EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF PIGMENT EXTRACTS AND 16S rRNA-BASED IDENTIFICATION OF PIGMENT-PRODUCING BACTERIA ISOLATED FROM SOIL

ALBAYRAK ISKENDER, N.

Programme of Medical Laboratory Techniques, Vocational School of Health Services, Artvin Çoruh University, Artvin, Türkiye
(e-mail: naiskender@artvin.edu.tr; phone: +90-466-215-6025)

(Received 3rd May 2025; accepted 30th Jun 2025)

Abstract. This study aimed to isolate pigment-producing bacteria from soil samples collected in the Artvin province for the first time, extract their pigments, and evaluate their antimicrobial activities. Fifteen bacterial isolates were obtained and identified through morphological and biochemical characterization, followed by 16S rRNA gene sequencing. The isolates were identified as *Arthrobacter ruber* (P1, P20), *Rhodococcus* sp. (P2, P6, P7), *Micrococcus yunnanensis* (P4), *Metabacillus indicus* (P5), *Arthrobacter* sp. (P9, P10), *Peribacillus muralis* (P12), *Chitinophaga arvensicola* (P16), *Streptomyces lincolnensis* (P17), *Arthrobacter agilis* (P18, P21), and *Nocardia tenerifensis* (P22). These isolates produced pigments in various hues such as red, pinkish-brown, yellow, yellowish-orange, light salmon-pink, and brownish tones. The antimicrobial activity of the pigment extracts was tested using dry disc diffusion method against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis*. Only the methanol and acetone extracts of pigments from *S. lincolnensis* and *N. tenerifensis* exhibited inhibitory activity, specifically against *S. aureus*. Notably, the acetone extract of *N. tenerifensis* (P22) demonstrated the highest antimicrobial effect with a 17.6 mm inhibition zone. High-Performance Liquid Chromatography (HPLC) analysis confirmed the presence of bioactive compounds in these extracts. The findings highlight the significant biotechnological potential of these pigment-producing soil bacteria as sources of natural antimicrobial agents.
Keywords: *soil bacteria, bacterial pigments, pigment extraction, antimicrobial activity, HPLC, bioactive compounds*

Introduction

Pigments are compounds that absorb light, resulting in the manifestation of specific colors. Natural pigments can be derived from a variety of sources, including ores, plants, insects, and microorganisms. Pigments synthesized by microorganisms are notable for their colorant properties as well as their bio-pharmacological effects, including antimicrobial, antioxidant, and anticancer activities. These characteristics expand the application potential of microbial pigments, not only in industrial fields but also in medical and biotechnological domains. Additionally, advantages such as stability, bioavailability, productivity, ease of processing, and high efficiency further enhance the preference for microbial pigments (Behera et al., 2021). In addition to these technical and biological advantages, microbial pigments offer significant benefits in terms of practical production compared to traditional natural pigment sources such as plants and animals. Their ability to avoid seasonal production issues and achieve high productivity makes them a sustainable and efficient option for natural pigment production (Venil and Lakshmanaperumalsamy, 2009; Goswami et al., 2010). Due to these characteristics, microorganisms present a strong economic and environmental potential in pigment production.

Pigment-producing microorganisms include groups such as bacteria, fungi, yeasts, and microalgae, and they are commonly found in nature. The pigments produced in high

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

quantities and with stability involve carotenoids, canthaxanthin, flavonoids, prodigiosin, tetrapyrroles, and astaxanthin (Goswami et al., 2010). Among the various pigment groups, carotenoids are the most commonly observed and extensively studied compounds. Their potential effects on the suppression of different cancer types and the enhancement of immune responses have also been documented (Krinsky et al., 2005).

Actinomycetes are microorganisms capable of producing endopigments retained in the mycelium or exopigments that can spread into the environment, in various colors such as blue, purple, red, pink, yellow, green, brown, and black (Thompson et al., 2002). Moreover, they have the potential to produce new antibiotics in addition to pigments, which are significant secondary metabolites from an industrial perspective. Many of these antibiotics contain pigments (Sarika, 2021). For example, *Streptomyces coelicolor* produces prodigiosin and actinorhodin, while *Chromobacterium violaceum* produces the violacein pigment (Ahmad et al., 2012). Examples of other pigment-producing bacterial species include; *Serratia marcescens* (prodigiosin), *Xanthophyllomyces dendrorhous* (astaxanthin), *Flavobacterium* species (zeaxanthin), *Bradyrhizobium* sp. (canthaxanthin), *Pseudomonas aeruginosa* (pyocyanin), *Staphylococcus aureus* (golden staphyloxanthin), and *Pseudomonas* spp. (blue-green phycocyanin) (Malik et al., 2012; Liu and Nizet, 2009).

Bacterial-derived pigments have several advantages, such as being biologically degradable, easy to extract, readily available, and producible on inexpensive substrates. Furthermore, these pigments can be evaluated in biopharmaceutical applications as anticancer, antibacterial, and antiproliferative agents (Choubey et al., 2021).

Microbial pigments have become a significant area of research in recent years due to all these potentials, particularly in the biotechnology, food, cosmetic, and pharmaceutical industries. However, studies on the isolation of microbial pigments from different geographical regions and the investigation of the pigment production capacities of microorganisms obtained from these soils are quite limited. Artvin province is a region with high biodiversity and rich ecosystems, and there have been no studies conducted on microbial pigment production from this soil.

This study aims to isolate microbial pigment-producing bacteria from the soils of Artvin province, determine their pigment production capacities, and investigate the antimicrobial activities of these pigments. Additionally, the analysis of the bioactive components of the pigments using high-performance liquid chromatography (HPLC) method, with the goal of identifying potential components that could contribute to biological activity, is also targeted.

Materials and methods

Collection of soil samples

Soil samples were collected during a field study conducted in Artvin province between September and October 2021. After removing the top 2-3 cm of soil, samples were taken from the lower 5-7 cm layer under aseptic conditions, placed in sterile 100 ml plastic containers, and stored at 5°C until use upon arrival at the laboratory. A total of 23 different soil samples were used in the study.

Isolation, purification and characterization of bacteria

Soil suspensions were prepared from the soil samples collected during the field study using sterile physiological saline, and the suspensions were diluted. Dilutions were spread

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

onto Nutrient agar (NA) plates for inoculation. The inoculated Petri dishes were incubated at 30°C for 3-6 days. Following incubation, only pigmented (colored) bacterial colonies were selected, purified, and stored at -20°C for use in subsequent studies.

The cultural, morphological (cell morphology, motility), biochemical (Gram reaction, catalase, oxidase) (Harley and Prescott, 2002), and other enzymatic activities, as well as phenotypic characteristics, were determined using the API 20E, API 20NE, API Coryne, API Staph, and API 50CH test kits (bioMérieux, France).

The biochemical characterization of the isolates was performed using API test systems commonly employed in the literature for the respective genera, taking into account the identifications based on 16S rRNA analysis. API 20E and API 20NE were used for *Arthrobacter* (See-Too et al., 2017), API 20NE and API Coryne for *Rhodococcus* (Li et al., 2015; Funke et al., 1997), API 50CHB and API 20E for *Metabacillus* and *Peribacillus* species (Kangale et al., 2021; Rodríguez et al., 2022), API Staph for *Micrococcus* (Choi et al., 2011), API 20E for *Streptomyces* (Espinoza et al., 2013), and API 20NE and API 20E for *Nocardia* (Zhou et al., 2020). This approach was employed to elucidate the biochemical profiles of the isolates. Additionally, the experimental workflow encompassing isolation, characterization, and test selection stages is schematically presented in *Figure 1*.

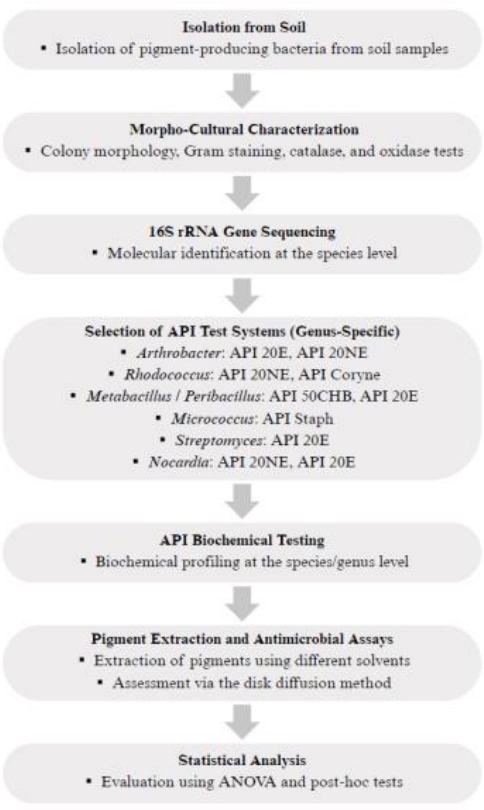


Figure 1. *Flowchart of experimental design and strain selection*

Molecular identification of bacteria and phylogenetic tree construction

Bacterial isolates were incubated in Nutrient broth (NB) at 30°C for 18 h. Genomic DNA was isolated from the bacteria using a DNA isolation kit (Norgen, Biotek Corp.) obtained from a commercial supplier, following the specified protocol.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

To amplify the 16S rRNA gene region, the primers 16SF (TCC GTA GGT GAA CCT GCG G) and 16SR (TCC TCC GCT TAT TGA TAT GC) were used. The PCR reaction mixture, with a total volume of 20 µl, was prepared according to the protocol of the commercial PCR Master Mix (A.B.TM). For each sample, a total of 20 µl PCR mixture was formulated, containing 10 µl Hs-Taq mm, 1 µl Forward primer (0.5 µM), 1 µl Reverse primer (0.5 µM), 4 µl template DNA, and 4 µl dH2O. The prepared mixtures were placed in a PCR thermal cycler and subjected to denaturation at 94°C for 5 min, followed by 35 cycles with the following steps: denaturation at 94°C for 30 s, annealing at 57°C for 45 s, and extension at 72°C for 45 s. The PCR process was completed with a final extension at 72°C for 7 min. The amplified DNA fragments were separated by electrophoresis in 1% 1 × TAE buffer, and the presence of the target gene region was confirmed. The obtained amplicons were then sent to a commercial company (Macrogen, Netherlands) for sequencing, and bidirectional sequencing was performed. The obtained sequences were compared with the closest related reference strains in the NCBI GenBank database to identify the species (Altschul et al., 1990). The sequences were edited using BioEdit software (version 7.09) and subsequently aligned with the ClustalW algorithm (Hall, 1999). Phylogenetic relationship analysis was performed using MEGA11 software (Tamura et al., 2021).

