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Article

Exploration of the Bioactivity of Pigmented Extracts from *Streptomyces* Strains Isolated along the Banks of the Guaviare and Arauca Rivers (Colombia)

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Abstract: Pigments are chemical compounds that impart color to materials via absorption, reflection, and refraction. Traditional sources of natural pigments include plant and insect tissues; however, microorganisms such as bacteria, yeasts, algae, and filamentous fungi also hold potential for pigment production. In this study, we investigated pigment production in 20 isolates from our in-house Actinobacteria strain collection, obtained from the banks of the Guaviare and Arauca Rivers in Colombia. Isolates were identified using 16S rRNA gene sequencing. We evaluated different culture media and determined their various bioactivities, including antioxidant, antibacterial, and cytotoxic properties. Promising pigmented hydroalcoholic extracts exhibiting the above-mentioned properties were identified and their chemical compositions were examined using LC-MS. Our findings highlighted a growth medium-dependent effect, as pigment production, coloration, and bioactivity outcomes were influenced by growth media. These results demonstrate the significant potential of *Streptomyces* isolates as sources of bioactive pigments for diverse applications.

Keywords: *Streptomyces*; antibacterial; pigment; antioxidant; cytotoxic

1. Introduction

Pigments are colored compounds that should not be physically or chemically affected by the medium in which they are incorporated [1], and have been used since ancient times by civilizations such as China, India, and Egypt [2]. Currently, they are widely employed in various industries, including food, textiles, paints, cosmetics, pharmaceuticals, and plastics [3]. The global market for natural pigments in the food industry is estimated to reach \$2.5 billion by 2025 [2].

Pigments can be classified, according to their origin, as natural or synthetic. Natural pigments are further categorized based on their specific origin (plant, mineral, microbial, or animal), color formation, chemical constitution, and application method. Examples of natural pigments include carotenoids, melanins, chlorophylls, flavonoids, anthocyanins, betalains and quinones. Synthetic pigments, on the other hand, are generally classified into cationic, anionic, and nonionic types, and by their applications as direct, acidic, basic, chrome mordants, azo, sulfur, reactive, and dispersed [4].

Despite their origins, pigments can be harmless or toxic to some degree. However, consumers often prefer natural pigments because they are considered safe, non-toxic, non-carcinogenic, and biodegradable [5]. In contrast, synthetic pigments can pose serious human health hazards and environmental risks [3,6] owing to their non-biodegradable nature [7]. They can cause adverse effects [8] such as decreased light penetration in water bodies [9] and increased chemical and biochemical

oxygen demand (BOD and COD), and some are bioaccumulative, toxic, mutagenic, or carcinogenic [10] even at very low concentrations.

Natural pigment production is widespread among plants, microalgae, fungi, bacteria, and insects. Among bacterial groups, pigment production is particularly notable in actinobacteria [11], which is highly profitable and biotechnologically valuable [12,13]. Actinobacteria produce metabolites with unique chemical structures and diverse bioactivities, including antibiotics, biopesticides, phytohormones, antitumor compounds, antiviral agents, enzymes, enzyme inhibitors, anti-inflammatory agents, and biosurfactants [14–16]. *Streptomyces*, in particular, is known to produce a large number of specialized metabolites with different properties [13,17–19]. In this regard, several genera such as *Streptomyces*, *Nocardia*, *Micromonospora*, *Thermomonospora*, *Actinoplanes*, *Microbispora*, *Streptosporangium*, *Actinomadura*, *Rhodococcus*, and *Kitasatospora* [20] include microorganisms that produce a wide variety of pigments. The most reported pigment-producing genus is *Streptomyces*, including species such as *S. griseus*, *S. griseoviridis*, *S. coelicolor* [21,22], *S. cyaneus* [23], *S. vietnamensis* [24], *S. echinoruber* [25], *S. shaanxiensis* [26], and *S. caeruleatus* [27].

Microbial pigments are emerging as alternatives to synthetic pigments, offering several advantages such as abundant raw materials independent of season, high yields, higher concentrations with simpler purification processes, regulatory approval, high stability to environmental factors [6,28–31], and biodegradability [32]. Additionally, some microbial pigments possess antioxidant, anti-inflammatory, anticancer, and antimicrobial properties [30,33] with potential applications in food colorants, nutraceuticals, and cosmetics [32].

Actinobacteria are widely distributed in diverse natural ecosystems, including soil, plants, limestone, fresh and seawater, sponges, volcanic caves, deserts, air, and insects [16]. Studies of actinobacteria from unexplored or underexploited habitats as new sources of specialized metabolites have increased [13]. Colombia, known for its megadiversity in fauna and flora, also exhibits a rich diversity of microorganisms [34].

Given this background, this study evaluated the bioactive potential of pigmented extracts from *Streptomyces* isolated from the Guaviare and Arauca rivers of Colombia. Hence, pigmented ethanolic extracts were prepared and evaluated for their antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, antibacterial activity by disc diffusion assay, and cytotoxic activity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against human dermal fibroblasts (HDFa), cervical-uterine cancer (HeLa), and MCF-7 (breast cancer) cell lines. Therefore, the aim of this study was to explore and characterize the bioactive properties of pigmented extracts of selected *Streptomyces* strains isolated from these unique Colombian habitats. By assessing their antioxidant, antibacterial, and cytotoxic activities, this study seeks to identify promising sources of natural pigments with potential applications in various industries, particularly cosmetics and pharmaceuticals. This study contributes to the growing field of natural product chemistry and highlights the significance of Actinobacteria as a valuable resource for biotechnological advancements.

2. Materials and Methods

2.1. Reagents and Culture Media

The components and culture media used were as follows: agar (DIBICO, Sta. Cruz Mexico), starch (Carlo Erba, Eure, France), glucose, CaCO_3 , K_2HPO_4 , $(\text{NH}_4)_2\text{SO}_4$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, KNO_3 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (PanReac, Barcelona Spain), yeast extract (OXOID, Hampshire UK), malt extract (CDH, New Delhi India), NaCl (Honeywell, Seelze Germany), KH_2PO_4 (DUKSAN, Kyungkido Korea), and TSA (Criterion, California USA). DPPH, ABTS, trolox, methanol, and ethanol were purchased from MilliporeSigma (St. Louis, MO, USA). MTT was acquired from Thermo Fisher Scientific Inc. (Waltham, MA USA).

2.2. Strains, Media and Culture Conditions

The Bioprospecting Research Group at Universidad de La Sabana has a collection including 380 isolates from sediments of the Guaviare riverbank and 780 isolates from sediments of the Arauca riverbank with *Streptomyces*-like morphological characteristics. These isolates have been cultured, isolated, and cryogenically preserved at -80°C in ISP2 medium. The colored isolates (20 isolates) were grown in four different culture media (see Table 1) at pH 7, in an incubator at 30°C for 7 days (Friocell, Querétaro, Mexico).

Table 1. Composition of the culture media used (g/L) [35–37].

Composition/Medium	ISP2	ISP4	modified ISP9	Starch nitrate
agar	15	15	15	15
starch	0	10	0	20
CaCO ₃	0	2	0	3
CuSO ₄ ·5H ₂ O	0	0	0.00064	0
yeast extract	4	0	0	0
malt extract	10	0	0	0
FeSO ₄ ·7H ₂ O	0	0.01	0.00011	0.01
glucose	4	0	10	0
K ₂ HPO ₄	0	1	5.65	1
KH ₂ PO ₄	0	0	2.38	0
KNO ₃	0	0	0	2
MgSO ₄ ·7H ₂ O	0	1	1	0.5
MnCl ₂ ·4H ₂ O	0	0.001	0.00079	0
NaCl	0	0.5	0	0
(NH ₄) ₂ SO ₄	0	2	2.64	0
ZnSO ₄ ·7H ₂ O	0	0.001	0.00015	1

The pathogens employed were *Klebsiella pneumoniae* ATCC11486, *Escherichia coli* ATCCBAA2469, *Pseudomonas aeruginosa* ATCC27853, *Bacillus subtilis* ATCC 55033, *Staphylococcus epidermidis* ATCC14990, and *Staphylococcus aureus* ATCCBAA44. These pathogens were cultured on trypticase soy agar (TSA) at 37°C for 24 h.

2.3. Fermentation of Isolates

The colored isolates were activated on the isolation medium (ISP2) by seeding the spores and incubating for 7 days at 30°C. They were then seeded on the other three culture media (ISP4, modified ISP9, and starch nitrate). Media in which the isolate did not grow or did not produce pigment were discarded and re-evaluated in liquid culture. A 1-cm² section was cut from each medium and placed in 3 mL of the same medium in liquid form, and these cultures were incubated at 30°C for 7 days with agitation at 150 rpm (New Brunswick Scientific, Connecticut, USA). The fermentation volume of the cultures was then increased by maintaining a proportion of 10% of the liquid culture to 90% of new liquid medium until a fermentation volume of 100 mL was achieved.

2.4. Preparation of extracts from pigmented strains

The final fermentation volume (100 mL) of each strain was distributed in 50 mL Falcon tubes and centrifuged (Thermo Scientific, Massachusetts, USA) at 6000 xg for 10 minutes. The phase (supernatant and/or sediment) containing the pigment was lyophilized for 24 hours (Labconco, Kansas, USA). A double extraction of the lyophilizate from each Falcon tube was performed: first, 75% ethanol (10 mL) was added, vortexed, and agitated for 24 h at 150 rpm. The mixture was then centrifuged at 6000 xg for 5 min, and the supernatants were placed in amber vials. The procedure was repeated with an additional volume of 75% ethanol (5 mL) for the second extraction.

2.5. Biological Activity Tests

2.5.1. Antioxidant Capacity

Two methods were performed to determine the antioxidant capacity, i.e, DPPH and ABTS-related procedures, using the respective stable radicals for scavenging capacity. In both cases, the antioxidant capacity of the extracts was evaluated at concentrations of 5000, 500, 50, and 5 ppm in 96-well plates in triplicate, using trolox as a positive control.

For DPPH, the extract (100 μ L) and 0.2 mM DPPH (100 μ L) were added to a 200- μ L well. The plate was incubated for 30 minutes in the dark, and absorbance was measured at 515 nm. For the ABTS method, aqueous solutions of 7 mM ABTS and 2.45 mM ammonium persulfate were separately prepared and subsequently mixed (1:1 ratio) and incubated in the dark for 16 h. The resulting ABTS⁺ radical was adjusted to an absorbance of 0.7. Finally, the extract (10 μ L) and ABTS⁺ radical solution (190 μ L) were added to a 200- μ L well. It was incubated for 30 min in the dark and the absorbance was measured at 734 nm (Bio-Rad, California USA) [38,39].

2.5.2. Antibacterial Activity

The pigmented extracts were evaluated against the pathogens *K. pneumoniae* ATCC11486, *E. coli* ATCCBAA2469, *P. aeruginosa* ATCC27853, *Bacillus subtilis* ATCC 55033, *S. epidermidis* ATCC14990, *S. aureus* ATCCBAA44 using antibiograms by disk diffusion. Solutions of the extract (30 mg/mL) were prepared in 75% ethanol, and pathogen dispersions were prepared using the MacFarland scale 0.5% in 0.85% saline solution. Mass seeding of pathogenic bacteria was performed using a sterile swab in TSA plates. The discs were impregnated with 5, 15, and 30 μ L of the pigment solutions to examine the variation of bioactivity depending on pigment amounts in the range of 150 μ g to 900 μ g. The tests were performed in triplicate. Finally, the TSA plates were incubated at 37°C for 24 h (Fricell, Querétaro Mexico) using the extraction solvent (75% ethanol) as a negative control and the respective antibiotic as a positive control. The antibiograms were photographed in the colony counter ProtoCOL3 (Microbiology International, Maryland USA) for the measurement of the inhibition halos using the ImageJ program [40].

