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Genomic Analysis and Characterization of Phenotypic Traits of Hydrocarbon-Degrading Actinobacterial Strains Isolated from a Sierozem Soil of Southern Kazakhstan

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Abstract

The self-purification capacity of oil-contaminated soils is largely determined by the metabolic activity of autochthonous oil-oxidizing microorganisms. This study examined three strains of actinobacteria, which are hydrocarbon degraders, isolated from oil-contaminated sierozem soil at the Daulet Asia landfill (Southern Kazakhstan). Functional gene analysis was conducted (genome completeness > 98%, and contamination < 3%), and the phenotypic properties of these bacterial strains were studied to assess their potential for bio- and phytoremediation technologies in the sharply continental arid climate of the Aral Sea region. The isolated strains *Rhodococcus kroppenstedtii* K18 and MF2 and *Kocuria rosea* K1 grow on oil (12–22% oil loss after 10 days of cultivation in liquid mineral salt medium) and diesel fuel and can utilize hexadecane and benzoate as the sole source of carbon and energy. Furthermore, *Rhodococcus* K18 and MF2 utilize dodecane and eicosane, while *K. rosea* K1 utilizes phenol and gentisate. A significant decrease in surface tension (to 31.4 mN/m) observed during cultivation of strains K18 and MF2 on minimal salt medium indicates the secretion of surfactants that increase the bioavailability of hydrophobic substrates. The studied strains possess a number of properties that promote plant growth: they produce auxins, solubilize calcium hydroxyapatite, and protect plants from infection by the phytopathogenic microorganisms *Fusarium oxysporum* and *Pectobacterium wasabiae*. Actinobacteria are compatible when co-cultivated, as no mutual growth inhibition was observed. This study expands our understanding of typical bacterial representatives of desert soil, and this may contribute to the development of bioremediation approaches for the restoration of disturbed biotopes under extreme environmental conditions.

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1. Introduction

Oil pollution poses a critical threat to vulnerable arid and hyperarid ecosystems, where natural self-purification is significantly limited by moisture deficits, extreme temperature fluctuations, high salinity, and intense UV radiation [1]. Bioremediation using indigenous hydrocarbonoclastic microorganisms has proven to be a sustainable and cost-effective alternative to physicochemical remediation methods [2]. Among oil-degrading microorganisms, actinobacteria stand out for their metabolic flexibility, resistance to stress factors, and ability to synthesize biosurfactants and extracellular enzymes that solubilize and stimulate hydrocarbon degradation [3]. Current research on the world's major desert regions is gradually revealing the ecological role and biotechnological potential of microorganisms in petrochemically contaminated arid soils [1].

Research conducted at the Yumen oil field in Gansu Province (Northwest China) revealed the adaptive potential of natural actinobacteria consortia to oil degradation in cold, alkaline, and oligotrophic desert conditions [4]. Moreover, long-term monitoring data in the Evrona hyperarid nature reserve (Israel) indicate that proteobacteria and actinobacteria become dominant during the long-term succession of microbial communities following large-scale oil spills [5,6].

Endophytic actinobacteria of the genus *Streptomyces*, associated with plants growing in oil-polluted areas of southern Algeria, were found to effectively utilize n-alkanes and mono- and polyaromatic hydrocarbons, synthesize biosurfactants and possessed a number of phytostimulating properties: they produce indole-3-acetic acid, ACC deaminase, siderophores, solubilize phosphates and are involved in nitrogen fixation [7].

The possibility of bioaugmentation of oil-contaminated desert soil collected from Mishrif (Kuwait) in model vessels in an open environment by reintroducing a consortium of four actinobacteria strains *Actinotalea ferrariae*, *Arthrobacter ginsengisoli*, *Dietzia cinnamea*, *Dietzia papillomatosis* and a strain of proteobacteria *Pseudomonas songnenensis* was demonstrated. Interestingly, bioaugmenting oil-polluted soil samples with microbial oil-degrading consortium did not drastically enhance soil bioremediation [8].