Extraction of bacterial pigments

Bacterial isolates producing pigments were incubated at 30°C on NA medium for 3-6 days to enhance pigment production. After incubation, the developed colonies were collected from the agar surface using a sterile inoculation loop and transferred to NB medium. The obtained suspensions were centrifuged at 5000 rpm for 10–15 min to remove the supernatant. For each isolate, two different solvents were added to the bacterial cell pellets for pigment extraction. These solvents, prepared in a 1:5 ratio, were 96% (v/v) methanol and 96% (v/v) acetone, with 5 ml of each added to the pellets. The mixtures were incubated in a shaking incubator for 24–48 h. After extraction, the tubes were left overnight in a refrigerator. The same procedure was repeated until the cell pellets became completely colorless. The extracts were sterilized using sterile syringe filters with a pore size of 0.45 µm. Subsequently, the solvents were removed using an evaporator operating at 40–50°C. The drying process was completed in a lyophilizer, and the obtained pigment extracts were converted into powder form. Finally, the extracts were stored at +4°C for use in antimicrobial analyses.

Antimicrobial activities of pigment extracts

The antimicrobial activity of bacterial pigment extracts was evaluated using the disc diffusion method against *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Proteus mirabilis* (ATCC 43071), and *Candida albicans* (ATCC 10231). The bacterial strains were incubated at 30°C on NA medium, and the yeast was incubated at 25°C on Sabouraud Dextrose Agar (SDA) for 18–24 h. The microbial density was adjusted to approximately $1x10^8$ cfu/mL according to the McFarland 0.5 standard, and 100 µL of the inoculum was transferred to Mueller-Hinton Agar (MHA) plates, spreading it with a sterile cotton swab. Subsequently, discs impregnated with the pigment extracts were placed onto the agar plates under aseptic conditions. Gentamicin (CN) and Fluconazole (FLU) were used as positive controls, while the solvents used in the extraction process served as negative controls. The bacteria and yeast strains were

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

incubated at 37°C and 27°C for 24–48 h, respectively. After incubation, the zone of inhibition surrounding the discs was measured with a ruler, and the results were evaluated. All experiments were performed in triplicate.

Analysis of pigment extracts by high-performance liquid chromatography (HPLC)

The analysis of pigment extracts was conducted using the AGILENT 1260 INFINITY II HPLC system (Agilent Technologies, Santa Clara, CA, USA). The HPLC-DAD (Diode Array Detector) technique was applied with a phenolic acetonitrile calibration method. Chromatographic separation was performed using an ACE 5 C18 column (250 × 4.6 mm inner diameter). The mobile phase consisted of a mixture of (A) acetonitrile and (B) a 1.5% acetic acid solution. The solvent gradient was applied as follows: initially 15% A and 85% B, and at 29 min, 40% A and 60% B. The analysis system was configured with a 1260 DAD WR detector (wavelengths of 250, 270, and 320 nm), a 1260 Quaternary pump (flow rate of 0.7 mL/min), a 1260 Vialsampler (10 µL injection volume), and a G7116A column oven (35°C). The quantities of phenolic compounds were determined using calibration curves prepared with standards at concentrations of 25, 50, 75, 100, 200, and 300 µg/mL. After dilution, the samples were analyzed using the HPLC-DAD system. The HPLC gradient acetonitrile was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), and methanol was sourced from Merck KGaA (Darmstadt, Germany). All phenolic standards were obtained from reliable commercial sources.

Scanning electron microscopy

The antimicrobial activity of the P17 and P22 isolates was evaluated by incubating them on nutrient agar plates at 30°C for 5-7 days. The colonies that grew were collected and homogenized in 1-2 µL of physiological saline on aluminum plates. The homogenized suspension was then spread in a thin layer, air-dried, and gold-coated. The prepared plates were examined under a Zeiss Evo LS10 (Germany) scanning electron microscope at a voltage of 10 kV, and electron micrographs were taken.

Results

Pigmented bacteria isolated from soil

In this study, a total of 15 bacterial isolates, belonging to eight different species capable of pigment production, were obtained from nine soil samples collected from the Artvin province. The soil sampling sites were randomly selected within the study area. GPS coordinates and elevation data of the sampling points are presented in *Table 1*.

The bacterial isolates formed red, pinkish-brown, yellow, yellowish-orange, light salmon pink, and brownish pigments on NA medium (*Table 2*). Isolate P17 produced extracellular pigments, whereas the other isolates produced intracellular pigments (*Fig. 2*). Except for isolate P16, all the other isolates were found to be Gram-positive. Isolates P2, P5, P6, P7, P12, and P16 exhibited rod morphology, while isolates P17 and P22 displayed filamentous and irregular rod shapes. Isolates P1, P9, P10, P18, P20, and P21 exhibited coccoid morphology, and isolate P4 exhibited coccus morphology. Isolates P5, P12, P16 (gliding motility), P18, and P21 were motile, whereas the other isolates were non-motile. Isolate P16 was weakly catalase-positive, while all other isolates were strongly catalase-positive. The oxidase test results were positive for isolates P12, P16, P18, P21, and P22.

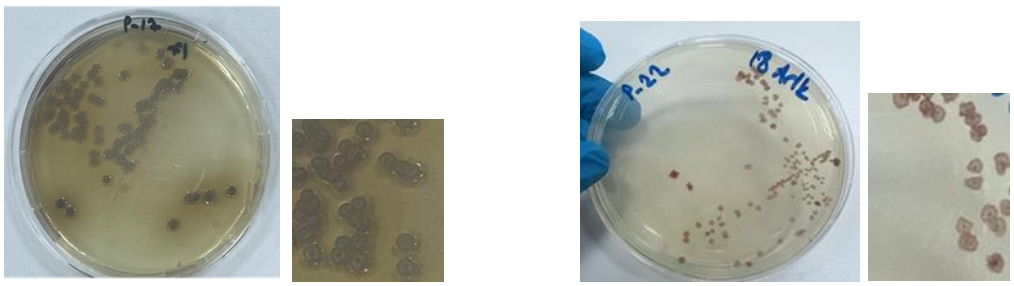
http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Table 1. *Location and elevation data of soil samples for pigment-producing bacterial isolates*

Isolate ID	Latitude (N)	Longitude (E)	Elevation (m)
P4, P18, P20	41° 14’ 23.8”	041° 21’ 0.56”	1274
P5, P6, P21	41° 14’ 0.57”	041° 51’ 13.0”	1152
P7, P17	41° 13’ 38.3”	041° 51’ 22.0”	1067
P16	41° 14’ 52.6”	041° 50’ 53.5”	1528
P9	41° 12’ 00.1”	041° 47’ 30.0”	540
P10	41° 11’ 57.7”	041° 43’ 33.4”	494
P12	41° 10’ 23.5”	041° 45’ 12.4”	449
P22	41° 12’ 42”	041° 46’ 58.4”	520

Table 2. *Pigment production of bacterial isolates isolated from soil*

Pigments	Isolate code	Isolates
Red	P1	*Arthrobacter ruber*
	P18	*Arthrobacter agilis*
	P20	*Arthrobacter ruber*
	P21	*Arthrobacter agilis*
Pinkish-brown	P22	*Nocardia tenerifensis*
Yellow	P4	*Micrococcus yunnanensis*
	P9	*Arthrobacter sp.*
	P10	*Arthrobacter sp.*
Yellowish-orange	P16	*Chitinophaga arvensicola*
	P2	*Rhodococcus sp.*
Orange	P5	*Metabacillus indicus*
	P6	*Rhodococcus sp.*
	P7	*Rhodococcus sp.*
Light salmon pink	P12	*Peribacillus muralis*
Brownish	P17	*Streptomyces lincolnensis*



(A) (B)

Figure 2. *Pigment production on NA medium. (A) Extracellular pigment production and diffusion in the medium by S. lincolnensis (P17) isolate. (B) Intracellular pigment accumulation in the N. tenerifensis (P22) isolate*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

According to the API 20E test results, all the isolates analyzed exhibited positive results for the L-tryptophan and VP (sodium pyruvate) tests. However, negative results were obtained for the lysine decarboxylase, ornithine decarboxylase, trisodium citrate, hydrogen sulfide (H_2S), and indole tests. The P17 isolate was the only one that demonstrated a positive reaction in the arginine dihydrolase and urease tests (*Table 3*).

Table 3. *API 20E test results of bacterial isolates*

Tests	P1	P5	P9	P10	P12	P16	P17	P18	P20	P21	P22
β-galaktosidase	+	+	+	-	+	-	+	-	+	-	-
Arjinin dihydrolase	-	-	-	-	-	-	+	-	-	-	-
Lysine dekarboxylase	-	-	-	-	-	-	-	-	-	-	-
Ornithine dekarboxylase	-	-	-	-	-	-	-	-	-	-	-
Trisodium citrate	-	-	-	-	-	-	-	-	-	-	-
H_2S (sodium thiosulfate)	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	+	-	-	-	-
L-tryptophan	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-
VP (sodium pyruvate)	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	wp	+	-	-	wp	+	+	wp	wp	-	-
Fermentation											
Glucose	-	+	-	+	+	+	+	-	-	-	+
D-mannitol	-	+	-	+	+	+	+	-	-	-	+
Inositol	-	+	-	+	+	+	+	-	-	-	+
D-sorbitol	-	+	-	+	+	+	+	-	-	-	+
L-rhamnose	-	+	-	+	+	+	+	-	-	-	+
D-saccharose	-	+	-	+	+	+	+	-	-	-	+
D-melibiose	-	+	-	+	+	+	+	-	-	-	+
Amygdaline	-	+	-	+	+	+	+	-	-	-	+
L- arabinose	-	+	-	+	+	+	+	-	-	+	+

+: positive result, wp: weakly positive, –: negative result

Based on the API 20NE test results, all examined isolates tested negative for indole production, arginine dihydrolase activity, and the utilization of capric acid and adipic acid. All isolates exhibited positive results for esculin hydrolysis. Utilization of phenylacetic acid was observed only in the P10 isolate, whereas urease activity was detected exclusively in the P6 isolate (*Table 4*).