2.5.3. Cytotoxic Activity

The cytotoxic activity of the pigmented extracts was evaluated against cancer lines such as HeLa (cervical-uterine cancer) and MCF-7 (breast cancer), and healthy cell lines like HDFa (human dermal fibroblasts), using the standard MTT assay. Cells were cultured in DMEM medium for HeLa and HDFa, and RPMI for MCF-7, with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin, and incubated at 37°C in a 5% CO₂ incubator. After incubation, cells were treated with different concentrations of the pigmented extracts in triplicate (5-500 ppm) and incubated for 24 h. Following the 24-h treatment, MTT solution (100 μ L) at 5 mg/mL was added and incubated for 4 h. MTT was removed, and dimethyl sulfoxide (DMSO) (100 μ L) was added to each well. The assay was measured at an absorbance of 570 nm using a plate reader (Bio-Rad, California, USA), with DMSO as the control [41].

2.6. Molecular Identification

At the end of the seven-day incubation period, a spore solution of each isolate was prepared, and genomic DNA was extracted using a Quick-DNA Fungal/Bacterial Microprep kit (Zymo Research Corporation, Irvine, CA, USA) according to the manufacturer's instructions. Molecular identification was carried out by sequencing a 1500 bp fragment of the 16S ribosomal RNA gene obtained by PCR amplification using the universal primers 1493R and 27F, under the following PCR cycling conditions: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes, with a final extension of 72°C for 7 minutes (Bio-Rad, California, USA). The amplification products were verified by agarose electrophoresis (Bio-Rad, California, USA). The sequence obtained was compared in the GenBank nucleotide sequence

database. A phylogenetic tree was constructed using the maximum likelihood method and Hasegawa-Kishino-Yano model [42] in MEGA X [43].

2.7. High-Performance Liquid Chromatography Coupled with Mass Spectrometry

The hydroalcoholic extracts were chemically characterized using a Shimadzu Corp LCMS-2020 system (Japan). This system featured a single quadrupole analyzer and a dual ion source (DUIS) that performs electrospray (ESI) and atmospheric pressure chemical (APCI) ionizations. The testing procedure employed a Phenomenex Synergy RP C18 column (150 × 4.4 mm, 2.6 µm). The column was heated to 30°C and maintained a flow rate of 0.7 mL/min. Two mobile phases, labeled A and B, consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. A gradient elution was conducted over 30 min, starting at 10% B and gradually increasing to 100% B until 25 min, and then returning to 10% B until 30 min. The DUIS was operated in both positive and negative ion modes, employing a scan range of 200–1000 *m/z*, a CDL temperature of 250°C, a detector voltage of 1.2 kV, a nebulizing gas flow rate of 1.3 L/min, and a drying gas flow rate of 8.0 L/min [44,45]. The characterization was supplemented by HRMS using a Shimadzu HPLC system coupled with a Bruker MicrOTOF-Q II spectrometer equipped with a Quadrupole-Time of Flight (QToF) analyzer and ESI. Chromatographic parameters identical to those previously mentioned were employed, operating ESI in both positive and negative ion modes, scanning between 100 to 1000 *m/z*, and setting capillary voltage at 4.5 kV, desolvation line temperature at 400 °C, nebulizer gas (N₂) at 4 Bar, drying gas at 8 L/min, and quadrupole and collision energies at 6 and 12 eV, respectively.

The raw data were pre-processed using MZmine 3 software [46], and the detected features were annotated (level 3) from the MS data using the StreptomeDB v3.0 database [47]. The possible metabolites responsible for the pigmentation or tested bioactivities were searched and additionally filtered using StreptomeDB and other databases such as PubChem [48], LOTUS [49] and the natural products atlas [50,51].

3. Results and Discussion

3.1. Pigmentation of the Strains in the Different Culture Media

Evaluating different culture media is crucial as the production of specialized metabolites, including pigments, is influenced by changes in the primary carbon and nitrogen sources. These changes can either increase the supply or limit access to key precursors [52–54], triggering the activation of biosynthetic enzymes or simultaneously affecting both processes [55,56]. Bacteria often prefer certain single carbon sources over others, preventing the synthesis of enzymes necessary for the catabolism of non-selected carbon sources through carbon catabolite repression (CCR) [57]. Similarly, nitrogen source preference (nitrogen repression) affects the expression of primary nitrogen metabolism and subsequently the biosynthesis of specialized metabolites, which require nitrogen compound precursors [56,58].

When evaluating pigment production of each strain in different culture media (see Table 1), it was observed that the highest number of pigments were produced in ISP2, which contains malt and yeast extract providing amino acids, peptides, vitamins, and minerals essential for bacterial growth [59–62], while glucose supplies the necessary energy for specialized metabolite synthesis like pigments [63,64]. This medium has previously been used to produce red pigments with anticancer activity from *Streptomyces* A 16-1 [65] and *Streptomyces* sp. PM4 [66], brown pigments with antibacterial and anti-HIV activities from *Streptomyces* sp. S45 [67], melanoid pigments like eumelanin with antiproliferative and antioxidant capacities from *S. parvus* BSB49[68], and pyomelanin from *Streptomyces* sp. MPPS2 [69].

The predominant colors produced were black and brown. These dark pigments, possibly melanin or melanoid, can be useful criteria for taxonomical studies [70]. However, varied colors such as yellow, pink, and purple were also observed in other culture media. The common nutrient for these particular pigments is starch as a carbon source, which can induce the expression of genes involved in specialized metabolite biosynthesis and ensure a sustained release of glucose for growth

and metabolite production [71–74]. This carbon source has been used in starch casein nitrate medium to produce red pigment with antimicrobial and anticancer activities from *Streptomyces* sp. JAR6 [75], yellow pigments with antimicrobial activity from *S. coeruleorubidus* [76] and *S. parvulus* C5-5Y [77] and with anticancer activity from *S. griseoaurantiacus* JUACTION 01 [41] and *S. aurantiacus* AAA5 [78].

It should be noted that pigment production in the starch nitrate culture medium is slow, but it has the advantage that pigment production is greater than the amount of biomass produced. This may be due to the fact that biosynthesis of specialized metabolites likely competes with biomass formation, slowing growth [56]. Additionally, some strains that produced pigment in agar lost their production in liquid medium, and when fermentation volumes were increased, a decrease in pigment production was observed, or the coloration became less visible compared to the culture medium color.

Table 2. Pigmentation produced by the strains in the different culture media. ((-) = no pigmentation).

Strain	ISP2	ISP4	modified ISP9	Starch nitrate
2		-	-	-
94		-		-
144			-	
197	-	-		-
220		-	-	-
246	-	-	-	
247		-	-	-
263		-	-	-
290	-		-	-
308		-	-	-
356		-	-	
381		-	-	-
443		-	-	-
626		-	-	-
864		-	-	-
1B18		-		-
1B247		-	-	-
3C110		-	-	-
4C168		-	-	-
4C171	-		-	-

3.2. Molecular Identification

The characterization of *Streptomyces* strains using 16S rRNA gene sequencing is a fundamental approach in microbial taxonomy and phylogenetics. This method provides a high level of accuracy in identifying and classifying bacterial species based on their genetic sequences [80,81]. In this study, a BLAST search of the GenBank database using the 16S rRNA gene sequences for each pigmented strain confirmed that all identified isolates belong to the genus *Streptomyces*. The 16S rRNA gene sequence similarities between the strains and type strains of the genus *Streptomyces* ranged from 98% to 100% (Table 4). This high similarity level indicated a close genetic relationship among the strains, aligning with previous findings in the literature where 16S rRNA gene sequencing has been effectively used to delineate species within the *Streptomyces* genus (Table 4).

A phylogenetic tree was constructed based on 16S rRNA gene sequences of *Streptomyces* species using the Maximum Likelihood method and Hasegawa-Kishino-Yano model [42] in MEGA X [43]. The bootstrap consensus tree, inferred from 1000 replicates, is presented in Figure 1, with the percentage of replicate trees supporting the clustered taxa shown next to the branches [79]. In the phylogenetic tree, some strains cluster closely together, such as strains *S. lacticiproducens* 864, *S.*

albospinus 381, and *S. humi* 144, and strains *S. murinus* 4C168, *S. humi* 308, and *S. mediolani* 1B247. However, there is no clear pattern in strain relationships, with the most promising yellow pigment-producing strains (*S. noursei* 290, *S. murinus* 246 and 4C171) being relatively distant from each other.

Some strains with identical BLAST identifications are found in different branches of the phylogenetic tree and exhibit varying bioactivities. This suggests that more extensive morphological, biochemical, or sequencing analyses might be necessary for accurate identification. Additionally, the novel sources of isolation could indicate that some strains represent new identifications. The use of 16S rRNA gene sequencing is well-documented in the literature for its robustness in identifying bacterial strains [80]. This gene serves as a reliable molecular marker due to its presence in all bacteria and the slow rate of evolutionary change in its sequence, making it ideal for phylogenetic studies. Similarly, it has been previously demonstrated the utility of 16S rRNA gene sequencing in the classification of *Streptomyces* species, highlighting its effectiveness in distinguishing between closely related strains [81]. However, despite its strengths, 16S rRNA gene sequencing has limitations [80]. The resolution of this method may not be sufficient to differentiate between very closely related species or strains with highly similar sequences. Consequently, additional genetic markers or whole-genome sequencing might be required for more precise identification and classification. Hence, while 16S rRNA gene sequencing is a powerful tool for the identification and characterization of *Streptomyces* strains, combining this method with other genetic and phenotypic analyses enhances the accuracy and depth of microbial taxonomy studies [82]. This integrative approach is crucial for uncovering the full potential of *Streptomyces* species in producing bioactive compounds.

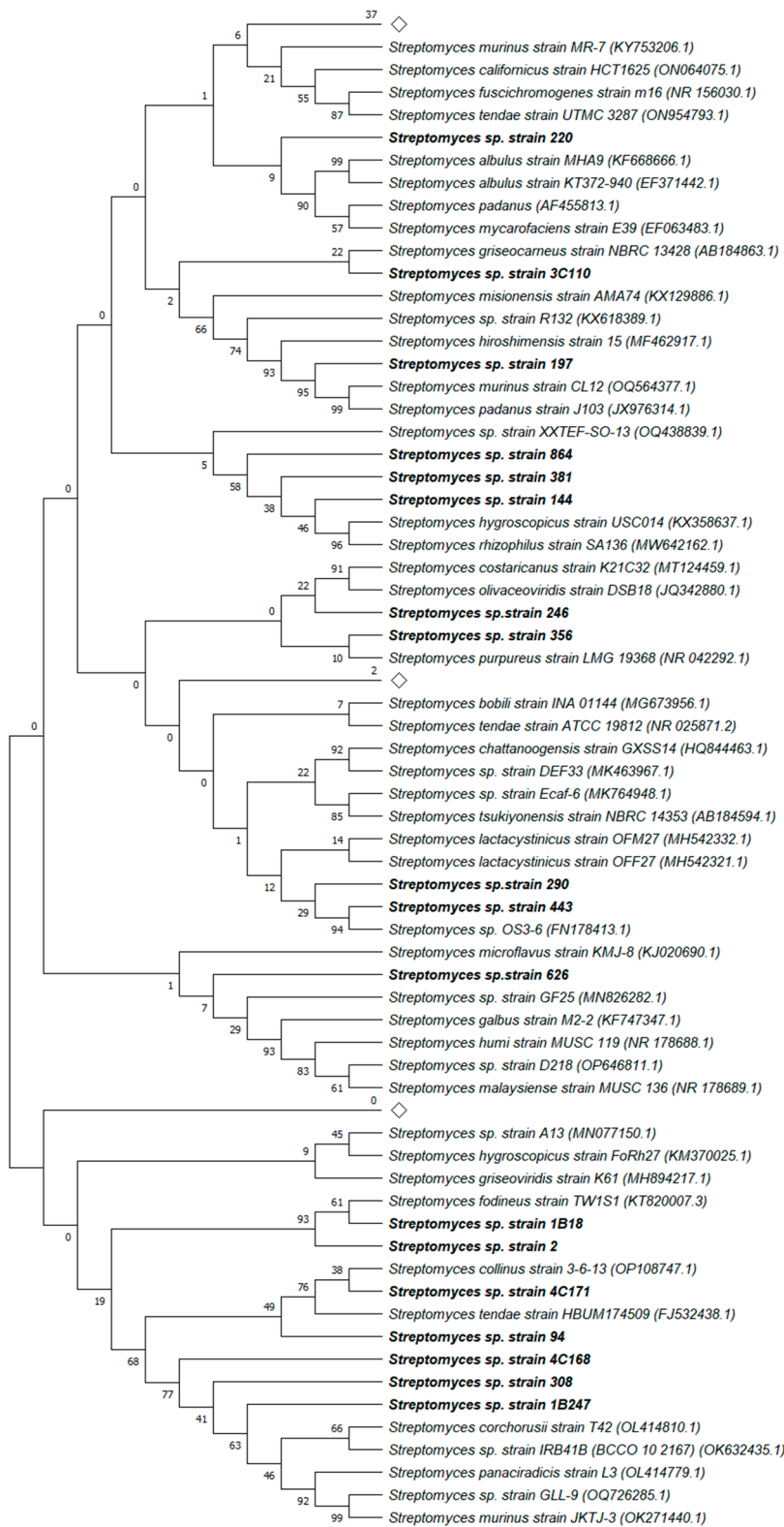


Figure 1. Phylogenetic tree of some the bioactive isolates.