Desert actinobacteria can mineralize a variety of hydrocarbon fractions. The oxidation of tetradecane, pristane, sec-octylbenzene, and other complex hydrocarbons by the *Nocardia cyriacigeorgica* strain SBUG 1472 isolated from the sands of Saudi Arabia had been demonstrated [9]. A strain of a new halotolerant actinobacteria species, *Prauserella oleivorans* PIRIT, isolated from oil-contaminated mud pits in Iran [10], capable of utilizing C_6–C_{30} alkanes, has been characterized. Ibrahim & Abdel-El-Haleem [11] demonstrated significant removal of crude oil in soil microcosms of *Streptomyces aurantiogriseus* NORA7, isolated from desert sand in western Egypt, and degradation of multiple PAHs (anthracene, naphthalene, phenanthrene, and azulene) in used motor oil by *Streptomyces ginkgonis* KM1-2 [12]. Similarly, *Streptomyces* sp. MB9 is capable of completely degrading fluorene, naphthalene, and, to a lesser extent, anthracene, pyrene, and phenanthrene [13].

Actinobacteria adapted to desert conditions demonstrate exceptional physiological resistance. *Nocardia mangyaensis* Y48^T grows actively in the range of 10–38 °C, tolerates NaCl concentrations up to 3%, and has a growth optimum at pH ~8, reflecting adaptation to the continental climate and alkaline soils of the Qaidam Basin (China) [14]. Among the extremophilic representatives of actinomycetes, *Bounagaea algeriensis* H8^T is a halophile

capable of growing at 15–25% NaCl in a temperature range of 20–45 °C [15] and *Thermobifida* sp. strain KB-T3 is a thermophile with activity at 40–65 °C [16]. Isolates of the genus *Streptomyces* prefer neutral or slightly alkaline conditions [7,11,12]. The morphologically filamentous growth pattern, branched mycelial networks, and thickened peptide-glycan cell walls characteristic of *Streptomyces* and *Nocardia* increase resistance to desiccation and facilitate penetration into oil-contaminated soil aggregates. Additionally, sporulation, melanin-like pigments, and compatible osmolytes (ectoine, hydroxyectoine, glycine betaine [17]) together provide high (robust tolerance) to UV radiation, heat stress, and dehydration [18,19]. Thus, literary data confirm the wide geographic distribution and functional diversity of desert-adapted actinobacteria.

Despite the progress made, significant gaps remain in our understanding of the genetic and enzymatic regulatory and biochemical mechanisms of hydrocarbon catabolism under extreme stress conditions, the field stability and efficiency of genetically modified actinobacteria consortia, and the integration of plant–microbe interactions into practical bioremediation schemes in desert environments. This study aims to analyze functional genes in the genomes of three hydrocarbon-degrading strains isolated from the sierozem soil of Southern Kazakhstan and to examine their phenotypic properties for potential use in bio- and phytoremediation technologies in the Aral Sea region.

2. Materials and Methods

2.1. Chemicals

All chemicals used in this study were of analytical grade. The reagents for the preparation of culture media used were obtained from the following companies: Reachim (Moscow, Russia), AppliChem (Darmstadt, Germany) and Panreac (Barcelona, Spain). Biochemical reagents were obtained from Sigma-Aldrich (Sigma, St. Louis, MO, USA), Serva (Heidelberg, Germany) and Fluka (Seelze, Germany), and molecular biology reagents were obtained from Thermo Fischer Scientific (Waltham, MA, USA), Zymo Research (Irvine, CA, USA), New England BioLabs (Hitchin, UK), Biocom (Moscow, Russia), Pronadisa (Conda, Spain), Difco Laboratories (Detroit, MI, USA).

2.2. Bacterial and Micromycetes Strains and Cultivation Conditions

The object of the study was the actinobacterial strains *Kocuria rosea* K1 and *Rhodococcus kroppenstedtii* K18 and MF2, previously isolated from samples of desert oil-contaminated poor, gray, and clayey soil (total petroleum hydrocarbon content 0.1–0.3% *w*/*w*) in Southern Kazakhstan [20].