In the API 50CH tests, the P5 and P12 isolates were able to utilize D-glucose, D-fructose, N-acetylglucosamine, D-melibiose, D-sucrose, and D-trehalose. D-mannose, D-mannitol, D-sorbitol, and arbutin gave positive results only in the P12 isolate, whereas methyl-α-D-glucopyranoside, esculin-iron citrate, D-raffinose, starch, and glycogen were positive solely in the P5 isolate. Glycerol, methyl-α-D-mannopyranoside, amygdalin, D-lactose, inulin, and D-melezitose showed weak positive reactions only in the P5 isolate, while L-arabinose, D-ribose, D-galactose, L-sorbose, inositol, and D-maltose showed weak positive results exclusively in the P12 isolate (*Table 5*).

According to the API Staph test results, the P4 isolate exhibited positive results for xylitol and melibiose utilization, alkaline phosphatase activity, and acetoin (Voges–Proskauer) production. Conversely, it tested negative for glucose and fructose utilization as well as nitrate reduction (*Table 6*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Table 4. *API 20NE test results of bacterial isolates*

Tests	P1	P2	P6	P7	P9	P10	P16	P18	P20	P21	P22
NO_3 - NO_2	-	+	+	-	+	+	+	-	-	-	+
Indole	-	-	-	-	-	-	-	-	-	-	-
Glucose fermentation	-	+	-	+	-	+	-	+	+	+	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	+	-	-	-	-	-	-	-	-
Esculin hidrolizi	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	wp	wp	-	-	-	+	+	wp	wp	-	-
β-Galactosidase	+	+	-	-	+	+	-	+	+	+	-
Assimilation											
D-Glucose	-	-	-	-	+	+	wp	-	-	-	-
L-Arabinose	-	+	+	+	-	+	-	-	-	-	-
D-Mannose	-	-	-	-	+	+	+	-	-	-	-
D-Mannitol	-	+	+	+	+	+	-	-	-	-	-
N-Acetyl-glucosamine	-	wp	wp	wp	+	-	+	-	-	-	-
D-Maltose	-	-	-	-	+	+	+	-	-	-	-
Potasyum Gluconate	-	+	+	+	+	+	-	-	-	-	-
Capric asit	-	-	-	-	-	-	-	-	-	-	-
Adipic asit	-	-	-	-	-	-	-	-	-	-	-
Malate	-	+	+	+	+	+	-	-	-	-	-
Citrate	-	+	+	+	+	wp	-	-	-	-	-
Phenyl acetic asit	-	-	-	-	-	+	-	-	-	-	-

+: positive result, wp: weakly positive, –: negative result

Regarding the API Coryne test, the P2, P6, and P7 isolates yielded positive results for esculin hydrolysis (ESC) and mannitol fermentation (MAN). D-ribose was utilized only by the P7 isolate, while lactose (LAC) and sucrose (SAC) showed weakly positive reactions exclusively in the P2 isolate. Additional phenotypic and biochemical characteristics of the isolates are presented in *Tables 2* and *3* (*Table 7*).

16S rRNA sequence-based bacterial identification and phylogenetic tree

16S rRNA gene sequencing analysis revealed the identification of the isolates as follows: *Arthrobacter ruber* (P1, P20), *Rhodococcus sp.* (P2, P6, P7), *Micrococcus yunnanensis* (P4), *Metabacillus indicus* (P5), *Arthrobacter sp.* (P9, P10), *Peribacillus muralis* (P12), *Chitinophaga arvensicola* (P16), *Streptomyces lincolnensis* (P17), *Arthrobacter agilis* (P18, P21), and *Nocardia tenerifensis* (P22). The sequencing data for each isolate were deposited in the GenBank database, with the following corresponding accession numbers: PV569727 (P1), PV569728 (P2), PV569729 (P4), PV569730 (P5), PV569731 (P6), PV569732 (P7), PV569733(P9), PV569734 (P10), PV569735 (P12), PV569736 (P16), PV569737 (P17), PV569738 (P18), PV569739 (P20), PV569740 (P21), and PV569741 (P22). The phylogenetic analysis demonstrated a high degree of similarity between the isolates and the respective reference strains. Phylogenetic relationships were inferred based on a phylogenetic tree constructed using the Neighbor-Joining (NJ) method with bootstrap analysis (1000 replicates), as shown in *Figure 3*.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Table 5. *API 50CH test results of P5 and P12 isolate*

Tests	P5/24 h	48 h	P12/24 h	48 h
Glycerol	-	wp	-	-
Erythritol	-	-	-	-
D-Arabinose	-	-	-	-
L-Arabinose	-	-	-	wp
D-Ribose	-	-	-	wp
D-Xylose	-	-	-	-
L-Xylose	-	-	-	-
D-Adonitol	-	-	-	-
Methyl-β-D-xylopyranoside	-	-	-	-
D-Galactose	-	-	-	wp
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	-	-	-	+
L-Sorbose	-	-	-	wp
L-Rhamnose	-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	wp
D-Mannitol	-	-	-	+
D-Sorbitol	-	-	-	+
Methyl-α-D-mannopyranoside	wp	wp	-	-
Methyl- α-D-glucopyranoside	+	+	-	-
N-Acetylglucosamine	-	+	+	+
Amygdalin	-	wp	-	+
Arbutin	-	-	-	+
Esculin-ferric citrate	+	+	-	-
Salisin	-	wp	-	wp
D-Celiobiose	-	wp	-	wp
D-Maltose	+	+	-	wp
D-Lactose (bovine origin)	-	wp	-	-
D-Melibiose	+	+	-	+
D-Saccharose	+	+	-	+
D-Trehalose	+	+	+	+
Inulin	-	wp	-	-
D-Melezitose	-	wp	-	-
D-Raffinose	+	+	-	-
Starch	+	+	-	-
Glycogen	+	+	-	-
Xylitol	-	-	-	-
Gentiobiose	-	-	-	-
D-Turanose	-	-	-	-
D-Lyxose	-	-	-	-
D-Tagatose	-	-	-	-
D-Fucose	-	-	-	-
L-Fucose	-	-	-	-
D-Arabitol	-	-	-	-
L-Arabitol	-	-	-	-
Potassium gluconate	-	-	-	wp
Potassium 2-ketogluconate	-	-	-	wp
Potassium 5-ketogluconate	-	-	-	+

+: positive result, wp: weakly positive, –: negative result

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Table 6. *API Staph test results of the P4 isolate*

Tests	P4
Glucose	-
Fructose	-
Mannose	wp
Maltose	wp
Lactose	wp
Trehalose	wp
Mannitol	wp
Xylitol	+
Melibiose	+
Nitrate to nitrite	-
Alkaline phosphatase production	+
Acetyl methyl carbinol production	+
Raffinose	wp
Xylose	wp
Saccharose	-
Acid from alpha-methylglucoside	-
Acid from N-acetylglucosamine	-
Arginine dihydrolase	-
Urease	+

+: positive result, wp: weakly positive, –: negative result

Table 7. *API Coryne test results of some bacterial isolates*

Tests	P2	P6	P7
NIT (Nitrate reduction)	-	-	-
PYZ (Pyrazinamidase)	-	-	-
PYRA (Pyrrolidonyl Arylamidase)	-	-	-
PAL (Alkaline phosphatase)	-	-	-
βGUR (β-glucuronidase)	-	-	-
βGAL (β-galactosidase)	-	-	-
αGLU (α-glucosidase)	-	-	-
βNAG (N-acetyl β-glucosaminidase)	-	-	-
ESC (Esculin hydrolysis)	+	+	+
URE (Urease)	-	+	-
GEL (Gelatin hydrolysis)	-	-	-
GLU (Glucose)	-	+	+
RIB (Ribose)	-	-	wp
XYL (Xylose)	-	-	-
MAN (Mannitol)	+	+	+
MAL (Maltose)	-	-	-
LAC (Lactose)	wp	-	-
SAC (Sucrose)	wp	+	+
GLYG (Glycogen)	-	-	-

+: positive result, wp: weakly positive, –: negative result

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

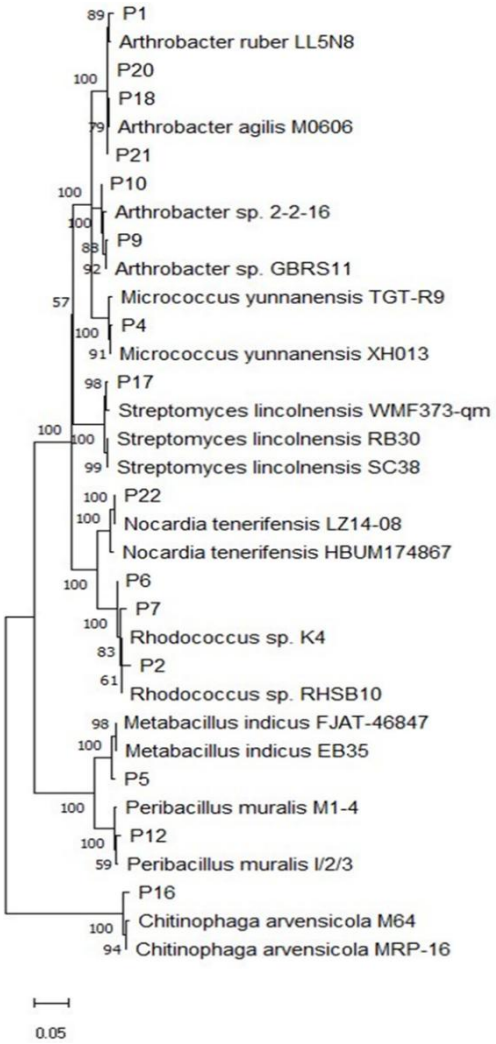


Figure 3. *Phylogenetic relationships of pigment-producing bacteria (MEGA Software 11)*

Extraction performance of bacterial pigments

Pigments from the bacterial isolates were extracted using methanol and acetone solvents. A total of twenty-five pigment extracts were successfully obtained through these procedures. No pigment production was observed in the P4, P12, and P17 isolates during acetone extraction. Additionally, the P7 isolate exhibited no pigment formation in either solvent treatment.