3.3. Biological Activity Assessment

3.3.1. Antioxidant Capacity

Antioxidants are crucial for preventing oxidative stress, which can cause damage to lipids, proteins, and DNA, leading to diseases such as cancer, neurodegeneration, and cardiovascular

conditions [83,84]. Various in-vitro and in-vivo methods are utilized to evaluate antioxidant capacity, playing a significant role in understanding how antioxidants function [85]. Two commonly used methods for evaluating antioxidant capacity are the DPPH and ABTS assays (see Figure 2). Differences between results from these methods may arise because the ABTS assay is applicable to both hydrophilic and lipophilic antioxidant systems, while the DPPH assay is applicable only to hydrophobic systems [86–88]. Additionally, it is suggested that the ABTS assay better reflects antioxidant contents than the DPPH assay [89], being highly sensitive, practical, fast, very stable [90] and useful for high-moisture or highly pigmented nutrients [91].

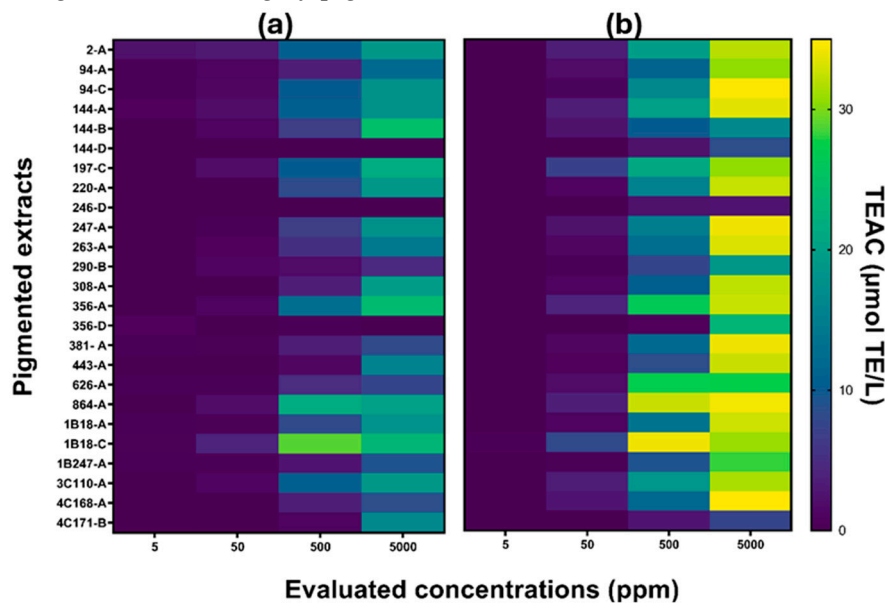


Figure 2. Antioxidant capacity of the pigmented extracts. (a) DPPH Method (b) ABTS Method. Extracts listed according to a code comprising the codified strain (Table 4) hyphenated to the culture medium type, i.e., ISP2 (A) ISP4 (B) modified ISP9 (C) starch nitrate (D). For more information, the mean data with standard deviation ($n = 3$) are presented in supplementary material (**Error! Reference source not found.** and **Error! Reference source not found.**).

In most pigmented extracts, a higher test concentration exhibits a higher antioxidant capacity, indicating a dose-dependent response [92]. Differences in antioxidant capacities determined by the two assays can be greater in highly pigmented sources [86]. According to the DPPH assay, extracts with the highest antioxidant potential ($TEAC > 20 \mu M$) are from strains *S. humi* 144 in ISP4, *S. misionensis* 197 in modified ISP9 (mISP9), *S. hygrosopicus* 356 in ISP2, and *S. lactacystinicus* 1B18 in mISP9. These extracts have dark brown or black colors, possibly indicating melanin pigments. Previously, *Streptomyces* strains have reported melanin with antioxidant capacity, such as *Streptomyces girseorubiginosus* D5 isolated from desert soil in India [93], *Streptomyces glaucescens* NEAE-H isolated from Gulbarga region in India [36], and *Streptomyces parvus* BSB49 from Bayburt province (Turkey) [68]. In the case of the ABTS assay, almost all extracts at maximum concentration exhibit good antioxidant capacities except for strains *S. murinus* 246 and *S. humi* 144 in starch nitrate medium, and strains *S. noursei* 290 and *S. murinus* 4C171 in ISP4, which resulted in $TEAC < 20 \mu M$. Notably, three of these are yellow pigments with low antioxidant capacity in both methods (see Table S1 and Table S2).

The use of different culture media affects the antioxidant capacities and specialized metabolite profiles [52–54], and explain the differences between the two methods, noting that a hydrophilic antioxidant may not be detected by the DPPH method, whereas the ABTS method could measure easier its scavenging capacity [86]. The strain *S. tendae* 94-derived extract shows slight changes in antioxidant capacity between ISP2 and mISP9 media in both methods. The strain *S. humi* 144-derived extract evaluated in three media shows significant differences: in ISP2 and ISP4, the DPPH-scavenging variation is slight ($6.86 \mu M$) and larger in ABTS ($16.95 \mu M$). However, in starch nitrate

medium, the change was considered drastic with null DPPH and low ABTS-scavenging ability. Similarly, strain *S. hygroscopicus* 356-derived extracts show high DPPH capacity when cultured in ISP 2 and almost null in starch nitrate, while ABTS assay shows antioxidant capacity with a difference of 9.59 µM.

3.3.2. Antibacterial Activity

The discovery and development of new antibiotics are crucial due to the increasing threat of antimicrobial resistance (AMR). This problem is exacerbated by the overuse and misuse of antibiotics, inappropriate waste management, and environmental transmission [94,95]. It is estimated that at least 700,000 people worldwide die each year as a result of drug-resistant infections [94]. Additionally, the high numbers of secondary infections during COVID-19 pandemic [96,97] and the massive use of antibiotics as (co)treatment [98] worsened the situation, adding to the ongoing emergence of AMR [99], with likely unfavorable outcomes in clinical, economic, and societal aspects [94,100,101].

Among the six pathogenic microorganisms tested, only three—*B. subtilis* ATCC 55033, *S. epidermidis* ATCC 14990, and *S. aureus* ATCC BAA44—demonstrated sensitivity to the pigmented extracts (see Table 3). The last two are of clinical interest. In this regard, *S. epidermidis* is part of the human skin microbiome but can cause opportunistic infections, such as nosocomial infections, particularly in immunocompromised patients [102,103]. It may outcompete other bacteria in skin wounds and can acquire antibiotic resistance through horizontal gene transfer from other *Staphylococcus* species [104,105]. On the other hand, *S. aureus* ATCC BAA44 is an Iberian MRSA clone isolated from a hospital. It can lead to a range of infections, including those affecting the skin, subcutaneous tissues, and potentially invasive infections such as osteomyelitis, meningitis, pneumonia, lung abscess, empyema, and endocarditis [106–108].

Table 3. Antibacterial activity of the pigmented extracts.

Pigmented Extracts	Tested bacterial strains						Pigmented Extracts	Tested bacterial strains					
	1	2	3	4	5	6		1	2	3	4	5	6
2-A	–	–	–	–	–	–	356-A	–	–	–	–	++	–
94-A	–	–	–	–	–	–	356-D	–	–	–	–	–	–
94-C	–	–	–	–	–	–	381-A	–	–	–	–	–	–
144-A	–	–	–	–	–	–	443-A	–	–	–	–	–	–
144-B	–	–	–	–	–	–	626-A	–	–	–	–	–	–
144-D	–	–	–	–	–	–	864-A	–	–	–	–	–	–
197-C	–	–	–	–	–	–	1B18-A	–	–	–	–	–	–
220-A	–	–	–	–	–	–	1B18-C	–	–	–	–	–	–
246-D	–	–	–	+	++	++	1B247-A	–	–	–	–	++	+++
246-A	–	–	–	–	+++	++	3C110-A	–	–	–	–	++	–
263-A	–	–	–	–	–	–	4C168-A	–	–	–	–	–	–
290-B	–	–	–	+++	++	++	4C171-B	–	–	–	++	+++	+++
308-A	–	–	–	–	–	–							

Pathogenic microorganisms: (1) *Klebsiella pneumoniae* ATCC11486 (2) *Escherichia coli* ATCCBAA2469 (3) *Pseudomonas aeruginosa* ATCC27853 (4) *Bacillus subtilis* ATCC 55033 (5) *Staphylococcus epidermidis* ATCC14990 (6) *Staphylococcus aureus* ATCCBAA44. +++ (inhibition diameter >15 mm), ++ (10-15 mm), + (1-10mm), – (inhibition diameter <1 mm). Pigmented extracts listed according to a code comprising the codified strain (Table 4) hyphenated to the culture medium type, i.e., ISP2 (A) ISP4 (B) modified ISP9 (C) starch nitrate (D). For more information, all the antibiograms are presented in supplementary material (Figures S1 to S15).

Table 4. Molecular identification of strains with pigmentation.

Strain code	Identification	% Similarity
2	<i>Streptomyces griseochromogenes</i>	99.93%
94	<i>Streptomyces tendae</i>	99.86%
144	<i>Streptomyces humi</i>	99.55%
197	<i>Streptomyces misionensis</i>	99.72%
220	<i>Streptomyces argillaceus</i>	100%
246	<i>Streptomyces murinus</i>	99.93%
290	<i>Streptomyces noursei</i>	99.24%
308	<i>Streptomyces humi</i>	99.64%
356	<i>Streptomyces hygroscopicus</i>	99.93%
381	<i>Streptomyces albospinus</i>	99.18%
443	<i>Streptomyces murinus</i>	98.96%
626	<i>Streptomyces albospinus</i>	99.85%
864	<i>Streptomyces lacticiproducens</i>	99.17%
1B18	<i>Streptomyces lactacystinicus</i>	99.35%
1B247	<i>Streptomyces mediolani</i>	99.64%
3C110	<i>Streptomyces fodineus</i>	99.86%
4C168	<i>Streptomyces murinus</i>	99.93%
4C171	<i>Streptomyces murinus</i>	99.86%

Pigmented extracts from strains *S. murinus* 246 grown in starch nitrate medium, *S. noursei* 290 and *S. murinus* 4C171 in ISP4 medium exhibited antimicrobial activity against these three bacteria and show promising potential as antibiotics. These strains are characterized by their yellow pigmentation and the use of starch as a carbon source. Notable, previously reported antibiotic yellow pigments include D-actinomycin from *S. parvulus* C5-5Y, isolated from the leather industry [77], a fraction from *S. hygroscopicus* subsp. *ossamyceticus* (D10), isolated from desert soil, which was active against vancomycin-resistant *S. aureus* [109], a crude pigment from *Streptomyces* sp. D25 with activity against *S. aureus* MTCC96 [110], grixazone A and B from *S. griseus* IFO13350, obtained from the Institute of Fermentation, Osaka, Japan (IFO), which was active against *B. subtilis* ATCC6633 and *S. aureus* 209P JC-1[111].

