOP Conference Series: Materials Science and Engineering*, 107(1), 0–9. https://doi.org/10.1088/1757-899X/107/1/012056.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, 45, 493–496.

Bauermeister, A., Calil, F. A., das CL Pinto, F., Medeiros, T. C., Almeida, L. C., Silva, L. J., de Melo, I. S., Zucchi, T. D., Costa-Lotufo, L. V., & Moraes, L. A. (2019). Pradimicin-IRD from *Amycolatopsis* sp. IRD-009 and its antimicrobial and cytotoxic activities. *Natural product research*, 33(12), 1713-1720, https://doi.org/10.1080/14786419.2018.1434639.

Bühler, R. M. M., Dutra, A. C., Vendruscolo, F., Moritz, D. E., & Ninow, J. L. (2013). *Monascus* pigment production in bioreactor using a co-product of biodiesel as substrate. *Ciência e Tecnologia de Alimentos*, 33, 9–13. https://doi.org/10.1590/s0101-20612013000500002

Bühler, R. M. M., Müller, B. L., Moritz, D. E., Vendruscolo, F., de Oliveira, D., & Ninow, J. L. (2015). Influence of light intensity on growth and pigment production by *Monascus ruber* in submerged fermentation. *Applied Biochemistry and Biotechnology*, 176(5), 1277–1289. https://doi.org/10.1007/s12010-015-1645-8.

Celedón, R. S., & Díaz, L. B. (2021). Natural Pigments of Bacterial Origin and Their Possible Biomedical Applications. *Microorganisms*, 9(4), 739. https://doi.org/10.3390/microorganisms9040739.

Challis, G. L. (2014). Exploitation of the *Streptomyces coelicolor* A3(2) genome sequence for discovery of new natural products and biosynthetic pathways. *Journal of Industrial Microbiology and Biotechnology*, 41(2), 219–232. https://doi.org/10.1007/s10295-013-1383-2.

Chatragadda, R., & Dufossé, L. (2021). Ecological and Biotechnological Aspects of Pigmented Microbes: A Way Forward in Development of Food and Pharmaceutical Grade Pigments. *Microorganisms*, 9(3), 637. https://doi.org/10.3390/microorganisms9030637.

Clinical and Laboratory Standards Institute (CLSI). (2017). Performance Standards for Antimicrobial Susceptibility Testing, 27th Edition. *Clinical and Laboratory Standards Institute*, Wayne, PA.

El-Naggar, N. E. A., & El-Ewasy, S. M. (2017). Bioproduction, characterization, anticancer and antioxidant activities of extracellular melanin pigment produced by newly isolated microbial cell factories *Streptomyces glaucescens* NEAE-H. *Scientific reports*, 7(1), 1-19. https://doi.org/10.1038/srep42129.

Elumalai, S., Santhose, B. I., & Kanna, G. R. (2014). Extraction of carotenoid and thin layer chromatography (TLC), GC-MS, FT-IR and HPLC analysis of pharmaceutically important pigment astaxanthin from a new strain of *Haematococcus pluvialis*. *Weekly Science Research Journal*, 2(8), 2321-7871. https://doi.org/10.9780/ 2321-7871/1202013/53

Goodfellow, M., Kämpfer, P., Busse, H. J., Trujillo, M. E., Suzuki, K. I., Ludwig, W., & Whitman, W. B. (2012). *Bergey's Manual® of Sistematic Bacteriology: Volume Five The Actinobacteria.* (Part A). ISBN: 978-1-4939-7916-5, New York: Springer.

Gordon, R. E., Barnett, D. A., Handerhan, J. E., & Pang, C. H. (1974). *Nocardia coeliaca, Nocardia autotrophica* and the Nocardin Strain. *International Journal of Systematic and Evolutionary Microbiology*, 24, 54-63, https://doi.org/10.1099/00207713-24-1-54.