The obtained extracts were subjected to solvent removal using an evaporator, followed by drying using a lyophilizer. The pigments, converted into powder form, were stored under appropriate conditions in Eppendorf tubes until use.

Antimicrobial activity of pigment extracts

In this investigation, antimicrobial activity was exclusively observed in the methanol extract of P17 (14.3 mm) and the acetone extract of P22 (17.6 mm) among the bacterial pigment extracts. These extracts demonstrated efficacy solely against *Staphylococcus aureus*, with no measurable inhibition zones against *Escherichia coli*, *Proteus mirabilis*, or *Candida albicans* (*Fig. 4*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

(A) (B) (C)

Figure 4. *Antimicrobial Effect of P17 Methanol and P22 Acetone Extracts on S. aureus. (A) Inhibition zones observed in the first trial. (B) Inhibition zones observed in the second trial. (C) Inhibition zones observed in the third trial. P17M: P17 Methanol extract, P22M: P22 Methanol extract, P22A: P22 Acetone extract, NK: Negative control, PK: Positive control (Gentamicin)*

Statistical analyses, including one-way ANOVA followed by Bonferroni and LSD post hoc tests, were performed to compare the antimicrobial activities of the tested treatments (P17 methanol extract, P22 acetone extract, and gentamicin) against *Staphylococcus aureus*. The results revealed statistically significant differences among the groups (ANOVA, $F = 208.385$, $p < 0.001$). The P22 extract demonstrated significantly greater antibacterial activity than the P17 extract ($p < 0.001$), but its effect remained significantly lower than that of gentamicin ($p < 0.001$). The results of the statistical comparisons, including mean inhibition zones and significance levels, are summarized in *Table 8*.

Table 8. *Antimicrobial activity observed only in two bacterial pigment extracts*

Microorganisms tested	P17 M	P22M	IZ (mm ± SD)* P22A	CN*	FLU*	NC*
S. aureus	14.3 ± 0.26[a]	-	17.6 ± 0.53[b]	20.3 ± 0.20[c]	nt	-
E. coli	-	-	-	20.3	nt	-
P. mirabilis	-	-	-	20	nt	-
C. albicans	-	-	-	nt	28.3	-

*IZ: Inhibition Zone; values shown as mean ± SD (standard deviation in mm), CN: Gentamicin, FLU: Fluconazole, NC: Negative control, –: No inhibition zone, nt: Not tested. All experiments were performed in triplicate

Bioactive compounds in antimicrobial active extracts

In this study, phenolic compounds, flavonoids, and ascorbic acid in the P22 acetone and P17 methanol pigment extracts were identified using HPLC-DAD. The HPLC analysis revealed that the P22 acetone extract contained approximately seven components, some of which could not be quantified due to their concentration being below the calibration point or present in trace amounts. The most abundant compounds identified in the P22 pigment extract were ascorbic acid, catechin, and rosmarinic acid, whereas in the P17 pigment extract, the predominant compounds were gallic acid and rosmarinic acid. Notably, the P22 extract contains ascorbic acid, catechin, and rosmarinic acid, but lacks gallic acid. Ascorbic acid was found to be the most abundant compound in the P22 extract, while gallic acid was the most prevalent compound in the P17 extract.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Rosmarinic acid, a phenolic compound, was present in both extracts, but the P22 extract contained a higher concentration of rosmarinic acid (3.41 ppm). The components in the P22 acetone and P17 methanol extracts are presented in *Table 9*.

Table 9. *Bioactive compounds in P22 acetone and P17 methanol pigment extracts*

Compound	P22 acetone extract ppm (mg/L)	P17 methanol extract ppm (mg/L)
Vitamin		
Ascorbic acid	59.87	-
Phenolics		
Gallic acid	-	10.3
Vanillic acid	nd	nd
Coumaric acid	nd	nd
Caffeic acid	-	nd
Ferulic acid	nd	nd
Rosmarinic acid	3.41	2.26
Flavonoids		
Catechin	18.52	nd
Epicatechin	-	nd
Myricetin	nd	nd
Apigenin	-	nd

–: negative result. nd: not determined, -: negative results

Scanning electron microscope imaging

The surface morphology of *S. lincolnensis* (P17) and *N. tenerifensis* (P22) isolates was examined using Scanning Electron Microscopy (SEM) within a magnification range of 5.00 KX to 10.00 KX, and at a voltage of 10.00 kV. The *S. lincolnensis* (P17) isolate exhibited typical actinomycete morphology, characterized by branched filamentous structures. Smooth-surfaced, oval-shaped spores were observed along the filaments. In contrast, the *N. tenerifensis* (P22) isolate displayed less branched filamentous structures, with no spore formation detected (*Fig. 5*). It is well known that *N. tenerifensis* is a species that does not produce spores.

Discussion

The isolation and characterization of pigment-producing bacterial strains from soil samples collected in the Artvin province contribute meaningfully to ongoing research on microbial secondary metabolites with biotechnological potential. In this study, a total of fifteen bacterial isolates were identified through morphological, biochemical, and molecular analyses. These isolates were taxonomically affiliated with the genera *Arthrobacter*, *Rhodococcus*, *Micrococcus*, *Metabacillus*, *Peribacillus*, *Chitinophaga*, *Streptomyces*, and *Nocardia*. Among them, only the methanolic extract of *Streptomyces lincolnensis* (P17) and the acetonic extract of *Nocardia tenerifensis* (P22) exhibited antimicrobial activity. These findings suggest that certain rare actinobacterial species may represent promising candidates for the discovery of novel natural products with antimicrobial properties.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

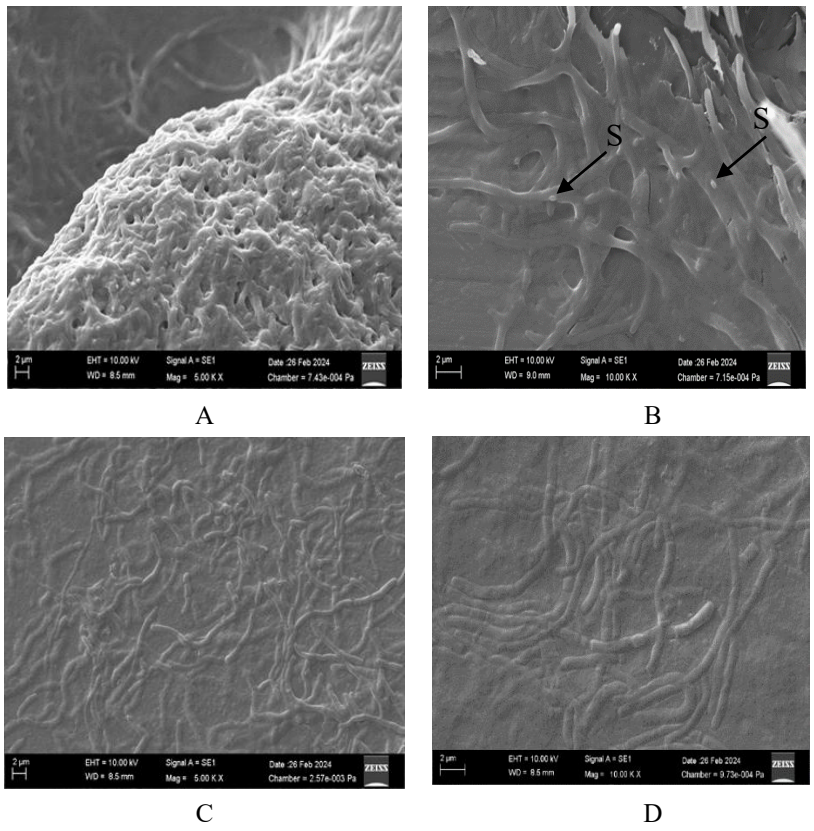


Figure 5. *Scanning electron microscopy (SEM) images of S. lincolnensis (P17) and N. tenerifensis (P22) strains. (A) S. lincolnensis (P17) at 5.00 KX magnification. (B) S. lincolnensis (P17) at 10.00 KX magnification. (C) N. tenerifensis (P22) at 5.00 KX magnification. (D) N. tenerifensis (P22) at 10.00 KX magnification. S: spore*

Within the order Actinomycetales, the genus *Streptomyces* stands out as the most diverse and ecologically widespread group. *Streptomyces* species are Gram-positive, filamentous, and obligately aerobic bacteria. These microorganisms are predominantly soil-dwelling and exhibit optimal growth in soils with neutral to alkaline pH conditions (Madigan and Martinko, 2010). In the taxonomic classification of this genus, spore morphology serves as a critical distinguishing criterion. Spore surfaces can vary among species, exhibiting smooth, warty, spiny, or hairy textures. Additionally, the production of soluble pigments on solid media is considered a significant parameter in phenotypic characterization. Pigment-producing or chromogenic strains typically synthesize diffusible pigments that range in color from dark brown to black, particularly in protein-rich media (Waksman, 1959).