To a lesser extent, extracts from strains *S. murinus* 246 and *S. mediolani* 1B247 grown in ISP2 showed activity against *S. epidermidis* ATCC 14990 and *S. aureus* ATCC BAA44. Extracts from strains *S. fodineus* 3C110 and *S. hygroscopicus* 356 in ISP2 exhibited activity only against *S. epidermidis* ATCC 14990. In most cases, changing the culture medium did not affect antibacterial activity. However, strain *S. hygroscopicus* 356 inhibited the growth of *S. epidermidis* ATCC 14990 in ISP2 medium but not in starch nitrate medium, suggesting that the same strain may produce different specialized metabolites with varying bioactivities depending on the medium.

3.3.3. Cytotoxic Activity

Identifying anti-cancer compounds is crucial due to the limitations of current chemotherapies, such as multidrug resistance and secondary effects. Natural products, including those from microorganisms, have shown potential in inhibiting cell proliferation and promoting apoptosis [112]. Bacteria are highlighted as sources of specialized metabolites with anti-cancer properties, potentially leading to the development of more effective cancer therapies [113,114]. *Streptomyces* species, known for producing clinically significant drugs like doxorubicin and bleomycin [115,116], also produce pigmented compounds with anticancer activity. Notable examples include red pigmented crude extracts from *Streptomyces* sp. PM4 [66] and *Streptomyces* sp. A 16-1 [65], pure compounds such as undecylprodigiosin from *Streptomyces* sp. JAR6 [75] and prodigiosin from *Streptomyces* sp. WMA-LM31 [117]. Other pigments include a yellow pigment from *Streptomyces griseoaurantiacus* JUACTION 01 [41] with activity against HeLa and liver cancer (HepG2), melanin from *Streptomyces glaucescens*

NEAE-H [36], and eumelanin from *Streptomyces parvus* BSB49 [68] with activity against skin cancer (HFB4) and HeLa, respectively.

When evaluating the pigmented extracts against three cell lines—human dermal fibroblasts (HDFa), cervical cancer cell line (HeLa), and breast cancer cell line (MCF-7) (see Figure 3)—the MCF-7 cell line was the most resistant to the extracts. Extracts from strains *S. murinus* 246 in starch nitrate and *S. murinus* 4C171 in ISP4 medium showed promising anticancer activity against HeLa and MCF-7 without being toxic to the HDFa cell line. Extract *Streptomyces noursei* 290 in ISP4 medium demonstrated good anticancer activity against HeLa at all concentrations evaluated and against MCF-7 at maximum concentration, although it was toxic to HDFa. Strain *S. murinus* 443 in ISP 2 also showed promise at all concentrations without being toxic to HDFa.

In general, tested extracts exhibited a dose-dependent response (Figure 3). However, some extracts exhibited atypical behavior. For instance, strain *S. tendae* 94 in mISP9 showed higher cytotoxicity against MCF-7 at intermediate concentrations than at the highest concentration, while strain *S. misionensis* 197 in mISP 9 and strain *S. tendae* 94 in ISP2 showed higher activity at lower concentrations. Half-maximal inhibitory concentrations (IC₅₀) were calculated for those extracts showing a dose-dependent response (see Figures S16-S20). Strain *S. murinus* 4C171 in starch nitrate had IC₅₀ values of 146.4 ppm against HeLa and 242.6 ppm against MCF-7, which were relatively higher than those reported in literature, ranging from 0.04 to 48.07 ppm [118]. Strain *S. murinus* 246 in starch nitrate had an IC₅₀ of 119.3 ppm against HeLa, which is lower than undecylprodigiosin from *Streptomyces* sp. JAR6 [75] but higher than a yellow pigment from *Streptomyces griseoaurantiacus* JUACT 01 [41]. For strains showing cytotoxicity, IC₅₀ values were 152.6 ppm for *S. mediolani* 1B247 in ISP2 and 104.8 ppm for *S. noursei* 290 in ISP4. These values are higher than those reported for melanin from *Streptomyces glaucescens* NEAE-H, which were 37.05 ppm and 48.07 ppm against noncancer cell lines [37]. This suggests that higher concentrations are required for cytotoxicity, though these extracts are not entirely safe.

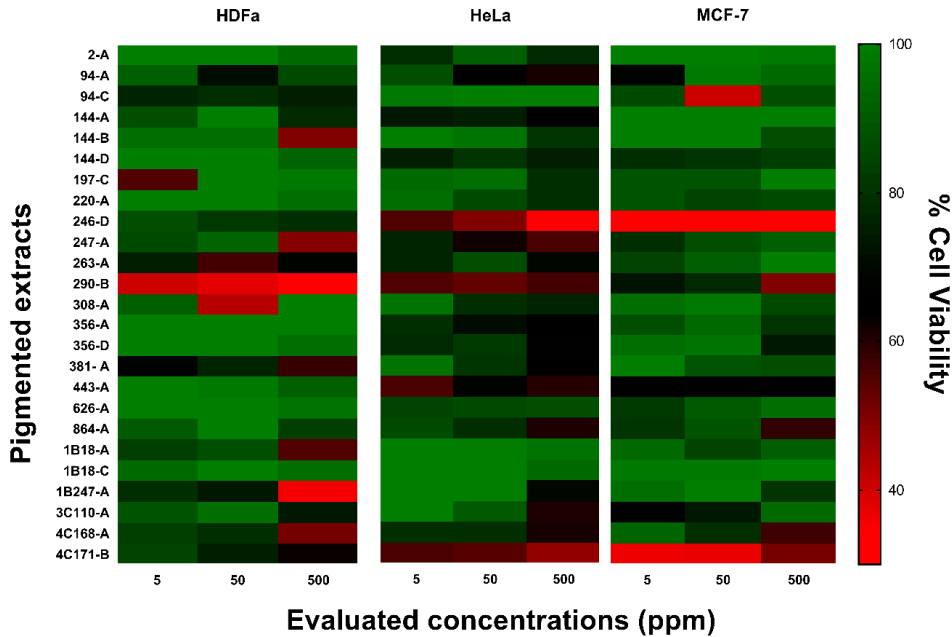


Figure 3. Cytotoxicity test of the pigmented extracts. Human Dermal Fibroblasts (HDFa), Cervical cancer cell line (HeLa), Breast cancer cell line (MCF-7). Extracts listed according to a code comprising the codified strain hyphenated to the culture medium type, i.e., ISP2 (A) ISP4 (B) modified ISP9 (C) starch nitrate (D). For more information, the mean data with standard deviation ($n = 3$) are provided in supplementary material (Tables S4 to S6).

3.4. HPLC-MS-Based Annotation (Level 3) of Metabolites Possibly Responsible for the Observed Activities by the Different *Streptomyces*-Derived Extracts

The most promising pigmented extracts were analyzed by HPLC-MS to chemically characterize such extracts and annotate those detected compounds. The annotated metabolites were searched in literature and StreptomeDB database to determine a plausible role in pigmentation and bioactivity, as well as to find previous sources related to *Streptomyces* species that produce them. In this context, strain *S. humi* 144, cultured on ISP2 and ISP4 media, exhibited antioxidant capacity, as evidenced by DDPH and ABTS assays. HPLC-MS-based characterization suggested that this activity may be associated with chlorogenic acid [49], a phenylpropanoid previously identified in *Streptomyces* sp. PM9 [119], known for its antioxidant efficacy comparable to L-ascorbic acid [120]. This effectiveness is often attributed to the hydroxyl groups in the aromatic ring structure [121]. Additionally, strain *S. humi* 144 in ISP2 medium demonstrated moderate cytotoxic activity against HeLa cells, possibly due to the presence of certain compounds, detected and annotated in this extract (Table S6), which previously reported to have cytotoxic activity. In contrast, strain *S. noursei* 290 in ISP4 showed antibacterial activity against *B. subtilis* ATCC 55033, *S. epidermidis* ATCC14990, and *S. aureus* ATCCBAA44, which may be attributed to the compounds listed in Table S7. This extract also had good anticancer activity against HeLa and MCF-7 cells, potentially due to the detected, annotated compounds listed in Table S8. Furthermore, HPLC-MS analysis suggested that the pigments responsible for coloration in this strain may include phenazine-1-carboxylate (also known as tubermycin B) with a greenish-yellow hue [122], or tetracenomycin D1, related to tetracenomycin C, a pale yellow antibiotic [123]. Other compounds with potential antimicrobial and cytotoxic activity included pentostatin, phenazine-1-carboxylate, yautomycetin, and tetracenomycin D1.

Strain *S. murinus* 246 in starch nitrate exhibited antibacterial activity against *B. subtilis* ATCC 55033, *S. epidermidis* ATCC14990, and *S. aureus* ATCCBAA44, possibly due to action of some of the annotated compounds (Table S9), which are related to antibiotics such as cephamycin C, lankamycin, and sisomicin. It also showed promising cytotoxic activity against HeLa and MCF-7 cells without toxicity to the normal cell line (HDFa), potentially due to the presence of fumiformamide, maculosin, and salinomycin as annotated compounds with reported cytotoxic activity (Table S10). The pigments responsible might include a xanthone-related compound, such as xantholipin B. Xanthones, derived from the greek word “xanthos” meaning yellow, are polyketide-derived compounds, firstly isolated from *Streptomyces* but also found in plants, with a C6-C1-C6 carbon skeleton [124]. Xanthones have various properties, including antimicrobial, antioxidant, anti-inflammatory, and antitumor activities [121–123]. Recently, a new polycyclic xanthone, namely sattahipmycin, was identified from marine *Streptomyces* sp. GKU 257-1, and exhibited antimicrobial and cytotoxic activity [125]. Remarkably, other *S. murinus* strain (i.e., 4C171) in ISP4 medium showed antibacterial activity against *B. subtilis* ATCC 55033, *S. epidermidis* ATCC14990, and *S. aureus* ATCCBAA44, potentially due to a diverse metabolite classess listed in Table S11. The extract of this strain exhibited low antioxidant capacity, possibly related to the low action or low concentration of annotated compounds in this extract (Table S12). However, it demonstrated promising anticancer activity against HeLa and MCF-7 cells without toxicity to the healthy cell line (HDFa), likely due to the presence of active metabolites (Table S13). Potential pigments include carotenoids like beta-carotene, γ -carotene, neurosporene, 1,2,1',2'-tetrahydrolycopene, and lycopene, which have been reported in *Streptomyces* species without photoinduction [126]. Other possible pigments include resistomycin, a natural antibiotic with cytotoxic activity [78,118,127]; flavonoids such as taxifolin or dihydroquercetin [118], phenazine-1-carboxylate which has a greenish-yellow coloration [122], tetracenomycin C which is pale yellow antibiotic [123], riboflavin, identified in species such as *Streptomyces* sp. TS-2-2 [128], and the red pigment roseoflavin from species like *Streptomyces davaonensis* [131,132].

On the other hand, strain *S. fodineus* 3C110 growth in ISP2 medium exhibited antibacterial activity against *S. epidermidis* ATCC14990, potentially due to those metabolites filtered as bioactives (Table S14). It also showed moderate antioxidant capacity, possibly related to salinomycin, the only antioxidant compound detected in this extract by HPLC-MS. This 3C110-derived extract was found to be active against HeLa cells at maximum dose, involving the presence of three cytotoxic

compounds (Table S15). Additionally, strain *S. hygroscopicus* 356 in ISP2 medium showed antibacterial activity against *S. epidermidis* ATCC14990, moderate antioxidant capacity, and reasonable cytotoxic activity against HeLa cells at maximum concentration, whose bioactivity were rationalized by the reported properties of those annotated compounds for this extract (Tables S16–S18). Remarkably, this strain *S. hygroscopicus* 356 grown in starch nitrate showed no antibacterial activity against the pathogens tested and lower antioxidant capacity compared to ISP2 medium, exhibiting a different profile and most compounds were not produced and accumulated in such a medium (i.e., starch nitrate), excepting 2-acetylpyrrole, previously produced by *Streptomyces* sp. MUM273b [129], which was additionally detected. This extract also showed cytotoxic activity against HeLa cells at maximum concentration but to a lesser degree than in ISP2, involving cytotoxic compounds such as chartreusin, jadomycin A, and pentalenolactone (Table S19).