Heo, Y. M., Kim, K., Kwon, S. L., Na, J., Lee, H., Jang, S., & Kim, J. J. (2018). Investigation of filamentous fungi producing safe, functional water-soluble pigments. *Mycobiology*, 46(3), 269–277. https://doi.org/10.1080/12298093.2018.1513114.

Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *Journal of bacteriology*, 57(2), 141-145, https://doi.org/10.1128/jb.57.2.141-145.1949.

Keekan, K. K., Hallur, S., Modi, P. K., & Shastry, R. P. (2020). Antioxidant activity and role of culture condition in the optimization of red pigment production by *Talaromyces purpureogenus* KKP through response surface methodology. *Current microbiology*, 77(8), 1780-1789. https://doi.org/10.1007/s00284-020-01995-4.

Koim-Puchowska, B., Kłosowski, G., Dróżdż-Afelt, J. M., Mikulski, D., & Zielińska, A. (2021). Influence of the medium composition and the culture conditions on surfactin biosynthesis by a native *Bacillus subtilis* natto BS19 strain. *Molecules*, 26(10), 2985. https://doi.org/10.3390/molecules26102985.

Kumar, A., Vishwakarma, H. S., Singh, J., Dwivedi, S., & Kumar, M. (2015). Microbial Pigments: Production And Their Applications In Various Industries. *International Journal of Pharmaceutical, Chemical & Biological Sciences*, 5(1). 203-212.

Lyra, F. D. A., Gonçalves de Lima, O., Coelho, J. S. B., Albuquerque, M. M. F., Maciel, G. M., Oliveira, L. L., & Maciel, M. C.N. (1964). Ciclamicina e ciclacidina: dois novos antibióticos produzidos pelo *Streptomyces capoamus* nov. sp. *Anais da Academia Brasileira de Ciências* 36(3), 323-334.

Manikkam, R., Venugopal, G., Ramasamy, B., & Kumar, V. (2015). Effect of critical medium components and culture conditions on antitubercular pigment production from novel *Streptomyces* sp D25 isolated from Thar desert, Rajasthan. *Journal of Applied Pharmaceutical Science*, 5(6), 015–019. https://doi.org/10.7324/JAPS.2015.50603

Metwally, R. A., El-Sersy, N. A., El Sikaily, A., Ghozlan, H. A., & Sabry, S. A. (2017). Statistical optimization and characterization of prodigiosin from a marine *Serratia rubidaea* RAM-Alex. *Journal of Pure and Applied Microbiology*, 11(3), 1259–1266. https://doi.org/10.22207/JPAM.11.3.04

Parmar, R. S., & Singh, C. (2018). A comprehensive study of eco-friendly natural pigment and its applications. *Biochemistry and Biophysics Reports*, 13, 22–26. https://doi.org/10.1016/j.bbrep.2017.11.002.

Ramesh, C., Vinithkumar, N. V., Kirubagaran, R., Venil, C. K., & Dufossé, L. (2019). Multifaceted applications of microbial pigments: current knowledge, challenges and future directions for public health implications. *Microorganisms*, 7(7), 186. https://doi.org/10.3390/microorganisms7070186.

Rana, B., Bhattacharyya, M., Patni, B., Arya, M., & Joshi, G. K. (2021). The Realm of Microbial Pigments in the Food Color Market. *Frontiers in Sustainable Food Systems*, 5, 54 1-14. https://doi.org/10.3389/fsufs.2021.603892.

Rodrigues, J. P., Prova, S. S., Moraes, L. A. B., & Ifa, D. R. (2018). Characterization and mapping of secondary metabolites of *Streptomyces* sp. from caatinga by desorption electrospray ionization mass spectrometry (DESI–MS). *Analytical and bioanalytical chemistry*, 410(27), 7135-7144. https://doi.org/10.1007/s00216-018-1315-0.

Saito, S., Kato, W., Ikeda, H., Katsuyama, Y., Ohnishi, Y., & Imoto, M. (2020). Discovery of "heat shock metabolites" produced by thermotolerant actinomycetes in high-temperature culture. *The Journal of antibiotics*, 73(4), 203-210, https://doi.org/10.1038/s41429-020-0279-4.