Bacterial pigments have found widespread applications as natural colorants in the food and cosmetic industries. In addition to their coloring properties, these pigments possess a range of biological activities, including anticancer, antioxidant, anti-inflammatory, and antimicrobial effects (Venil and Lakshmanaperumalsamy, 2009). To date, more than sixty antibiotics derived from *Streptomyces* species have been identified, many of which are used across diverse fields such as human and veterinary medicine, agriculture, and industry (Waksman, 1959; Madigan and Martinko, 2010). Consequently, *Streptomyces* strains isolated from both terrestrial and aquatic environments represent valuable sources for the discovery of novel bioactive compounds (Al-Shaibani et al., 2021). It is widely recognized that many natural products within this genus remain unexplored and await characterization.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Numerous studies have been conducted on the antimicrobial activities of *Streptomyces* species isolated from soil (Narayana et al., 2008; Sarika et al., 2021; Chanthasena et al., 2022; Al-Joubori et al., 2023), and these microorganisms are recognized for their capacity to produce a wide variety of secondary metabolites, including antibiotics and pigments. The pigments produced by *Streptomyces* can be classified as endopigments (associated with specific cellular structures) or exopigments (released into the environment). In some cases, different antibiotics produced by Actinobacteria are also considered as pigments. These pigments, particularly in protein-rich environments, exhibit characteristics that range in color from dark brown to black and can diffuse into the surrounding medium (Waksman, 1959). There is no previous research reported in the literature on pigment extraction from *Streptomyces lincolnensis* using methanol and ethanol solvents. This study represents the first investigation into the extraction of pigments from *S. lincolnensis* using methanol and ethanol solvents. The resulting extracts were found to generate a 14.3 mm inhibition zone against *Staphylococcus aureus*, demonstrating the potential antimicrobial activity of the pigment.

Previous studies have reported that the ethyl acetate and methanol extracts of various *Streptomyces* species exhibit strong inhibitory activity, particularly against Gram-positive bacteria such as *Staphylococcus aureus* (Kekuda et al., 2013; Kazi et al., 2022). These findings are in agreement with the results obtained in the present study and further support the antimicrobial potential of the pigments.

Moreover, the lincosamide antibiotic lincomycin is effective against Gram-positive bacteria and presents a significant therapeutic option in clinical settings (Yang, 2020). In this context, it is suggested that the *Streptomyces lincolnensis* (P17) isolate should be further investigated in detail for its potential to produce lincomycin in future studies. The potential use of the pigments obtained, particularly against clinically significant pathogens such as *Staphylococcus aureus*, offers new opportunities for biotechnological applications.

The genus *Nocardia*, belonging to the Actinobacteria group, typically exists as a saprophyte in soils rich in organic matter. *Nocardia* species have been successfully isolated from various soil samples (Ajello et al., 1979; Valero-Guillén et al., 1984; Van Gelderen de Komaid et al., 1987; Kämpfer et al., 2004; Ping et al., 2006), and these species are reported to have the capacity to produce bioactive compounds with antimicrobial activity (Mikami et al., 2000; Mukai et al., 2006). In particular, *Nocardia* sp. CS682 has been noted to exhibit higher antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) compared to *Micrococcus luteus* (Sohng et al., 2008). Additionally, a soil-derived isolate of *Nocardia pseudobrasiliensis* has been reported to demonstrate significant antimicrobial activity against both Gram-positive and Gram-negative bacteria (Seratnahaei et al., 2023).

Nocardia tenerifensis was first described by Kämpfer et al. (2004) and isolated from soil samples. It was reported that this isolate formed orange-colored vegetative mycelium on Medium 65 at 25°C. In contrast, in this study, it was observed that the isolate developed pink-brown colonies on nutrient agar at 30°C. This discrepancy suggests that pigment production may vary depending on the composition of the medium and environmental conditions. On the other hand, a literature review reveals that there are no studies that have subjected isolates of this species to pigment extraction using solvents such as methanol or acetone and evaluated the antimicrobial properties of these extracts. This study presents the antimicrobial potential of compounds extracted from the *Nocardia tenerifensis* P22 isolate using acetone. The analysis revealed that the acetone extract of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

the P22 isolate exhibited a significant antimicrobial activity, forming an inhibition zone of 17.6 mm against the Gram-positive *Staphylococcus aureus*. However, the isolate did not show any inhibitory effect against Gram-negative bacteria or *Candida albicans*. These findings suggest that the biologically active metabolites of *N. tenerifensis* P22 could have potential applications, particularly in the treatment of *S. aureus*-related infections.

Previous studies have shown that species belonging to the genera *Arthrobacter*, *Rhodococcus*, *Micrococcus*, and *Bacillus* have been isolated from soil samples (Kageyama et al., 2008; Hoang et al., 2014; Belov et al., 2018). The genus *Rhodococcus* is a significant group of microorganisms known for its ability to degrade various natural and xenobiotic compounds. Moreover, these bacteria are also highly valuable for producing antibiotics that contain a limited number of characterized secondary metabolites (Borisova, 2011). For instance, an antimicrobial agent produced by a *Rhodococcus* strain isolated from soil has been reported to exhibit antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, but no antibacterial effects were observed (Chiba et al., 1999). On the other hand, a *Rhodococcus rhodochrous* strain, isolated from soil containing manganese ore in Iran, has been identified as a carotenoid pigment producer. The methanol extract of this microorganism was found to exhibit antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Haddad et al., 2017).

In the present study, it was determined that the *Rhodococcus* strains P2, P6, and P7 were unsuitable for pigment extraction using methanol and acetone solvents, with strain P7 failing to yield pigments under both conditions. Additionally, the methanol and acetone extracts obtained from strains P2 and P6 did not demonstrate any antibacterial or antifungal activity against the tested microorganisms. This finding contrasts with previous reports, highlighting a potential divergence in the bioactivity profiles of *Rhodococcus* strains.

The *Arthrobacter* genus, belonging to the *Actinobacteria* class, comprises Gram-positive, aerobic bacteria known for their versatile pigment production. These bacteria are commonly found in diverse environments, including soil, water, air, and food, and are capable of thriving in extreme conditions. Indeed, *Arthrobacter* strains have been successfully isolated from challenging environments such as sewage, radioactive waste tanks, and contaminated water (Sutthiwong et al., 2014). For instance, *Arthrobacter ruber* was isolated from glacial ice (Liu et al., 2018), while *Arthrobacter agilis*, sourced from Antarctic sea ice, produces carotenoid pigments containing a bacterioruberin backbone (Fong et al., 2001).

Bacterioruberin, a natural antioxidant synthesized by numerous species within the *Haloferacaceae* family of halophilic archaea, possesses antimicrobial, antiviral, anticancer, and antihaemolytic properties (Caimi et al., 2022). Given its potent antioxidant activity, bacterioruberin holds substantial promise for diverse applications in the pharmaceutical and food industries. The pigment production potential of *Arthrobacter* strains positions them as valuable candidates for biotechnological applications, particularly as natural colorants (Noby et al., 2023). Furthermore, *A. agilis* has demonstrated a remarkable ability to produce Indole-3-acetic acid (IAA) with a maximum concentration of 75 mg/L, highlighting its biotechnological significance, particularly in studies related to microbial immobilization (Ozdal et al., 2017).

The ethyl acetate pigment extract from *Arthrobacter gandavensis*, a yellow-pigmented strain, has exhibited a diverse range of biological activities, including antibacterial, anticancer, anti-leishmanial, hemolytic, protein kinase inhibition, and antioxidant effects.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

Its antibacterial properties have been specifically demonstrated against *Klebsiella pneumonia*, *Enterobacter sakazakii*, and *Salmonella typhi* (Numan et al., 2018). Furthermore, the methanol extract of several *Arthrobacter* strains has shown exceptional antibacterial activity against various bacterial pathogens, including *Micrococcus flavus*, *Bacillus subtilis*, and *Salmonella typhi*. These strains hold significant promise as potential natural sources for the treatment of infections caused by multidrug-resistant microorganisms within the community (Pathak et al., 2011).

In the present study, the *Arthrobacter* isolates identified as P1, P9, P10, P18, P20, and P21 did not exhibit any antimicrobial activity against the test microorganisms, which contrasts with previously reported findings in the literature. Nevertheless, the thorough characterization of pigments derived from *Arthrobacter* isolates holds significant potential for the identification of new natural food colorant sources. Moreover, the *Arthrobacter ruber* isolates coded P1 and P20 were isolated from soil for the first time in this study.

Smitha and Nath (2017) identified a yellow-pigment-producing bacterium isolated from soil, which they classified as *Micrococcus yunnanensis* S-CSR-0010. The pigment extracted from this isolate using acetone exhibited inhibitory effects against *Pseudomonas aeruginosa* and multidrug-resistant *Staphylococcus aureus* (MDRSA). It was reported that the purification of this pigment holds promise for the discovery of new therapeutic agents. In contrast, our study found that the *M. yunnanensis* isolate coded P4 did not demonstrate antimicrobial activity against the microorganisms tested. This suggests that further testing of the pigment against different pathogens is necessary.

Bacteria of the *Chitinophaga* genus possess the ability to degrade chitin and can be isolated from various environments, including freshwater, decaying plant material, animal feces, and soil (Kämpfer et al., 2006; Meliah et al., 2018; Weon et al., 2009). The biological activity of the isolate coded P16 was not detected against the tested bacteria. However, considering the chitin-degrading capabilities of these bacteria, their potential insecticidal properties against harmful insects should be further investigated.

The *Metabacillus* genus, belonging to the *Bacillaceae* family, is characterized by chromogenic bacteria that synthesize various pigments and exhibit fluorescent properties under UV light. These bacteria hold significant potential in the field of biotechnology, as the production of naturally sourced pigments can be beneficial for various applications (Kharkhota et al., 2022). Previous studies have isolated *Metabacillus indicus* from brown algae (Nurhilaliyah et al., 2023) and motor oil-contaminated soils (George et al., 2023). In the present study, the orange pigment obtained from the *Metabacillus indicus* isolate coded P5 did not exhibit antimicrobial activity. However, this bacterium may still hold promise for utilization in various biotechnological fields.