HPLC-MS-based characterization of *S. hygroscopicus* 356-derived extracts obtained from two culture media (ISP2 and starch nitrate) revealed the plausible production of annotated aromatic polyketide diphenyl ethers, i.e., Violaceol I and II, purple pigments known from co-cultures of *Streptomyces rapamycinicus* and *Aspergillus nidulans* [130], or endophytic fungi *Aspergillus austroafricanus* and *Streptomyces lividans* [131]. These pigments or related compounds might account for the pinkish-purple color of strain *S. hygroscopicus* 356 in starch nitrate and its darker shade in ISP2 medium. However, the coloration of this strain 356 in ISP2 differs slightly from purple, possibly due to the presence of other pigments like β -isorenieratene, luteothin, and xanthone-like metabolites.

Particularly, strain *S. misionensis* 197 in mISP9 medium shows no antibacterial activity against evaluated pathogens or cytotoxicity against cell lines but exhibits certain toxicity against HDFa. It also showed moderate antioxidant activity, potentially due to antioxidant compounds (Table S20). Similarly, strain *S. murinus* 443 in ISP2 medium showed no antibacterial activity against evaluated pathogens and low antioxidant capacity but it was active against HeLa and MCF-7 cells, possibly related to the presence of previously reported *Streptomyces*-derived compounds (Table S21–S22). Detected pigments in this extract included carotenoids such as β -carotene, γ -carotene, β -isorenieratene, phytoene, 1,2,1',2'-tetrahydrolycopene, and lycopene [126]. Finally, strain *S. laticiproducens* 864 in ISP2 medium exhibits no antibacterial activity against evaluated pathogens, moderate antioxidant capacity, and cytotoxic activity against HeLa and MCF-7 cells at the highest tested concentrations, whose properties could be explained by the detection of bioactive metabolites listed in Tables S23 and S24. In the case of strain *S. humi* 308 in ISP2 medium exhibits only antioxidant capacity, possibly due to the production of phenolic-like antioxidant metabolites [132–134].

4. Conclusions

The genus *Streptomyces* stands out as a remarkable source of diverse pigments with a spectrum of bioactivities, including antioxidant, antibacterial, and cytotoxic properties. This study underscores the significant potential of pigmented extracts from various *Streptomyces* strains to be active against pathogenic microorganisms and exhibit anticancer properties. Among the six pathogenic microorganisms tested, three (*B. subtilis*, *S. epidermidis*, and *S. aureus*) showed antibiotic sensitivity to certain pigmented extracts, while six *Streptomyces*-derived extracts exhibited cytotoxic activity (cell viability < 40%) against two cancer cell lines (HeLa and MCF-7), and various pigmented extracts demonstrated antioxidant capacity at different levels (TEAC > 20 μ M) through DPPH and ABTS methods. Notably, strains *S. noursei* 290, *S. murinus* 246 and 4C171, which produce yellow pigments and utilize starch as a carbon source, exhibited promising antibacterial and anticancer activities. Extracts from strains *S. murinus* 4C171 and 246 also showed potent anticancer properties across various concentrations, exhibiting selectivity without compromising the viability of healthy HDFa cell lines. Molecular identification through 16S rRNA gene sequencing confirmed that all isolated strains belong to the *Streptomyces* genus, with similarities ranging from 98% to 100%. The HPLC-MS-based chemical characterization of active extracts identified several bioactive compounds responsible for the observed activities. Strain *S. humi* 144's antioxidant capacity is likely linked to the presence of chlorogenic acid, while strain *S. noursei* 290's broad-spectrum bioactivities might be attributed to compounds such as phenazine-1-carboxylate and tetracenomycin D1. Similarly, *S. murinus* strains'

activities (strains 246 and 4C17) are possibly due to carotenoids and xanthone-related compounds. This study also highlighted the importance of culture media in influencing the chemical composition of resulting extracts. Thus, the exploration of distinct culture mediums is crucial in characterizing the variations on the bioactivity associated with the specialized metabolite production, as their synthesis is intricately linked to primary carbon and nitrogen sources. For instance, strain *S. hygroscopicus* 356 exhibited different antibacterial and antioxidant capacities depending on the employed medium, suggesting that the same strain can produce different specialized metabolites with varying bioactivities. Notably, starch is a particularly favorable carbon source, driving the biosynthesis of yellow pigmented extracts that showcase the most pronounced potential as both antibacterial and anticancer agents. These findings underscore the multifaceted nature of *Streptomyces*-derived compounds (e.g., pigments) and extracts and their promising applications in the pharmaceutical and therapeutic industries. Hence, the pigmented extracts from these *Streptomyces* strains show reasonable promise as sources of novel antibiotics and anticancer agents. Further research, including the purification of these compounds and exploration of their mechanisms of action, is warranted to fully realize their therapeutic potential.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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References

1. Marcano, D. *Introducción a La Química de Los Colorantes*; 2018; ISBN 9789806195592.
2. Lyu, X.; Lyu, Y.; Yu, H.; Chen, W.; Ye, L.; Yang, R. Biotechnological Advances for Improving Natural Pigment Production: A State-of-the-Art Review. *Bioresour. Bioprocess.* **2022**, *9*, 8, doi:10.1186/s40643-022-00497-4.
3. Ahmad, W.A.; Yusof, N.Z.; Nordin, N.; Zakaria, Z.A.; Rezali, M.F. Production and Characterization of Violacein by Locally Isolated *Chromobacterium Violaceum* Grown in Agricultural Wastes. *Appl. Biochem. Biotechnol.* **2012**, *167*, 1220–1234, doi:10.1007/s12010-012-9553-7.
4. Sajda .S. Affat Classifications, Advantages, Disadvantages, Toxicity Effects of Natural and Synthetic Dyes: A Review. *Univ. Thi-Qar J. Sci.* **2021**, *8*, 130–135.
5. Venil, C.K.; Zakaria, Z.A.; Ahmad, W.A. Bacterial Pigments and Their Applications. *Process Biochem.* **2013**, *48*, 1065–1079, doi:10.1016/j.procbio.2013.06.006.
6. Paul, T.; Bandyopadhyay, T.K.; Mondal, A.; Tiwari, O.N.; Muthuraj, M.; Bhunia, B. A Comprehensive Review on Recent Trends in Production, Purification, and Applications of Prodigiosin. *Biomass Convers. Biorefinery* **2020**, doi:10.1007/s13399-020-00928-2.
7. Husain, Q. Potential Applications of the Oxidoreductive Enzymes in the Decolorization and Detoxification of Textile and Other Synthetic Dyes from Polluted Water: A Review. *Crit. Rev. Biotechnol.* **2006**, *26*, 201–221, doi:10.1080/07388550600969936.
8. Khattab, T.A.; Abdelrahman, M.S.; Rehan, M. Textile Dyeing Industry: Environmental Impacts and

- Remediation. *Environ. Sci. Pollut. Res.* **2020**, *27*, 3803–3818, doi:10.1007/s11356-019-07137-z.
9. Sarayu, K.; Sandhya, S. Current Technologies for Biological Treatment of Textile Wastewater—A Review. *Appl. Biochem. Biotechnol.* **2012**, *167*, 645–661, doi:10.1007/s12010-012-9716-6.
 10. Lellis, B.; Fávaro-Polonio, C.Z.; Pamphile, J.A.; Polonio, J.C. Effects of Textile Dyes on Health and the Environment and Bioremediation Potential of Living Organisms. *Biotechnol. Res. Innov.* **2019**, *3*, 275–290, doi:10.1016/j.biori.2019.09.001.
 11. Sanchez-Marroquín, A.; Zapata, M. Observations on the Pigment of *Streptomyces Coelicolor*. **1954**, *2*.
 12. Manivasagan, P.; Venkatesan, J.; Sivakumar, K.; Kim, S.-K. Actinobacterial Melanins: Current Status and Perspective for the Future. *World J. Microbiol. Biotechnol.* **2013**, *29*, 1737–1750, doi:10.1007/s11274-013-1352-y.
 13. Dharmaraj, S. Marine *Streptomyces* as a Novel Source of Bioactive Substances. *World J. Microbiol. Biotechnol.* **2010**, *26*, 2123–2139, doi:10.1007/s11274-010-0415-6.
 14. Mann, J. Natural Products as Immunosuppressive Agents. *Nat. Prod. Rep.* **2001**, *18*, 417–430, doi:10.1039/b001720p.
 15. Olano, C.; Méndez, C.; Salas, J.A. Antitumor Compounds from Marine Actinomycetes. *Mar. Drugs* **2009**, *7*, 210–248, doi:10.3390/md7020210.
 16. Selim, M.S.M.; Abdelhamid, S.A.; Mohamed, S.S. Secondary Metabolites and Biodiversity of Actinomycetes. *J. Genet. Eng. Biotechnol.* **2021**, *19*, doi:10.1186/s43141-021-00156-9.
 17. Pathom-aree, W.; Stach, J.E.M.; Ward, A.C.; Horikoshi, K.; Bull, A.T.; Goodfellow, M. Diversity of Actinomycetes Isolated from Challenger Deep Sediment (10,898 m) from the Mariana Trench. *Extremophiles* **2006**, *10*, 181–189, doi:10.1007/s00792-005-0482-z.
 18. Bull, A.T. *Microbial Diversity and Bioprospecting*; Bull, A.T., Ed.; ASM Press: Washington, DC, USA, 2003; ISBN 9781683672173.
 19. Berdi, J. Bioactive Microbial Metabolites. *J. Antibiot. Antibiot.* **2005**, *58*, 1–26, doi:10.1038/ja.2005.1.
 20. Devi Salam, M. Antimicrobial Potential of Actinomycetes Isolated from Soil Samples of Punjab, India. *J. Microbiol. Exp.* **2014**, *1*, doi:10.15406/jmen.2014.01.00010.
 21. Darshan, N.; Manonmani, H.K. Prodigiosin and Its Potential Applications. *J. Food Sci. Technol.* **2015**, *52*, 5393–5407.
 22. Zhang, H.C.; Zhan, J.X.; Su, K.M.; Zhang, Y.X. A Kind of Potential Food Additive Produced by *Streptomyces Coelicolor*: Characteristics of Blue Pigment and Identification of a Novel Compound, Lambda-Actinorhodin. *Food Chem.* **2006**, *95*, 186–192.
 23. Petinate, S.D.; Martins, R.M.; Coelho, R.R.; Meirelles, M.N.L.; Branquinha, M.H.; Vermelho, A.B. Influence of Growth Medium in Proteinase and Pigment Production by *Streptomyces Cyaneus*. *Mem. Inst. Oswaldo Cruz* **1999**, *94*, 173–177, doi:10.1590/S0074-02761999000200008.
 24. Zhu, H.-H.; Guo, J.; Yao, Q.; Yang, S.-Z.; Deng, M.-R.; Le Phuong, T.B.; Hanh, V.T.; Ryan, M.J. *Streptomyces Vietnamensis* Sp. Nov., a Streptomycete with Violet Blue Diffusible Pigment Isolated from Soil in Vietnam. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1770–1774, doi:10.1099/ijs.0.64774-0.
 25. Gupta, C.; Garg, A.P.; Prakash, D.; Goyal, S. Microbes as Potential Sources of Biocolours. *Pharmacologyonline* **2011**, 1309–1318.
 26. Lin, Y.B.; Wang, X.Y.; Fang, H.; Ma, Y.N.; Tang, J.; Tang, M.; Wei, G.H. *Streptomyces Shaanxiensis* Sp. Nov., a Blue Pigment-Producing Streptomycete from Sewage Irrigation Soil. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 1725–1730, doi:10.1099/ijs.0.029959-0.
 27. Zhu, H.-H.; Guo, J.; Yao, Q.; Yang, S.-Z.; Deng, M.-R.; Li, T.-H. *Streptomyces Caeruleatus* Sp. Nov., with Dark Blue Diffusible Pigment. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 507–511.
 28. Malik, K.; Tokkas, J.; Goyal, S. Microbial Pigments : A Review. *Int. J. Microb. Resour. Technol.* **2012**, 361–365.
 29. Morales-Oyervides, L.; Oliveira, J.; Sousa-Gallagher, M.; Méndez-Zavala, A.; Montañez, J.C. Assessment of the Dyeing Properties of the Pigments Produced by *Talaromyces* Spp. *J. Fungi* **2017**, *3*, doi:10.3390/jof3030038.
 30. Narsing Rao, M.P.; Xiao, M.; Li, W. Fungal and Bacterial Pigments : Secondary Metabolites with Wide Applications. *Front. Microbiol.* **2017**, *8*, 1–13, doi:10.3389/fmicb.2017.01113.
 31. Pombeiro-Sponchiado, S.R.; Sousa, G.S.; Andrade, J.C.R.; Lisboa, H.F.; Gonçalves, R.C.R. Production of Melanin Pigment by Fungi and Its Biotechnological Applications. *Melanin* **2017**, doi:10.5772/67375.
 32. Paillière-Jiménez, M.E.; Stincone, P.; Brandelli, A. Natural Pigments of Microbial Origin. *Front. Sustain. Food Syst.* **2020**, *4*, 1–8, doi:10.3389/fsufs.2020.590439.
 33. Ramesh, C.; Vinithkumar, N.V.; Kirubakaran, R.; Venil, C.K.; Dufossé, L. *Multifaceted Applications of Microbial Pigments: Current Knowledge, Challenges and Future Directions for Public Health Implications*; 2019; Vol. 7; ISBN 3326221754.
 34. Uribe Vélez, D. Metagenómica ¿Una Oportunidad Para El Estudio de La Diversidad Microbiana En