The bacteria were grown in a complete LB medium [21] and a mineral salt medium (MSM, g/L: Na_2HPO_4—0.73, KH_2PO_4—0.35, $MgSO_4 \times 7H_2O$—0.1, $NaHCO_3$—0.25, $MnSO_4 \times 5H_2O$—0.002, NH_4NO_3—0.75, $FeSO_4 \times 7H_2O$—0.02, pH 7.0). Solid media were obtained by adding agar (Pronadisa) at a concentration of 20 g/L. The prepared media were sterilized by autoclaving for 30 min at 116 °C (additional pressure 0.5 atm). Batch cultivation was carried out at 30 °C and 180 rpm for 24–72 h using an Infors HT Multitron rotary shaker (Infors AG, Bottmingen, Switzerland).

Phytopathogenic fungi *Fusarium graminearum* VKM F-1668, *Fusarium oxysporum* VKM F-140 and *Fusarium culmorum* VKM F-2303 and a bacterium *Pectobacterium wasabiae* (previously *Erwinia carotovora*) B15 were used as test cultures to assess the antagonistic activity of the studied actinobacteria strains. For the cultivation of fungi, Kanner's medium was used [22].

In microvegetation experiments rapeseed plants (*Brassica napus* L. variety Galant) were cultivated on an agar hormone-free Murashige–Skoog medium [23] at a temperature of

22–24 °C, under a 16 h photoperiod, a light intensity of 190 µmol m^{-2} s^{-1} and a relative air humidity of 65%.

2.3. DNA Isolation, Genome Sequencing and Analysis

For DNA extraction, strains K1, K18, and MF2 were grown in liquid LB medium for 24 h. The biomass was precipitated by centrifugation (12,000 rpm, 5 min). DNA was isolated using the Vazyme Fastpure kit (Cat. No. DC112-01). Libraries were prepared using the Raissol kit (Cat. No SG GM plus 192) according to the manufacturer's protocol. Sequencing was performed on a SurfSeq 5000 system (GeneMind, Shenzhen, China). Paired-end reads of 150 bp in length were obtained. Read quality control was performed using FastQC v. 0.12.0 [24], and filtration was performed using Trimmomatic v. 0.39 [25], using the following parameters: ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 LEADING:15 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:100.

Assembly was performed using the following number of filtered, high-quality paired-end reads: K1—2,442,001, K18—3,438,096, MF2—2,150,333.

Assembly was performed from filtered reads using Spades v.3.15.4 [26] with a k-mer search of 21–127. Contigs shorter than 1000 bp were removed using the reformat.sh script from the BBmap package v. 38.95 [27]. Assembly metrics are presented in Table 1. Completeness and contamination parameters were calculated using CheckM ver. 1.2.4 [28].

Table 1. Strain genome assembly metrics.

Assembly Parameter	*K. rosea* K1	*R. kroppenstedtii* K18	*R. kroppenstedtii* MF2
Genome size, bp	4,034,491	4,346,793	4,382,462
Contigs number	188	227	61
Longest contig size, bp	116,827	215,970	435,296
N50, bp	34,722	35,093	176,221
N75, bp	21,943	18,827	83,698
N90, bp	11,692	10,055	48,530
Genome coverage	231×	237×	147×
Completeness, %	98.62	97.99	98.85
Contamination, %	2.8	0.82	0.74

Strains were annotated using Prokka v. 1.14.6 [29] and eggnog-mapper v. 2 [30]. A digital DNA–DNA hybridization (dDDH) parameter was used to determine the taxonomic position of the strains. dDDH values were calculated using the TYGS service [31] and a formula d4 by taking the sum of all identical base pairs in homologous regions and dividing it by the overall length of those homologous regions. Formula d4 is independent of overall genome size (https://tygs.dsmz.de/faqs, accessed on 20 May 2026). This makes it robust—and the standard recommended choice—when using draft genomes.

Functional analysis was performed with KEGG [32] and dbCAN3 [33], and the search for clusters of secondary metabolites production was performed using antiSMASH v. 7.0 [34]. Mobile elements and prophages in the genomes were predicted using the instruments mobileOG-db v. 2.0 [35], PHIGARO v. 2.4.0 [36], VirSorter v. 2 [37], and PHASTEST v. 3.0 [38].

2.4. Determination of a Range of Substrates Utilized

The studied actinobacteria strains were tested for the ability to utilize different aromatic and aliphatic compounds as the sole sources of carbon and energy. For this, the strains were grown in test tubes in 10 mL of MSM containing a growth substrate at 30 °C and 180 rpm for 3–7 days.