Peribacillus, a genus within the *Bacillaceae* family, consists of endospore-forming bacteria that exhibit either aerobic or facultative anaerobic properties. Based on molecular taxonomy, this genus was distinguished from the previously identified *Bacillus* species and recognized as a separate genus (Patel and Gupta, 2020). The species *Bacillus muralis* was previously isolated from deteriorating wall paintings (Heyrman et al., 2005). In the present study, no inhibitory effect was observed from the *Peribacillus muralis* isolate against the test organisms. Furthermore, there are no existing reports in the literature indicating that *Peribacillus muralis* has been isolated from soil. In this regard, our study provides a novel finding as the first soil-derived isolation of this species.

Microbial pigments exhibit a variety of biological activities. HPLC analysis of the acetone and methanol extracts revealed the presence of several compounds that could play a role in the aforementioned biological activities, particularly antimicrobial activity.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

The results of the HPLC analysis identified the presence of rosmarinic acid. Rosmarinic acid, a natural antimicrobial agent typically found in plants, is one of the significant bioactive compounds that can be isolated and is commonly used in infections caused by microorganisms, including bacteria, fungi, and viruses. Additionally, the combination of rosmarinic acid and its derivatives with antibiotics has been shown to produce a synergistic effect (Kernou et al., 2023). This compound has demonstrated antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, and *Bacillus subtilis*. It is believed that these effects result from rosmarinic acid's ability to disrupt bacterial cells and cellular proteins, thereby inhibiting the activity of the Na^+/K^+-ATPase enzyme (Zhang et al., 2022). Furthermore, a study has shown that rosmarinic acid causes significant detrimental effects on *Pseudomonas aeruginosa* and induces damage to the cellular structure of *Aspergillus niger*. Rosmarinic acid has even been described as a natural antimicrobial compound released into the rhizosphere in response to microbial threats (Bais et al., 2002).

The HPLC analysis of the acetone extract from *Nocardia tenerifensis* revealed that ascorbic acid constitutes the main compound, accounting for 59.87% of the extract. Indeed, numerous studies have demonstrated and evaluated the antibacterial effects of ascorbic acid. Inhibition of growth has been reported against a range of microorganisms, including *Staphylococcus aureus*, *Escherichia coli* (Abu-Ghazaleh, 2013), *Streptococcus mutans*, *Staphylococcus aureus*, *Porphyromonas gingivalis*, *Candida albicans*, *Enterococcus faecalis* (Isela et al., 2013), *E. coli*, *Klebsiella pneumoniae* (Verghese et al., 2017), *Mycobacterium tuberculosis* (Vilchèze et al., 2013), and *Helicobacter pylori* (Namiot et al., 2020). In this study, the acetone pigment extract of the P22 strain exhibited significant antimicrobial activity exclusively against *S. aureus*, suggesting that this compound may be directly responsible for the observed effect.

The second major compound identified in the extract was catechin, which constitutes 18.52% of the composition. Catechins, polyphenolic compounds found in various plants, are particularly abundant in tea leaves and are well-known for their potent antioxidant properties (Bae et al., 2020). The antimicrobial effects of catechins are primarily mediated by their ability to disrupt bacterial cell membranes. Gram-negative bacteria, however, exhibit greater resistance to these effects compared to Gram-positive bacteria, as the negatively charged lipopolysaccharides in Gram-negative bacterial membranes contribute to their resistance (Ikigai et al., 1993). In contrast, Gram-positive bacteria, such as *Staphylococcus aureus*, tend to show higher sensitivity to these compounds. Additionally, catechins exert antimicrobial activity by disrupting the protein structures and functions of pathogenic cells (Shimamura et al., 2007). Furthermore, the combination of catechins with various antibiotics (e.g., norfloxacin and gentamicin) has been shown to produce a synergistic effect against *S. aureus*, facilitating more effective antibiotic therapy (Gomes et al., 2018). This synergy may help overcome the resistance mechanisms developed by Gram-positive bacteria against antibiotics (Stapleton et al., 2007).

Another compound identified in the methanol extract profile of *Streptomyces lincolnensis* (P17) is gallic acid, which was found as the major component at 10.03%. Gallic acid, a phenolic compound, is naturally occurring in many plants and is known for its diverse biological functions. It has been reported to possess antimicrobial activity against various bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The antimicrobial effects of gallic acid are thought to result from its ability to alter the hydrophobic structure of bacterial cell membranes, disrupting membrane permeability and leading to leakage of cellular

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

contents (Borges et al., 2013). These findings underscore the potential of gallic acid as a broad-spectrum and natural antimicrobial agent (Tian et al., 2022).

In this study, only the methanol extract of *S. lincolnensis* (P17) and the acetone extract of *N. tenerifensis* (P22) exhibited antimicrobial activity. Both extracts were effective solely against *Staphylococcus aureus*, showing no antimicrobial potential against the other tested microorganisms. To date, there have been no reports of any compound produced by *N. tenerifensis* (P22) exhibiting antimicrobial activity. The observed inhibitory effect of the P22 extract, which produced the highest inhibition zone against *S. aureus*, is likely associated with the high concentration of ascorbic acid (59.87%) present in the extract, as reported in the literature.

Therefore, this isolate may represent a promising microbial source for biotechnological applications, particularly in the pharmaceutical industry. The high concentration of catechins found in the *N. tenerifensis* (P22) extract likely contributes to its antimicrobial activity. In contrast, the high concentration of gallic acid observed in the methanol extract of *S. lincolnensis* (P17) is considered the primary component responsible for the detected antimicrobial activity. Notably, it has been reported that *Streptomyces* culture supernatants tend to exhibit inhibitory effects more frequently against Gram-positive bacteria, such as *Enterococcus faecium* and *S. aureus*, compared to Gram-negative bacteria (Li et al., 2019). Similar effects are produced by rosmarinic acid, which was detected in both extracts. While phenolic compounds from bacterial sources have been less studied compared to plant-based ones, this opens up the possibility of exploring these microorganisms as alternative sources in biotechnological production processes.

Based on the literature presented above, the combined use of phenolic compounds such as rosmarinic acid and catechins with antibiotics has the potential to develop more effective antimicrobial formulations against infections caused by microorganisms. This is particularly crucial in combating multi-drug-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

In this study, methanol and acetone solvents exhibited differential efficiencies in pigment extraction; pigments from isolates P4, P12, and P17 were extracted exclusively with methanol. Nonetheless, although pigmented colonies were observed on solid media for isolate P7, pigment extraction was unsuccessful using either methanol or acetone. This phenomenon may result from pigments being tightly bound to cell wall or membrane structures (Meckel and Kester, 1980). Solvent selection and extraction methodologies critically affect extraction yield. Therefore, further studies employing physical (e.g., ultrasonication) and chemical (e.g., lysozyme treatment) pretreatments, alongside alternative solvents, are recommended to enhance extraction efficiency.

Conclusions

In conclusion, this study investigated the pigment production potential and antimicrobial activities of various bacterial species isolated from soil samples in Artvin Province. The pigments of *Streptomyces lincolnensis* and *Nocardia tenerifensis*, extracted with methanol and acetone, respectively, exhibited inhibitory effects against *Staphylococcus aureus*. However, no inhibitory activity was observed against other Gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis*) or *Candida albicans*. Notably, the acetone extract of *N. tenerifensis* (P22) exhibited the highest antimicrobial activity, with an inhibition zone of 17.6 mm. HPLC analysis revealed that these pigments

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

contain phenolic compounds, such as gallic acid, ascorbic acid, catechins, and rosmarinic acid, which are likely contributors to their antimicrobial properties. These findings suggest that the studied species have significant potential as sources of microbial pigments and natural antimicrobial agents for biotechnological applications. However, further investigation of their effects on a broader range of microorganisms is warranted to better understand their potential in biotechnological contexts.

Future studies will provide additional insights into enhancing the pharmaceutical and biotechnological applicability of these isolates and their bioactive compounds. This study represents the first literature report on the antimicrobial properties of pigments extracted from *N. tenerifensis* and *S. lincolnensis* using acetone and methanol solvents, respectively. Moreover, *A. ruber* and *P. muralis* were isolated from soil for the first time in this study.

Acknowledgements. This work was supported by the Artvin Çoruh University Research Foundation (Project Number: ACU-BAP 2020.F83.02.02). The author would like to thank the Artvin Çoruh University (AÇÜ) - Science and Technology Application and Research Center for conducting the experimental stages of this study, and the Artvin Çoruh University (AÇÜ) - Medical and Aromatic Plants Application and Research Center for their contributions to the HPLC analysis.

REFERENCES

[1] Abu-Ghazaleh, B. M. (2013): Effects of ascorbic acid, citric acid, lactic acid, NaCl, potassium sorbate and *Thymus vulgaris* extract on *Staphylococcus aureus* and *Escherichia coli*. – African Journal of Microbiology Research 7(1): 7-12.

[2] Ahmad, W. A., Ahmad, W. Y. W., Zakaria, Z. A., Yusof, N. Z. (2012): Application of Bacterial Pigments as Colorant. – In: Application of Bacterial Pigments as Colorant. Springer Briefs in Molecular Science. Springer, Berlin, pp. 57-74.

[3] Ajello, G., Brown, J., Mahgoub, E., Ajello, L. (1979): A note on isolation of pathogenic aerobic actinomycetes from Sudanese soils. – Current Microbiology 2: 25-26.

[4] Al-Joubori, B., Saadoun, I., Hotchin, N., Cunningham, D., Alderwick, L. (2023): The isolation of novel terrestrial *Streptomyces* strains with antimicrobial and cytotoxic properties. – Arabian Journal of Basic and Applied Sciences 30: 285-298.

[5] Al-Shaibani, M. M., Mohamed, R. M. S. R., Sidik, N. M., El Enshasy, H. A., Al-Gheethi, A., Noman, E., Al-Mekhlafi, N. A., Zin, N. M. (2021): Biodiversity of secondary metabolites compounds isolated from phylum Actinobacteria and its therapeutic applications. – Molecules 26: 4504.