- Colombia? *Rev. Colomb. Biotechnol.* **2009**, *XI*, 4–7.
35. Shirling, E.B.; Gottlieb, D. Methods for Characterization of *Streptomyces* Species. *Int. J. Syst. Bacteriol.* **1966**, *16*, 313–340, doi:10.1099/00207713-16-3-313.
 36. El-Naggar, N.E.-A.; El-Ewasy, S.M. Bioproduction, Characterization, Anticancer and Antioxidant Activities of Extracellular Melanin Pigment Produced by Newly Isolated Microbial Cell Factories *Streptomyces Glaucescens* NEAE-H. *Sci. Rep.* **2017**, *7*, 42129, doi:10.1038/srep42129.
 37. Benouaguene, S.; Ranque, S.; Gacemi Kirane, D. A Non-Polyenic Antifungal Produced by a *Streptomyces Yatensis* Strain Isolated from Mellah Lake in El Kala, North-East of Algeria. *J. Mycol. Med.* **2015**, *25*, 2–10, doi:10.1016/j.mycmed.2014.10.002.
 38. Siddharth, S.; Vittal, R.R. Evaluation of Antimicrobial , Enzyme Inhibitory , Antioxidant and Cytotoxic Activities of Partially Purified Volatile Metabolites of Marine *Streptomyces* Sp.S2A. *Microorganisms* **2018**, *6*, 72, doi:10.3390/microorganisms6030072.
 39. Francenia Santos-Sánchez, N.; Salas-Coronado, R.; Villanueva-Cañongo, C.; Hernández-Carlos, B. Antioxidant Compounds and Their Antioxidant Mechanism. In *Antioxidants*; IntechOpen, 2019; pp. 1–29.
 40. Taroco, R.; Seija, V.; Vignoli, R. Métodos de Estudio de La Sensibilidad Antibiótica. In *Temas de Bacterología y Virología Médica*; 2008; pp. 663–671.
 41. Prashanthi, K.; Suryan, S.; Varalakshmi, K.N. In Vitro Anticancer Property of Yellow Pigment from *Streptomyces Griseoaurantiacus* JUACTION 01. *Brazilian Arch. Biol. Technol.* **2015**, *58*, 869–876, doi:10.1590/S1516-89132015060271.
 42. Hasegawa, M.; Kishino, H.; Yano, T. Dating of the Human-Ape Splitting by a Molecular Clock of Mitochondrial DNA. *J. Mol. Evol.* **1985**, *22*, 160–174, doi:10.1007/BF02101694.
 43. Kumar, S.; Stecher, G.; Li, M.; Niyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549, doi:10.1093/molbev/msy096.
 44. Ramirez-Rodriguez, L.; Stepanian-Martinez, B.; Morales-Gonzalez, M.; Diaz, L. Optimization of the Cytotoxic Activity of Three *Streptomyces* Strains Isolated from Guaviare River Sediments (Colombia, South America). *Biomed Res. Int.* **2018**, *2018*, 1–14, doi:10.1155/2018/2839356.
 45. Bernal, F.A.; Orduz-Díaz, L.L.; Coy-Barrera, E. Application of Parafac and OPLS-DA Analyses on HPLC Fingerprints for the Characterization of *Hibiscus Sabdariffa* Calyxes. *Quim. Nova* **2016**, doi:10.5935/0100-4042.20160007.
 46. Schmid, R.; Heuckeroth, S.; Korf, A.; Smirnov, A.; Myers, O.; Dyrland, T.S.; Bushuiev, R.; Murray, K.J.; Hoffmann, N.; Lu, M.; et al. Integrative Analysis of Multimodal Mass Spectrometry Data in MZmine 3. *Nat. Biotechnol.* **2023**, *41*, 447–449, doi:10.1038/s41587-023-01690-2.
 47. Mounib, A.F.A.; Gao, M.; Qaseem, A.; Li, J.; Kirchner, P.A.; Ndingkokhar, B.; Bekono, B.D.; Simoben, C. V.; Babiaka, S.B.; Malange, Y.I.; et al. StreptomeDB 3.0: An Updated Compendium of Streptomycetes Natural Products. *Nucleic Acids Res.* **2021**, *49*, D600–D604, doi:10.1093/nar/gkaa868.
 48. Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B.A.; Thiessen, P.A.; Yu, B.; et al. PubChem 2023 Update. *Nucleic Acids Res.* **2023**, *51*, D1373–D1380, doi:10.1093/nar/gkac956.
 49. Rutz, A.; Sorokina, M.; Galgonek, J.; Mietchen, D.; Willighagen, E.; Gaudry, A.; Graham, J.G.; Stephan, R.; Page, R.; Vondrášek, J.; et al. The LOTUS Initiative for Open Knowledge Management in Natural Products Research. *Elife* **2022**, *11*, doi:10.7554/eLife.70780.
 50. van Santen, J.A.; Poynton, E.F.; Isakova, D.; McMann, E.; Alsup, T.A.; Clark, T.N.; Fergusson, C.H.; Fewer, D.P.; Hughes, A.H.; McCadden, C.A.; et al. The Natural Products Atlas 2.0: A Database of Microbially-Derived Natural Products. *Nucleic Acids Res.* **2022**, *50*, D1317–D1323, doi:10.1093/nar/gkab941.
 51. van Santen, J.A.; Jacob, G.; Singh, A.L.; Aniebok, V.; Balunas, M.J.; Bunsko, D.; Neto, F.C.; Castaño-Espriu, L.; Chang, C.; Clark, T.N.; et al. The Natural Products Atlas: An Open Access Knowledge Base for Microbial Natural Products Discovery. *ACS Cent. Sci.* **2019**, *5*, 1824–1833, doi:10.1021/acscentsci.9b00806.
 52. Wink, J.; Fatemeh, M.; Javad, H. *Biology and Biotechnology of Actinobacteria*; Wink, J., Mohammadipanah, F., Hamed, J., Eds.; Springer International Publishing: Cham, 2017; ISBN 978-3-319-60338-4.
 53. Anzai, K.; Ohno, M.; Nakashima, T.; Kuwahara, N.; Suzuki, R.; Tamura, T.; Komaki, H.; Miyadoh, S.; Harayama, S.; Ando, K. Taxonomic Distribution of *Streptomyces* Species Capable of Producing Bioactive Compounds among Strains Preserved at NITE/NBRC. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 287–295, doi:10.1007/s00253-008-1510-6.
 54. Ryu, Y.-G.; Butler, M.J.; Chater, K.F.; Lee, K.J. Engineering of Primary Carbohydrate Metabolism for Increased Production of Actinorhodin in *Streptomyces Coelicolor*. *Appl. Environ. Microbiol.* **2006**, *72*, 7132–7139, doi:10.1128/AEM.01308-06.
 55. Demain, A.L.; Sanchez, S. Microbial Drug Discovery: 80 Years of Progress. *J. Antibiot. (Tokyo)*. **2009**, *62*, 5–16, doi:10.1038/ja.2008.16.
 56. Romero-Rodríguez, A.; Maldonado-Carmona, N.; Ruiz-Villafán, B.; Koirala, N.; Rocha, D.; Sánchez, S.