Sayed, A. M., Hassan, M. H., Alhadrami, H. A., Hassan, H. M., Goodfellow, M., & Rateb, M. E. (2020). Extreme environments: microbiology leading to specialized metabolites. *Journal of applied microbiology*, 128(3), 630-657, https://doi.org/10.1111/jam.14386.

Sen, T., Barrow, C. J., & Deshmukh, S. K. (2019). Microbial Pigments in the Food Industry—Challenges and the Way Forward. *Frontiers in Nutrition*, 6(March), 1–14. https://doi.org/10.3389/fnut.2019.00007.

Sharma, P., Singh, T. A., Bharat, B., Bhasin, S., & Modi, H. A. (2018). Approach towards different fermentative techniques for the production of bioactive actinobacterial melanin. *Beni-Suef University journal of basic and applied sciences*, 7(4), 695-700. https://doi.org/10.1016/j.bjbas.2018.08.002.

Shirling, E. B., Gottlieb, D. (1996). Methods for characterization of *Streptomyces* species. *International Journal of Systematic and Evolutionary Microbiology*, 16(3), 313-340. https://doi: 10.1099/00207713-16-3-313.

Sierra, G. (1957) A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substrates. *Antonie van Leeuwenhoek*, 23, 15-22, https://doi.org/10.1007/BF02545855.

Subramanian, P., & Gurunathan, J. (2020). Differential Production of Pigments by Halophilic Bacteria Under the Effect of Salt and Evaluation of Their Antioxidant Activity. *Applied biochemistry and biotechnology*, 190(2), 391-409. https://doi.org/10.1007/s12010-019-03107-w.

Tang, X., Zhao, J., Li, K., Chen, Z., Sun, Y., & Gao, J. (2019). *Streptomyces cyaneochromogenes* sp. nov., a blue pigment-producing actinomycete from manganese-contaminated soil. *International journal of systematic and evolutionary microbiology*, 69(8), 2202-2207, https://doi.org/10.1099/ijsem.0.003406.

Terán Hilares, R., de Souza, R. A., Marcelino, P. F., da Silva, S. S., Dragone, G., Mussatto, S. I., & Santos, J. C. (2018). Sugarcane bagasse hydrolysate as a potential feedstock for red pigment production by *Monascus ruber*. *Food Chemistry*, 245(May 2017), 786–791. https://doi.org/10.1016/j.foodchem.2017.11.111.

Trivedi, N., Tandon, S., & Dubey, A. (2017). Fourier transform infrared spectroscopy (FTIR) profiling of red pigment produced by *Bacillus subtilis* PD5. *African Journal of Biotechnology*, 16(27), 1507-1512. https://doi.org/10.5897/AJB2017.15959.

Vendruscolo, F., Luise Müller, B., Esteves Moritz, D., De Oliveira, D., Schmidell, W., & Luiz Ninow, J. (2013). Thermal stability of natural pigments produced by *Monascus ruber* in submerged fermentation. *Biocatalysis and Agricultural Biotechnology*, 2(3), 278–284. https://doi.org/10.1016/j.bcab.2013.03.008

Venil, C. K., Dufossé, L., & Renuka Devi, P. (2020). Bacterial pigments: sustainable compounds with market potential for pharma and food industry. *Frontiers in Sustainable Food Systems*, 4, 100, https://doi.org/10.3389/fsufs.2020.00100.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of bacteriology*, 173(2), 697-703, https://doi.org/10.1128/jb.173.2.697-703.1991.

Zhou, Y., Han, L. R., He, H. W., Sang, B., Yu, D. L., Feng, J. T., & Zhang, X. (2018). Effects of agitation, aeration and temperature on production of a novel glycoprotein GP-1 by *Streptomyces kanasenisi* ZX01 and scale-up based on volumetric oxygen transfer coefficient. *Molecules*, 23(1), 125, 1-14. https://doi.org/10.3390/molecules23010125.