The substrates were used in the following concentrations: naphthalene, salicylate, gentisate, *o*-phthalate, benzoate, phenol, and catechol—0.1% (*w*/*v*); benzene, toluene, dodecane, hexadecane, eicosane, diesel fuel, and petroleum (from Kumkol oil deposit)—1.0% (*v*/*v*). Water-insoluble aromatic acids were added to the growth medium as sodium salts. Microbial growth was assessed spectrophotometrically by measuring optical density (OD) at 590 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). When culturing strains on agar medium, volatile compounds (naphthalene, benzene, toluene, hexadecane, and diesel fuel) were applied to the lid of an inverted Petri dish. The experiments were performed in triplicate for each strain/substrate combination.

2.5. Determination of the Level of Resistance of Actinobacteria to Heavy Metals and Antibiotics

Bacterial resistance to heavy metals (HM) and antibiotics was determined using the replica method on LB agar medium. A total of 100 µL of bacterial suspension was added to the replicator wells, and replicas were imprinted onto the medium with different concentrations of antibiotics and HM. Bacterial growth was assessed after 2–5 days. HM solutions in the form of cobalt sulfate ($CoSO_4 \times 7H_2O$), nickel chloride ($NiCl_2 \times 6H_2O$), copper chloride ($CuCl_2 \times 5H_2O$), zinc nitrate ($Zn(NO_3)_2 \times 6H_2O$), cadmium acetate ($Cd(CH_3COO)_2 \times 2H_2O$), and lead nitrate ($Pb(NO_3)_2$) were added to the medium to final concentrations of 1.5, 3.0, and 5.0 mM from stock (100 mM) solutions after autoclaving.

Antibiotic solutions were added to the medium to final concentrations, µg/mL: chloramphenicol, rifampicin, streptomycin, kanamycin—50, 100; penicillin—100, gentamicin—10, 20; tetracycline, chlortetracycline, trimethoprim, neomycin, amikacin, novobiocin, carbenicillin, cefotaxime, ceftriaxone, ceftazidime, meropenem—20.

2.6. Colonization of Plants with Strains

Rapeseeds were sterilized for 1 min in 70% ethanol and then for 2 min in 20% sodium hypochlorite solution and washed three times (10 min each) in sterile distilled water. The seeds were germinated in vitro on agar Murashige–Skoog medium. The plants were then cultivated for 14 days. For inoculation, cell suspensions (10^4 CFU/mL) of the studied bacterial strains were applied to the leaves using a brush. The plants were then cultivated under the conditions described above. On the 14th day after inoculation with the bacterial strains, the growth of the rapeseed shoots was measured. Six plants were applied for each variant. Measurements were performed in three analytical and three biological replicates.

2.7. Study of Resistance of Colonized Plants to Fungal and Bacterial Pathogens

Two-weeks-old rape plants were inoculated by the strains under study (in accordance with Section 2.6) and then six-week-old plants were infected with phytopathogenic microorganisms. The micromycete pathogens used were *Fusarium graminearum* VKM F-1668, *Fusarium oxysporum* VKM F-140, and *Fusarium culmorum* VKM F-2303. Plants were infected by placing fungal mycelium onto leaf internodes. *Pectobacterium wasabiae* B15 was used as a bacterial phytopathogen. Plants were infected with *P. wasabiae* B15 by pricking leaves with a sterile needle dipped in a *P. wasabiae* B15 cell suspension at a density of 10^6 CFU/mL. Infected plants were grown at 22–24 °C. The degree of tissue damage was assessed 1–14 days after inoculation.

2.8. Determination of Auxin Concentration in Bacterial Culture Broth

Bacteria were grown in test tubes in 10 mL MSM at 30 °C and 180 rpm for 5 days. Glycerol (final concentration 0.1%, *v*/*v*), hexadecane (1.0%, *v*/*v*), and diesel fuel (1.0%, *v*/*v*) served as carbon sources. Furthermore, 1.0 mM L-tryptophan (Fluka, Seelze, Germany) was added to the culture medium as an auxin precursor. The level of auxin production was determined colorimetrically [39]. For this purpose, cells were pelleted via centrifugation

at 12,000 rpm for 5 min. Moreover, 0.5 volumes of Salkowski's reagent (0.05 M $FeCl_3$ in 35% perchloric acid) were added to 1 volume of the supernatant. Optical density was determined after 1 h at a wavelength of 540 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). Auxin content in the supernatant was determined using a calibration curve constructed using a standard solution of indole-3-acetic acid (IAA) (Sigma-Aldrich, Burlington, MA, USA).