- Interplay between Carbon, Nitrogen and Phosphate Utilization in the Control of Secondary Metabolite Production in *Streptomyces*. *Antonie Van Leeuwenhoek* **2018**, *111*, 761–781, doi:10.1007/s10482-018-1073-1.
57. Görke, B.; Stülke, J. Carbon Catabolite Repression in Bacteria: Many Ways to Make the Most out of Nutrients. *Nat. Rev. Microbiol.* **2008**, *6*, 613–624, doi:10.1038/nrmicro1932.
 58. Omura, S.; Tanaka, Y.; Mamada, H.; Masuma, R. Effect of Ammonium Ion, Inorganic Phosphate and Amino Acids on the Biosynthesis of Protylonolide, a Precursor of Tylosin Aglycone. *J. Antibiot. (Tokyo)*. **1984**, *37*, 494–502, doi:10.7164/antibiotics.37.494.
 59. Tomé, D. Yeast Extracts: Nutritional and Flavoring Food Ingredients. *ACS Food Sci. Technol.* **2021**, *1*, 487–494, doi:10.1021/acsfoodscitech.0c00131.
 60. Narayana, K.J.P.; Kumar, K.G.; Vijayalakshmi, M. L-Asparaginase Production by *Streptomyces Albidoflavus*. *Indian J. Microbiol.* **2008**, *48*, 331–336, doi:10.1007/s12088-008-0018-1.
 61. Khopade, A.; Ren, B.; Liu, X.-Y.; Mahadik, K.; Zhang, L.; Kokare, C. Production and Characterization of Biosurfactant from Marine *Streptomyces* Species B3. *J. Colloid Interface Sci.* **2012**, *367*, 311–318, doi:10.1016/j.jcis.2011.11.009.
 62. Langheinrich, W.; Ring, K. Regulation of Amino Acid Transport in Growing Cells of *Streptomyces Hydrogenans*. *Arch. Microbiol.* **1976**, *109*, 227–235, doi:10.1007/BF00446633.
 63. Van Wezel, G.P.; Mahr, K.; König, M.; Traag, B.A.; Pimentel-Schmitt, E.F.; Willimek, A.; Titgemeyer, F. GlcP Constitutes the Major Glucose Uptake System of *Streptomyces Coelicolor* A3(2). *Mol. Microbiol.* **2004**, *55*, 624–636, doi:10.1111/j.1365-2958.2004.04413.x.
 64. Čihák, M.; Kameník, Z.; Šmídová, K.; Bergman, N.; Benada, O.; Kofroňová, O.; Petříčková, K.; Bobek, J. Secondary Metabolites Produced during the Germination of *Streptomyces Coelicolor*. *Front. Microbiol.* **2017**, *8*, doi:10.3389/fmicb.2017.02495.
 65. Saengkhae, C.; Srivibool, R.; Watanadilok, R.; Enomoto, K. Partially Purified Pigment Extract from *Streptomyces* A 16-1 Induces Apoptosis of Human Carcinoma of Nasopharynx Cell (KB Cells) via the Mitochondrial and Caspase-3 Pathway. *Walailak J. Sci. Technol.* **2017**, *14*, 51–63, doi:10.14456/vol14iss1pp.
 66. Karuppiyah, V.; Aarthi, C.; Ramohan; Sivakumar, K.; Kannan, L. Statistical Optimization and Anticancer Activity of a Red Pigment Isolated from *Streptomyces* Sp. PM4. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 650–656, doi:10.1016/S2221-1691(13)60131-8.
 67. Vaishnavi, M.; Manigundan, K.; Smalia, T.; N; Hini, S.U.; Gopikrishnan, V.; Kumar, A.; Hanna, L.E.; Radhakrishnan, M.; Aruni, W. Antibacterial and Anti-HIV Activity of Extracellular Pigment from *Streptomyces* Sp. S45 Isolated from Sabarimala Forest Soil, India. *Indian J. Exp. Biol.* **2020**, *58*, 861–868, doi:10.56042/ijeb.v58i12.44575.
 68. Bayram, S.; Dengiz, C.; Gerçek, Y.C.; Cetin, I.; Topcul, M.R. Bioproduction, Structure Elucidation and in Vitro Antiproliferative Effect of Eumelanin Pigment from *Streptomyces Parvus* BSB49. *Arch. Microbiol.* **2020**, *202*, 2401–2409, doi:10.1007/s00203-020-01956-2.
 69. Bayram, S. Production, Purification, and Characterization of *Streptomyces* Sp. Strain MPPS2 Extracellular Pyomelanin Pigment. *Arch. Microbiol.* **2021**, doi:10.1007/s00203-021-02437-w.
 70. Dastager, S.G.; Wen-Jun, L.; Dayanand, A.; Shu-Kun, T.; Xin-Peng, T.; Xiao-Yang, Z.; Li-Hua, X.; Cheng-Lin, J. Separation, Identification and Analysis of Pigment (Melanin) Production in *Streptomyces*. *African J. Biotechnol.* **2006**, *5*, 1131–1134.
 71. Tang-um, J.; Niamsup, H. Extracellular Amylase Activity from Endophytic *Streptomyces Griseoflavus* P4. *Chiang Mai J. Sci.* **2012**, *39*, 346–350.
 72. Singh, R.; Tiwari, M.; Singh, R.; Lee, J.-K. From Protein Engineering to Immobilization: Promising Strategies for the Upgrade of Industrial Enzymes. *Int. J. Mol. Sci.* **2013**, *14*, 1232–1277, doi:10.3390/ijms14011232.
 73. Elibol, M. Optimization of Medium Composition for Actinorhodin Production by *Streptomyces Coelicolor* A3(2) with Response Surface Methodology. *Process Biochem.* **2004**, *39*, 1057–1062, doi:10.1016/S0032-9592(03)00232-2.
 74. Elyasi Far, B.; Ahmadi, Y.; Yari Khosroshahi, A.; Dilmaghani, A. Microbial Alpha-Amylase Production: Progress, Challenges and Perspectives. *Adv. Pharm. Bull.* **2020**, *10*, 350–358, doi:10.34172/apb.2020.043.
 75. Abraham, J.; Chauhan, R. Profiling of Red Pigment Produced by *Streptomyces* Sp. JAR6 and Its Bioactivity. *3 Biotech* **2018**, *8*, 22, doi:10.1007/s13205-017-1044-7.
 76. Azimi, S.; Baserisalehi, M.; Bahador, N. Evaluation of Antimicrobial Pigment Produced by *Streptomyces Coeruleorubidus*. *Nat. Environ. Pollut. Technol.* **2014**, *13*, 641–644.
 77. Soundari, A. Parimala Gnana Mani, V.M.; Bose, V.S.C.; Jabastin, J.; Priyadarisini, V.B. A Preliminary Assessment of Yellow Pigment from *Streptomyces Parvulus* C5-5Y. *J. Pure Appl. Microbiol.* **2017**, *11*, 197–203, doi:10.22207/JIPAM.11.1.25.
 78. Vijayabharathi, R.; Bruheim, P.; Andreassen, T.; Raja, D.S.; Devi, P.B.; Sathyabama, S.; Priyadarisini, V.B. Assessment of Resistomycin, as an Anticancer Compound Isolated and Characterized from *Streptomyces*

- Aurantiacus* AAA5. *J. Microbiol.* **2011**, 49, 920–926, doi:10.1007/s12275-011-1260-5.
79. Felsenstein, J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* (N. Y). **1985**, 39, 783, doi:10.2307/2408678.
 80. Abellan-Schneyder, I.; Matchado, M.S.; Reitmeier, S.; Sommer, A.; Sewald, Z.; Baumbach, J.; List, M.; Neuhaus, K. Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. *mSphere* **2021**, 6, 10.1128/msphere.01202-20, doi:10.1128/msphere.01202-20.
 81. Labeda, D.P.; Goodfellow, M.; Brown, R.; Ward, A.C.; Lanoot, B.; Vannanneyt, M.; Swings, J.; Kim, S.-B.; Liu, Z.; Chun, J.; et al. Phylogenetic Study of the Species within the Family Streptomycetaceae. *Antonie Van Leeuwenhoek* **2012**, 101, 73–104, doi:10.1007/s10482-011-9656-0.
 82. Nicault, M.; Tidjani, A.-R.; Gauthier, A.; Dumarcay, S.; Gelhaye, E.; Bontemps, C.; Leblond, P. Mining the Biosynthetic Potential for Specialized Metabolism of a Streptomyces Soil Community. *Antibiotics* **2020**, 9, 271.
 83. Zou, Z.; Xi, W.; Hu, Y.; Nie, C.; Zhou, Z. Antioxidant Activity of Citrus Fruits. *Food Chem.* **2016**, 196, 885–896, doi:10.1016/j.foodchem.2015.09.072.
 84. Pisoschi, A.M.; Pop, A. The Role of Antioxidants in the Chemistry of Oxidative Stress: A Review. *Eur. J. Med. Chem.* **2015**, 97, 55–74, doi:10.1016/j.ejmech.2015.04.040.
 85. Alam, M.N.; Bristi, N.J.; Rafiquzzaman, M. Review on in Vivo and in Vitro Methods Evaluation of Antioxidant Activity. *Saudi Pharm. J.* **2013**, 21, 143–152, doi:10.1016/j.jsps.2012.05.002.
 86. Kim, D.-O.; Lee, K.W.; Lee, H.J.; Lee, C.Y. Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Phenolic Phytochemicals. *J. Agric. Food Chem.* **2002**, 50, 3713–3717, doi:10.1021/jf020071c.
 87. Arnao, M.B.; Cano, A.; Acosta, M. The Hydrophilic and Lipophilic Contribution to Total Antioxidant Activity. *Food Chem.* **2001**, 73, 239–244, doi:10.1016/S0308-8146(00)00324-1.
 88. Hyun, H.B.; Shrestha, S.; Boo, K.H.; Cho, S.K. Evaluation of Antioxidant Potential of Ethyl Acetate Fraction of *Rosmarinus Officinalis* L. and Its Major Components. *J. Korean Soc. Appl. Biol. Chem.* **2015**, 58, 715–722, doi:10.1007/s13765-015-0097-8.
 89. Floegel, A.; Kim, D.-O.; Chung, S.-J.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH Assays to Measure Antioxidant Capacity in Popular Antioxidant-Rich US Foods. *J. Food Compos. Anal.* **2011**, 24, 1043–1048, doi:https://doi.org/10.1016/j.jfca.2011.01.008.
 90. Arnao, M.B. Some Methodological Problems in the Determination of Antioxidant Activity Using Chromogen Radicals: A Practical Case. *Trends Food Sci. Technol.* **2000**, 11, 419–421, doi:https://doi.org/10.1016/S0924-2244(01)00027-9.
 91. Floegel, A.; Kim, D.-O.; Chung, S.-J.; Chun, O.K. Comparison of ABTS/DPPH Assays for the Detection of Antioxidant Capacity in Foods. *FASEB J.* **2010**, 24, 535.9–535.9, doi:10.1096/fasebj.24.1_supplement.535.9.
 92. Kuskoski, E.M.; Asuero, A.G.; Troncoso, A.M.; Mancini-filho, J.; Fett, R. Aplicación de Diversos Métodos Químicos Para Determinar Actividad Antioxidante En Pulpa de Frutos. *Ciência e Tecnol. Aliment.* **2005**, 25, 726–732.
 93. Thirumalai, D.; Manikkam, R.; Ramasamy, B. Antioxidant Activity of Melanin Pigment from *Streptomyces* Species D5 Isolated from Desert Soil, Rajasthan, India. *Drug Invent. Today* **2010**, 2, 12–13.
 94. Miethke, M.; Pieroni, M.; Weber, T.; Brönstrup, M.; Hammann, P.; Halby, L.; Arimondo, P.B.; Glaser, P.; Aigle, B.; Bode, H.B.; et al. Towards the Sustainable Discovery and Development of New Antibiotics. *Nat. Rev. Chem.* **2021**, 5, 726–749, doi:10.1038/s41570-021-00313-1.
 95. Byrne, M.K.; Miellet, S.; McGlinn, A.; Fish, J.; Meedya, S.; Reynolds, N.; van Oijen, A.M. The Drivers of Antibiotic Use and Misuse: The Development and Investigation of a Theory Driven Community Measure. *BMC Public Health* **2019**, 19, 1425, doi:10.1186/s12889-019-7796-8.
 96. Zhou, P.; Liu, Z.; Chen, Y.; Xiao, Y.; Huang, X.; Fan, X.-G. Bacterial and Fungal Infections in COVID-19 Patients: A Matter of Concern. *Infect. Control Hosp. Epidemiol.* **2020**, 41, 1124–1125, doi:10.1017/ice.2020.156.
 97. Langford, B.J.; So, M.; Raybardhan, S.; Leung, V.; Westwood, D.; MacFadden, D.R.; Soucy, J.-P.R.; Daneman, N. Bacterial Co-Infection and Secondary Infection in Patients with COVID-19: A Living Rapid Review and Meta-Analysis. *Clin. Microbiol. Infect.* **2020**, 26, 1622–1629, doi:10.1016/j.cmi.2020.07.016.
 98. Vaughn, V.M.; Gandhi, T.N.; Petty, L.A.; Patel, P.K.; Prescott, H.C.; Malani, A.N.; Ratz, D.; McLaughlin, E.; Chopra, V.; Flanders, S.A. Empiric Antibacterial Therapy and Community-Onset Bacterial Coinfection in Patients Hospitalized With Coronavirus Disease 2019 (COVID-19): A Multi-Hospital Cohort Study. *Clin. Infect. Dis.* **2021**, 72, e533–e541, doi:10.1093/cid/ciaa1239.
 99. Hsu, J. How Covid-19 Is Accelerating the Threat of Antimicrobial Resistance. *BMJ* **2020**, m1983, doi:10.1136/bmj.m1983.
 100. Bassetti, M.; Giacobbe, D.R. A Look at the Clinical, Economic, and Societal Impact of Antimicrobial Resistance in 2020. *Expert Opin. Pharmacother.* **2020**, 21, 2067–2071, doi:10.1080/14656566.2020.1802427.
 101. Nieuwlaat, R.; Mbuagbaw, L.; Mertz, D.; Burrows, L.L.; Bowdish, D.M.E.; Moja, L.; Wright, G.D.;