[6] Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. (1990): Basic local alignment search tool. – Journal of Molecular Biology 215: 403-410.

[7] Bae, J., Kim, N., Shin, Y., Kim, S. Y., Kim, Y. J. (2020): Activity of catechins and their applications. – Biomedicine & Dermatology 4: 1-10.

[8] Bais, H. P., Walker, T. S., Schweizer, H. P., Vivanco, J. M. (2002): Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. – Plant Physiology and Biochemistry 40(11): 983-995.

[9] Behera, H. T., Mojumdar, A., Nivedita, S., Ray, L. (2021): Microbial Pigments: Secondary Metabolites with Multifaceted Roles. – In: Vaishnav, A., Choudhary, D. K. (eds.) Microbial Polymers. Springer, Singapore, pp. 631-654.

[10] Belov, A. A., Cheptsov, V. S., Vorobyova, E. A. (2018): Soil bacterial communities of Sahara and Gibson deserts: physiological and taxonomical characteristics. – AIMS Microbiology 4: 685-710.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

[11] Borges, A., Ferreira, C., Saavedra, M. J., Simões, M. (2013): Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. – Microbial Drug Resistance 19(4): 256-265.
[12] Borisova, R. B. (2011): Isolation of a Rhodococcus soil bacterium that produces a strong antibacterial compound. – Ph. D. Thesis, East Tennessee State University, Johnson City, TN.
[13] Caimi, A. T., Yasynska, O., Rojas, P. C. R., Romero, E. L., Morilla, M. J. (2022): Improved stability and biological activity of bacterioruberin in nanovesicles. – Journal of Drug Delivery Science and Technology 77: 103896.
[14] Chanthasena, P., Hua, Y., Rosyidah, A., Pathom-Aree, W., Limphirat, W., Nantapong, N. (2022): Isolation and identification of bioactive compounds from *Streptomyces actinomycinicus* PJ85 and their in vitro antimicrobial activities against methicillin-resistant *Staphylococcus aureus*. – Antibiotics 11: 1797.
[15] Chiba, H., Agematu, H., Kaneto, R., Terasawa, T., Sakai, K., Kazuyuki, D., Yoshioka, T. (1999): Rhodopeptins (Mer-N1033): novel cyclic tetrapeptides with antifungal activity from *Rhodococcus* sp. I. Taxonomy, fermentation, isolation, physicochemical properties and biological activities. – Journal of Antibiotics 52: 695-699.
[16] Choi, H. J., Hong, J. B., Park, J. J., Chi, W. J., Kim, M. C., Chang, Y. K., Hong, S. K. (2011): Production of agarase from a novel Micrococcus sp. GNUM-08124 strain isolated from the East Sea of Korea. – Biotechnology and Bioprocess Engineering 16: 81-88.
[17] Choubey, S., Chapade, S. V., Garud, S. A. (2021): Optimization of pigment production by *Micrococcus* and *Arthrobacter* species isolated from soil and water. – International Journal of Research in Pharmaceutical Sciences 12(3): 1902-1907.
[18] Espinoza, L. E., Davelos Baines, A. L., Lowe, K. L. (2013): Biochemical, nutrient and inhibitory characteristics of *Streptomyces* cultured from a hypersaline estuary, the Laguna Madre (Texas). – OnLine Journal of Biological Sciences.
[19] Fong, N. J. C., Burgess, M. L., Barrow, K. D. (2001): Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. – Applied Microbiology and Biotechnology 56: 750-756.
[20] Funke, G., Von Graevenitz, A., Clarridge, J. E., Bernard, K. A. (1997): Clinical microbiology of coryneform bacteria. – Clinical Microbiology Reviews 10(1): 125-159.
[21] George, A. E., Antia, U. E., Adeleke, A. J., Fatunla, O. K. (2023): Optimisation of polyhydroxybutyrate production by *Lysinibacillus fusiformis* and *Metabacillus indicus* isolated from spent engine-oil contaminated soil. – UMYU Journal of Microbiology Research 8(2): 30-39.
[22] Gomes, F. M. S., da Cunha Xavier, J., Dos Santos, J. F. S., de Matos, Y. M. L. S., Tintino, S. R., de Freitas, T. S., Coutinho, H. D. M. (2018): Evaluation of antibacterial and modifying action of catechin antibiotics in resistant strains. – Microbial Pathogenesis 115: 175-178.
[23] Goswami, G., Chaudhuri, S., Dutta, D. (2010): Effect of pH and temperature on pigment production from an isolated bacterium. – Chemical Engineering Transactions 20: 127-132.
[24] Haddad, M., Aghaei, S., Zargar, M. (2017): Antimicrobial and antioxidant activity of carotenoid pigment produced by native *Rhodococcus* spp. isolated from soil. – International Journal of Molecular and Clinical Microbiology 7(1): 809-815.
[25] Hall, T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95-98.
[26] Harley, J. P., Prescott, L. M. (2002): Laboratory Exercises in Microbiology. 5th Ed. – McGraw-Hill, New York.
[27] Heyrman, J., Logan, N. A., Rodríguez-Díaz, M., Scheldeman, P., Lebbe, L., Swings, J., Heyndrickx, M., De Vos, P. (2005): Study of mural painting isolates, leading to the transfer of *Bacillus maroccanus* and *Bacillus carotarum* to *Bacillus simplex*, emended description of *Bacillus simplex*, re-examination of the strains previously attributed to *Bacillus*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

macroides and description of *Bacillus muralis* sp. nov. – International Journal of Systematic and Evolutionary Microbiology 55: 119-131.

[28] Hoang, V. A., Kim, Y. J., Nguyen, N. L., Yang, D. C. (2014): *Arthrobacter gyeryongensis* sp. nov., isolated from soil of a *Gynostemma pentaphyllum* field. – International Journal of Systematic and Evolutionary Microbiology 64: 420-425.

[29] Ikigai, H., Nakae, T., Hara, Y., Shimamura, T. (1993): Bactericidal catechins damage the lipid bilayer. – Biochimica et Biophysica Acta-Biomembranes 1147(1): 132-136.

[30] Isela, N. N. R., Sergio, N. C., Martínez-Sanmiguel, J. J., Hernandez-Delgadillo, R., Cabral-Romero, C. (2013): Ascorbic acid on oral microbial growth and biofilm formation. – Pharmaceutical Innovation 2(4: Part A): 103.

[31] Kageyama, A., Morisaki, K., Omura, S., Takahashi, Y. (2008): *Arthrobacter oryzae* sp. nov. and *Arthrobacter humicola* sp. nov. – International Journal of Systematic and Evolutionary Microbiology 58: 53-56.

[32] Kämpfer, P., Buczolits, S., Jackel, U., Grün-Wollny, I., Busse, H. J. (2004): *Nocardia tenerifensis* sp. nov. – International Journal of Systematic and Evolutionary Microbiology 54(2): 381-383.

[33] Kämpfer, P., Young, C. C., Sridhar, K. R., Arun, A. B., Lai, W. A., Shen, F. T., Rekha, P. D. (2006): Transfer of [*Flexibacter*] *sancti*, [*Flexibacter*] *filiformis*, [*Flexibacte*r] *japonensis* and [*Cytophaga*] *arvensicola* to the genus *Chitinophaga* and description of *Chitinophaga skermanii* sp. nov. – International Journal of Systematic and Evolutionary Microbiology 56(9): 2223-2228.

[34] Kangale, L. J., Raoult, D. A., Ghigo, E., Fournier, P. E. (2021): *Metabacillus schmidteae* sp. nov., cultivated from planarian *Schmidtea mediterranea* microbiota. – Microbiology Research 12(2): 299-316.

[35] Kazi, Z., Hungund, B., Yaradoddi, J., Banapurmath, N., Yusuf, A., Kishore, K. L., Soudagar, M. E. M., Yunus Khan, T. M., El-fasakhany, A., Buyonda, K. A. (2022): Production, characterization, and antimicrobial activity of pigment from *Streptomyces* species. – Journal of Nanomaterials 8.

[36] Kekuda, P. T. R., Dileep, N., Junaid, S., Rakesh, K. N., Mesta, S. C., Onkarappa, R. (2013): Biological activities of *Streptomyces* species SRDP-07 isolated from soil of Thirthahalli, Karnataka, India. – International Journal of Drug Development & Research 5(3): 268-285.

[37] Kernou, O. N., Azzouz, Z., Madani, K., Rijo, P. (2023): Application of rosmarinic acid with its derivatives in the treatment of microbial pathogens. – Molecules 28(10): 4243.

[38] Kharkhota, M., Hrabova, H., Kharchuk, M., Ivanytsia, T., Mozhaieva, L., Poliakova, A.; Avdieieva, L. (2022): Chromogenicity of aerobic spore-forming bacteria of the Bacillaceae family isolated from different ecological niches and physiographic zones. – Brazilian Journal of Microbiology 53(3): 1395-1408.

[39] Krinsky, N. I., Johnson, E. J. (2005): Carotenoid actions and their relation to health and disease. – Molecular Aspects of Medicine 26: 459-516.

[40] Li, F., Liu, S., Lu, Q., Zheng, H., Osterman, I. A., Lukyanov, D. A., Sergiev, P. V., Dontsova, O. A., Liu, S., Ye, J., Huang, D., Sun, C. (2019): Studies on antibacterial activity and diversity of cultivable actinobacteria isolated from mangrove soil in Futian and Maoweihai of China. – Evidence-Based Complementary and Alternative Medicine 3476567.

[41] Liu, G. Y., Nizet, V. (2009): Color me bad: microbial pigments as virulence factors. – Trends in Microbiology 17: 406-413.

[42] Liu, Q., Xin, Y. H., Chen, X. L., Liu, H. C., Zhou, Y. G., Chen, W. X. (2018): *Arthrobacter ruber* sp. nov., isolated from glacier ice. – International Journal of Systematic and Evolutionary Microbiology 68(5): 1616-1621.