2.9. Analysis of Phosphate Solubilization Capacity

The bacterial strains were screened for their ability to solubilize phosphates using NBRIP agar medium [40] containing insoluble calcium hydroxyapatite $Ca_5(OH)(PO_4)_3$ (Sigma, St. Louis, MO, USA) (0.5% *w*/*v*) as the single source of phosphorus based on the formation of clear zones around bacterial colonies.

2.10. Measurement of Culture Broth Surface Tension

Microorganisms were cultivated in liquid MSM medium supplemented with hexadecane (1.0% *v*/*v*) in 750 mL Erlenmeyer flasks at 30 °C and 180 rpm for three days. Measurements were carried out using a semi-automatic surface tensiometer model 21 (Fisher Scientific, Pittsburgh, PA, USA) at 25 °C according to the manufacturer's protocol. The surface tension of the reference solution (carbon-free MSM) was 77 mN/m. To avoid the effect of residual hexadecane on the measurement results, experimental flasks were cooled at a temperature of 4 °C until hexadecane becomes solid, and the samples of culture broth were taken from the flasks with high accuracy.

2.11. Determination of Compatibility of the Studied Strains

To study the compatibility of the test strains, a fourfold dilution of LB agar medium with glucose added to a final concentration of 1.2 g/L was used. Strain compatibility was determined by the absence of a growth inhibition zone (antibacterial activity) relative to each other. For this purpose, 100 µL of a culture of one of the test strains (density 1–3 × 10^8 CFU/mL) was spread over the agar surface with a spatula. Then, 10 µL of the remaining strains were added to the lawn, and the plates were incubated at room temperature for 7 days. The inhibition zone was measured from the edge of the bacterial colony on days 2–7.

2.12. Data Analysis

The built-in analytical tool in MS Excel 2007 was used for statistical processing of biometric data. Measurements were performed in three analytical and three biological replicates. Graphs and tables show mean values and their standard deviations. The significance of differences was assessed using the nonparametric Mann–Whitney test [41]. Comparisons of data among multiple groups were performed via two-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Determination of the Taxonomic Position of Strains

Previously [20], the three studied strains were identified based on 16S rRNA gene sequence analysis as belonging to the genera *Rhodococcus* (strains K18 and MF2) and *Kocuria* (strain K1). The complete genome analysis performed in this study confirmed the preliminary identification. The strains are reliable representatives of the species *K. rosea* and *R. kroppenstedtii* (Table 2).

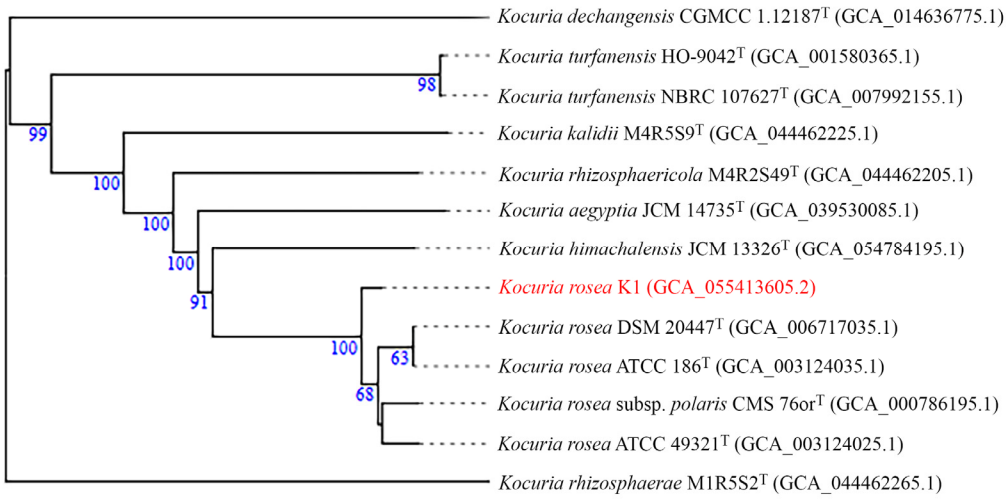
In the phylogenomic trees, they also cluster with representatives of the aforementioned species (Figure 1). Strains K18 and MF2, which are representatives of the same species, are

closely related (their DDH value is 99.7%) but differ in physiological properties, suggesting that they are two distinct strains, albeit isolated from the same biotope.