- Schünemann, H.J. Coronavirus Disease 2019 and Antimicrobial Resistance: Parallel and Interacting Health Emergencies. *Clin. Infect. Dis.* **2021**, *72*, 1657–1659, doi:10.1093/cid/ciaa773.
102. Rupp, M.E.; Fey, P.D.; Heilmann, C.; Götz, F. Characterization of the Importance of *Staphylococcus Epidermidis* Autolysin and Polysaccharide Intercellular Adhesin in the Pathogenesis of Intravascular Catheter-Associated Infection in a Rat Model. *J. Infect. Dis.* **2001**, *183*, 1038–1042, doi:10.1086/319279.
 103. Otto, M. *Staphylococcus Epidermidis* — the “accidental” Pathogen. *Nat. Rev. Microbiol.* **2009**, *7*, 555–567, doi:10.1038/nrmicro2182.
 104. Forbes, B.A.; Schaberg, D.R. Transfer of Resistance Plasmids from *Staphylococcus Epidermidis* to *Staphylococcus Aureus*: Evidence for Conjugative Exchange of Resistance. *J. Bacteriol.* **1983**, *153*, 627–634, doi:10.1128/jb.153.2.627-634.1983.
 105. Loggenberg, S.R.; Twilley, D.; De Canha, M.N.; Lall, N. Chapter 4 - Medicinal Plants Used in South Africa as Antibacterial Agents for Wound Healing. In *Medicinal Plants as Anti-Infectives*; Chassagne, F.B.T., Ed.; Academic Press, 2022; pp. 139–182 ISBN 978-0-323-90999-0.
 106. Khan, T.M.; Kok, Y.L.; Bukhsh, A.; Lee, L.-H.; Chan, K.-G.; Goh, B.-H. Incidence of Methicillin Resistant *Staphylococcus Aureus* (MRSA) in Burn Intensive Care Unit: A Systematic Review. *Germes* **2018**, *8*, 113–125, doi:10.18683/germes.2018.1138.
 107. Clebak, K.T.; Malone, M.A. Skin Infections. *Prim. Care Clin. Off. Pract.* **2018**, *45*, 433–454, doi:10.1016/j.pop.2018.05.004.
 108. Baddour, L.M.; Wilson, W.R.; Bayer, A.S.; Fowler, V.G.; Tleyjeh, I.M.; Rybak, M.J.; Barsic, B.; Lockhart, P.B.; Gewitz, M.H.; Levison, M.E.; et al. Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications. *Circulation* **2015**, *132*, 1435–1486, doi:10.1161/CIR.0000000000000296.
 109. Selvameenal, L.; Radhakrishnan, M.; Balagurunathan, R. Antibiotic Pigment from Desert Soil Actinomycetes; Biological Activity, Purification and Chemical Screening. *Indian J. Pharm. Sci.* **2009**, *71*, 499–504, doi:10.4103/0250-474X.58174.
 110. Manikkam, R.; Venugopal, G.; Ramasamy, B.; Kumar, V. Effect of Critical Medium Components and Culture Conditions on Antitubercular Pigment Production from Novel *Streptomyces* Sp D25 Isolated from Thar Desert, Rajasthan. *J. Appl. Pharm. Sci.* **2015**, *5*, 15–19, doi:10.7324/JAPS.2015.50603.
 111. Ohnishi, Y.; Furusho, Y.; Higashi, T.; Chun, H.K.; Furihata, K.; Sakuda, S.; Horinouchi, S. Structures of Grixazone A and B, A-Factor-Dependent Yellow Pigments Produced under Phosphate Depletion by *Streptomyces Griseus*. *J. Antibiot. (Tokyo)*. **2004**, *57*, 218–223, doi:10.7164/antibiotics.57.218.
 112. Lu, J.-J.; Wang, Y.-T. Identification of Anti-Cancer Compounds from Natural Products. *Chin. J. Nat. Med.* **2020**, *18*, 481–482, doi:10.1016/S1875-5364(20)30057-1.
 113. Mohan, C.D.; Rangappa, S.; Nayak, S.C.; Jadimurthy, R.; Wang, L.; Sethi, G.; Garg, M.; Rangappa, K.S. Bacteria as a Treasure House of Secondary Metabolites with Anticancer Potential. *Semin. Cancer Biol.* **2022**, *86*, 998–1013, doi:10.1016/j.semcancer.2021.05.006.
 114. Schabel, F.M.; Pittillo, R.F. Screening for and Biological Characterization of Antitumor Agents Using Microorganisms. In; 1961; pp. 223–256.
 115. Tan, L.T.H.; Chan, K.G.; Pusparajah, P.; Yin, W.F.; Khan, T.M.; Lee, L.H.; Goh, B.H. Mangrove Derived *Streptomyces* Sp. MUM265 as a Potential Source of Antioxidant and Anticancer Agents. *BMC Microbiol.* **2019**, *19*, doi:10.1186/s12866-019-1409-7.
 116. Chen, H.; Cui, J.; Wang, P.; Wang, X.; Wen, J. Enhancement of Bleomycin Production in *Streptomyces Verticillus* through Global Metabolic Regulation of N-Acetylglucosamine and Assisted Metabolic Profiling Analysis. *Microb. Cell Fact.* **2020**, *19*, 32, doi:10.1186/s12934-020-01301-8.
 117. Sajjad, W.; Ahmad, S.; Aziz, I.; Azam, S.S.; Hasan, F.; Shah, A.A. Antiproliferative, Antioxidant and Binding Mechanism Analysis of Prodigiosin from Newly Isolated Radio-Resistant *Streptomyces* Sp. Strain WMA-LM31. *Mol. Biol. Rep.* **2018**, *45*, 1787–1798, doi:10.1007/s11033-018-4324-3.
 118. Sarmiento-Tovar, A.A.; Silva, L.; Sánchez-Suárez, J.; Diaz, L. *Streptomyces*-Derived Bioactive Pigments: Ecofriendly Source of Bioactive Compounds. *Coatings* **2022**, *12*, 1858, doi:10.3390/coatings12121858.
 119. Salla, T.D.; Astarita, L. V.; Santarém, E.R. Defense Responses in Plants of Eucalyptus Elicited by *Streptomyces* and Challenged with *Botrytis Cinerea*. *Planta* **2016**, *243*, 1055–1070, doi:10.1007/s00425-015-2460-8.
 120. Kim, S.S.; Lee, C.K.; Kang, S.S.; Jung, H.A.; Choi, J.S. Chlorogenic Acid, an Antioxidant Principle from the Aerial Parts of *Artemisia Iwayomogi* That Acts on 1,1-Diphenyl-2-Picrylhydrazyl Radical. *Arch. Pharm. Res.* **1997**, *20*, 148–154, doi:10.1007/BF02974002.
 121. Saqib, M.; Iqbal, S.; Mahmood, A.; Akram, R. Theoretical Investigation for Exploring the Antioxidant Potential of Chlorogenic Acid: A Density Functional Theory Study. *Int. J. Food Prop.* **2016**, *19*, 745–751, doi:10.1080/10942912.2015.1042588.
 122. Keudell, K.C.; Huang, J.K.; Wen, L.; Klopfenstein, W.E.; Bagby, M.O.; Lanser, A.C.; Plattner, R.D.; Peterson,

- R.E.; Weisleder, D. Fatty Acids Enhanced Tubermycin Production by *Pseudomonas* Strain 2HS. *Microbios* **2000**, *102*, 27–38.
123. Weber, W.; Zähler, H.; Siebers, J.; Schröder, K.; Zeeck, A. [Metabolic products of microorganisms. 175. Tetracenomycin C (author's transl)]. *Arch. Microbiol.* **1979**, *121*, 111–116, doi:10.1007/BF00689973.
 124. Remali, J.; Sahidin, I.; Aizat, W.M. Xanthone Biosynthetic Pathway in Plants: A Review. *Front. Plant Sci.* **2022**, *13*, doi:10.3389/fpls.2022.809497.
 125. Leetanasaksakul, K.; Koomsiri, W.; Suga, T.; Matsuo, H.; Hokari, R.; Wattana-Amorn, P.; Takahashi, Y.; Shiomi, K.; Nakashima, T.; Inahashi, Y.; et al. Sattahipmycin, a Hexacyclic Xanthone Produced by a Marine-Derived *Streptomyces*. *J. Nat. Prod.* **2022**, *85*, 1211–1217, doi:10.1021/acs.jnatprod.1c00870.
 126. Matselyukh, B.P.; Matselyukh, D.Y.; Golembiovska, S.L.; Polishchuk, L. V; Lavrinchuk, V.Y. Isolation of *Streptomyces Globisporus* and *Blakeslea Trispora* Mutants with Increased Carotenoid Content. *Mikrobiol. Z.* **2013**, *75*, 10–16.
 127. Han, Z.; Zhao, X.; Zhang, E.; Ma, J.; Zhang, H.; Li, J.; Xie, W.; Li, X. Resistomycin Induced Apoptosis and Cycle Arrest in Human Hepatocellular Carcinoma Cells by Activating P38 MAPK Pathway In Vitro and In Vivo. *Pharmaceuticals* **2021**, *14*, doi:10.3390/ph14100958.
 128. Shaaban, K.A.; Singh, S.; Elshahawi, S.I.; Wang, X.; Ponomareva, L. V; Sunkara, M.; Copley, G.C.; Hower, J.C.; Morris, A.J.; Kharel, M.K.; et al. Venturicidin C, a New 20-Membered Macrolide Produced by *Streptomyces* Sp. TS-2-2. *J. Antibiot. (Tokyo)*. **2014**, *67*, 223–230, doi:10.1038/ja.2013.113.
 129. Tan, L.T.; Mahendra, C.K.; Yow, Y.; Chan, K.; Khan, T.M.; Lee, L.; Goh, B. *Streptomyces* Sp. MUM273b: A Mangrove-derived Potential Source for Antioxidant and UVB Radiation Protectants. *Microbiologyopen* **2019**, *8*, doi:10.1002/mbo3.859.
 130. Berger, H.; Bacher, M.; Labuda, R.; Eppel, I.M.; Bayer, F.; Sulyok, M.; Gasparotto, E.; Zehetbauer, F.; Doppler, M.; Gratzl, H.; et al. Polaramycin B, and Not Physical Interaction, Is the Signal That Rewires Fungal Metabolism in the *Streptomyces–Aspergillus* Interaction. *Environ. Microbiol.* **2022**, *24*, 4899–4914, doi:10.1111/1462-2920.16118.
 131. Ebrahim, W.; El-Neketi, M.; Lewald, L.-I.; Orfali, R.S.; Lin, W.; Rehberg, N.; Kalscheuer, R.; Daletos, G.; Proksch, P. Metabolites from the Fungal Endophyte *Aspergillus Austroafricanus* in Axenic Culture and in Fungal–Bacterial Mixed Cultures. *J. Nat. Prod.* **2016**, *79*, 914–922, doi:10.1021/acs.jnatprod.5b00975.
 132. Kemung, H.M.; Tan, L.T.-H.; Chan, K.-G.; Ser, H.-L.; Law, J.W.-F.; Lee, L.-H.; Goh, B.-H. Antioxidant Activities of *Streptomyces* Sp. Strain MUSC 14 from Mangrove Forest Soil in Malaysia. *Biomed Res. Int.* **2020**, *2020*, 6402607, doi:https://doi.org/10.1155/2020/6402607.
 133. Lima, S.M.A.; Pereira, P.S.; Silva, B.I.M. da; Ribeiro, N.E.; Borba, E.F. de O.; Oliveira Tintino, C.D. de M.; Randau, K.P.; Coutinho, H.D.M.; Lima-Gomes, G.M. de S.; Chávez-González, M.L.; et al. Antioxidant, Antimicrobial and Cytotoxic Activities of Secondary Metabolites from *Streptomyces* Sp. Isolated of the Amazon-Brazil Region. *Res. Soc. Dev.* **2021**, *10*, e366101018974, doi:10.33448/rsd-v10i10.18974.
 134. de Moura, P.A.; de Albuquerque Lima, T.; Ferreira, M.R.A.; Soares, L.A.L.; de Souza Lima, G.M.; Napoleão, T.H.; da Silva, M.V.; de Oliveira, A.P.S.; Paiva, P.M.G. The Relevance of Actinobacteria as Sources of Antioxidant Compounds: Evaluation of *Streptomyces* Isolates from Rhizosphere Collected at Brazilian Caatinga. In *Microbial and Natural Macromolecules - Synthesis and Applications*; Das, S., Dash, H.R.B.T.-M. and N.M., Eds.; Academic Press: London UK, 2021; pp. 401–418 ISBN 978-0-12-820084-1.

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