[43] Madigan, M. T., Martinko, J. M. (2010): Brock Biology of Microorganisms. 11th Ed. – Palme Yayınevi, Ankara.

[44] Malik, K., Tokkas, J., Goyal, S. (2012): Microbial pigments: a review. – International Journal of Microbial Research & Technology 1(4): 361-365.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

[45] Meckel, R. A., Kester, A. S. (1980): Extractability of carotenoid pigments from non-photosynthetic bacteria with solvents and detergents: implications for the location and binding of the pigments. – Journal of General Microbiology 120(1): 111-116.
[46] Meliah, S.; Kusumawati, D. I.; Lisdiyanti, P. (2018): The genus *Chitinophaga* isolated from Wanggameti National Park and their lytic activities. – Journal of Biological Indonesia 14(2).
[47] Mikami, Y., Komaki, H., Imai, T., Yazawa, K., Nemoto, A., Tanaka, Y., Gräefe, U. (2000): A new antifungal macrolide component, Brasilicardin B produced by *Nocardia brasiliensis*. – Journal of Antibiotics 53: 70-74.
[48] Mukai, A., Fukai, T., Matsumoto, Y., Ishikawa, J., Hoshino, Y., Yazawa, K., Harada, K., Mikami, Y. (2006): Transvalencin Z, a new antimicrobial compound with salicylic acid residue from *Nocardia transvalensis* IFM 10065. – Journal of Antibiotics 59: 366-369.
[49] Namiot, A., Namiot, D. B., Bucki, R., Kemona, A., Kurylonek, A. J., Namiot, Z. (2020): Vitamin C does not improve the efficacy of *Helicobacter pylori* eradication in smokers. – Progress in Health Sciences 10: 77-81.
[50] Narayana, K. J., Prabhakar, P., Vijayalakshmi, M., Venkateswarlu, Y., Krishna, P. S. (2008): Study on bioactive compounds from *Streptomyces* sp. ANU 6277. – Polish Journal of Microbiology 57(1): 35-39.
[51] Noby, N.; Khattab, S. N.; Soliman, N. A. (2023): Sustainable production of bacterioruberin carotenoid and its derivatives from *Arthrobacter agilis* NP20 on whey-based medium: optimization and product characterization. – Bioresource Technology and Bioprocessing 10(1): 46.
[52] Numan, M., Bashir, S., Mumtaz, R., Tayyab, S., Ullah, I., Khan, A. L., Khan, S. Z., Al-Harrasi, A. (2018): Chemical profile and in-vitro pharmacological activities of yellow pigment extracted from *Arthrobacter gandavensis*. – Process Biochemistry 75: 74-82.
[53] Nurhilaliyah, N., Zilda, D. S., Wijanarka, W., Sulistyaningtyas, A. R., Ethica, S. N. (2023): Endophytic bacteria isolated from brown seaweed *Chnoospora* sp. as potential producer of therapeutic protease. – Aquaculture, Aquarium, Conservation and Legislation 16(3): 1372-1383.
[54] Ozdal, M.; Ozdal, O. G.; Sezen, A.; Algur, O. F.; Kurbanoglu, E. B. (2017): Continuous production of indole-3-acetic acid by immobilized cells of *Arthrobacter agilis*. – 3 Biotech 7(1): 23.
[55] Patel, S.; Gupta, R. S. (2020): A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov., and *Alkalihalobacillus* gen. nov. – International Journal of Systematic and Evolutionary Microbiology 70(1): 406-438.
[56] Pathak, R., Singh, R., Singh, A., Gautam, H., Dhaker, A. S., Kumar, R., Arora, R.; Gautam, H. K. (2011): Assessment of antibacterial and free radical scavenging activity in psychrophilic *Arthrobacter* sp. – Pharmacology Online 1: 344-355.
[57] Ping, X., Wen-Jun, L., Shu-Kun, T., Yi, J., Hui-Ying, G., Li-Hua, X., Cheng-Lin, J. (2006): *Nocardia lijiangensis* sp. nov., a novel actinomycete strain isolated from soil in China. – Systematic and Applied Microbiology 29(4): 308-314.
[58] Rodríguez, M., Reina, J. C., Sampedro, I., Llamas, I., Martínez-Checa, F. (2022): *Peribacillus castrilensis* sp. nov.: a plant-growth-promoting and biocontrol species isolated from a river otter in Castril, Granada, southern Spain. – Frontiers in Plant Science 13: 896728.
[59] Sarika, K., Sampath, G., Govindarajan, R. K., Ameen, F., Alwakeel, S., Al Gwaiz, H. I., Komuraiah, T. R., Ravi, G. (2021): Antimicrobial and antifungal activity of soil actinomycetes isolated from coal mine sites. – Saudi Journal of Biological Sciences 28(6): 3553-3558.
[60] See-Too, W. S., Ee, R., Lim, Y. L., Convey, P., Pearce, D. A., Mohidin, T. B. M., Yin, W-F., Chan, K. G. (2017): Complete genome of *Arthrobacter alpinus* strain R3.8:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

bioremediation potential unraveled with genomic analysis. – Standards in Genomic Sciences 12: 1-7.

[61] Seratnahaei, M., Eshraghi, S. S., Pakzad, P., Zahraei-Ramazani, A., Yaseri, M. (2023): Antibacterial effects of bioactive metabolites extracted from *Nocardia pseudobrasiliensis*. – Caspian Journal of Environmental Sciences 21(3): 483-492.

[62] Shimamura, T., Zhao, W. H., Hu, Z. Q. (2007): Mechanism of action and potential for use of tea catechin as an antiinfective agent. – Anti-Infective Agents in Medicinal Chemistry 6(1): 57-62.

[63] Smitha, K., Nath, S. S. (2017): Activity of novel yellow pigment produced by *Micrococcus yunnanensis* S-CSR-0010 against multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. – Research Journal of Pharmaceutical, Biological and Chemical Sciences 8(2): 715-722.

[64] Sohng, J. K., Yamaguchi, T., Seong, C. N., Baik, K. S., Park, S. C., Lee, H. J., Jang, S. Y., Simkhada, J. R., Yoo, J. C. (2008): Production, isolation and biological activity of nargenicin from *Nocardia* sp. CS682. – Archives of Pharmacal Research 31: 1339-1345.

[65] Stapleton, P. D., Shah, S., Ehlert, K., Hara, Y., Taylor, P. W. (2007): The β-lactam-resistance modifier (−)-epicatechin gallate alters the architecture of the cell wall of *Staphylococcus aureus*. – Microbiology 153(7): 2093-2103.

[66] Sutthiwong, N., Fouillaud, M., Valla, A., Caro, Y., Dufosse, L. (2014): Bacteria belonging to the extremely versatile genus *Arthrobacter* as novel source of natural pigments with extended hue range. – Food Research International 65: 156-162.

[67] Tamura, K., Stecher, G., Kumar, S. (2021): MEGA11: Molecular Evolutionary Genetics Analysis version 11. – Molecular Biology and Evolution 38: 3022-3027.

[68] Thompson, C. J., Fink, D., Nguyen, L. D. (2002): Principles of microbial alchemy: insights from the *Streptomyces coelicolor* genome sequence. – Genome Biology 3(7): 1020.1-1020.4.

[69] Tian, Q., Wei, S., Su, H., Zheng, S., Xu, S., Liu, M., Bo, R., Li, J. (2022): Bactericidal activity of gallic acid against multi-drug resistance *Escherichia coli*. – Microbial Pathogenesis 173: 105824.

[70] Valero-Guillen, P. L., Martín-Luengo, F. (1984): *Nocardia* in soils of southeastern Spain: abundance, distribution, and chemical characterisation. – Canadian Journal of Microbiology 30: 1088-1092.

[71] Van Gelderen de Komaid, A., Runco de Laborda, R., Salim, R. (1987): Natural occurrence of Nocardia in soil of Tucumán: physiological characteristics. – Mycopathologia 99: 15-19.

[72] Venil, C. K., Lakshmanaperumalsamy, P. (2009): An insightful overview on microbial pigment, prodigiosin. – Electronic Journal of Biology 5: 49-61.

[73] Verghese, R. J., Mathew, S. K., David, A. (2017): Antimicrobial activity of vitamin C demonstrated on pathogenic *Escherichia coli* and *Klebsiella pneumoniae*. – Journal of Current Research in Scientific Medicine 3: 88-93.

[74] Vilchèze, C., Hartman, T., Weinrick, B., Jacobs Jr., W. R. (2013): *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. – Nature Communications 4(1): 1881.

[75] Waksman, S. A. (1959): The Actinomycetes (Öner, M., Trans. 1989). – Ege Üniversitesi Basımevi, Bornova–İzmir.

[76] Weon, H. Y., Yoo, S. H., Kim, Y. J., Son, J. A., Kim, B. Y., Kwon, S. W., Koo, B. S. (2009): Chitinophaga niabensis sp. nov. and Chitinophaga niastensis sp. nov., isolated from soil. – International Journal of Systematic and Evolutionary Microbiology 59(6): 1267-1271.

[77] Yang, J., Ye, R., Zhang, H., Liu, Y. (2020): Amplification of lmbB1 gene in *Streptomyces lincolnensis* improves quantity and quality of lincomycin A fermentation. – Preparative Biochemistry & Biotechnology 50(6): 529-537.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389

[78] Zhang, J., Cui, X., Zhang, M., Bai, B., Yang, Y., Fan, S. (2022): The antibacterial mechanism of perilla rosmarinic acid. – Biotechnology and Applied Biochemistry 69(4): 1757-1764.

[79] Zhou, T., Wang, X. Y., Deng, D. Q., Xu, L. H., Li, X. L., Guo, Y., Li, W. H., Xie, H., Zhang, P. L., Zhou, X. H. (2020): *Nocardia colli* sp. nov., a new pathogen isolated from a patient with primary cutaneous nocardiosis. – International Journal of Systematic and Evolutionary Microbiology 70(5): 2981-2987.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_83658389