Table 2. dDDH values calculated to determine the taxonomic position of the strains.

Strain	Type Strain	DDH Value (Formula d4), %
K1	*Kocuria rosea* ATCC 186 (GCA_006094695.1)	88.7
K18	*Rhodococcus kroppenstedtii* DSM 44908	81.1
MF2	*Rhodococcus kroppenstedtii* DSM 44908	81.2

(**a**)



(**b**)

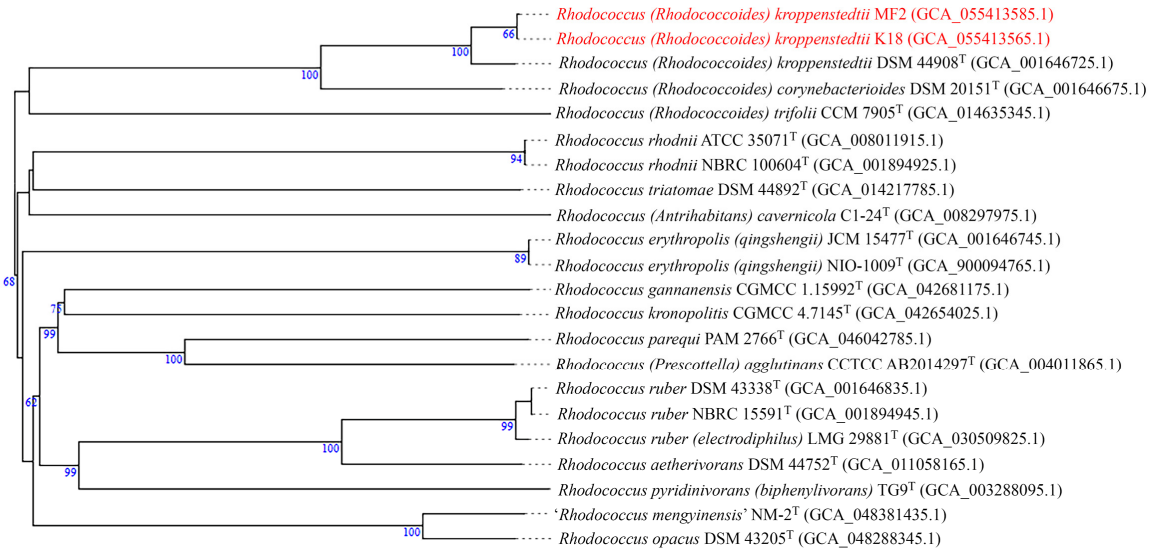


Figure 1. Phylogenomic tree showing the positions of strains K1 (**a**), K18, and MF2 (**b**) relative to the type strains of related species. The trees were constructed using the GBDP (Genome Blast Distance Phylogeny) approach; intergenomic distances were calculated using the trimming algorithm and the d5 distance formula [42]. Branch support was determined using bootstrap analysis (100 replicates). The trees were rooted at the midpoint and visualized using PhyD3 v. 1.3 [43].

3.1.1. The Position of Strain K1 Within the Species *Kocuria rosea*

Currently (June 2026), 28 full genomes of various *K. rosea* strains are deposited in the Genbank database [44]. Of these, two genomes (SMAG_U15784 (GCA_035474715.1) and RL56_bin.039 (GCA_031180065.1)) have a genome length atypical for this species (3.135 and 2.826 Mbp, respectively) and are therefore excluded from the analysis. Also,

two other genomes (AF099C18 (GCA_004799725.1) and MS50-16 (GCA_023896275.1)) are contaminated and therefore excluded from the analysis. The *K. rosea* strains with good quality genomes deposited in the Genbank database are arranged in the dendrogram as follows (Figure 2).

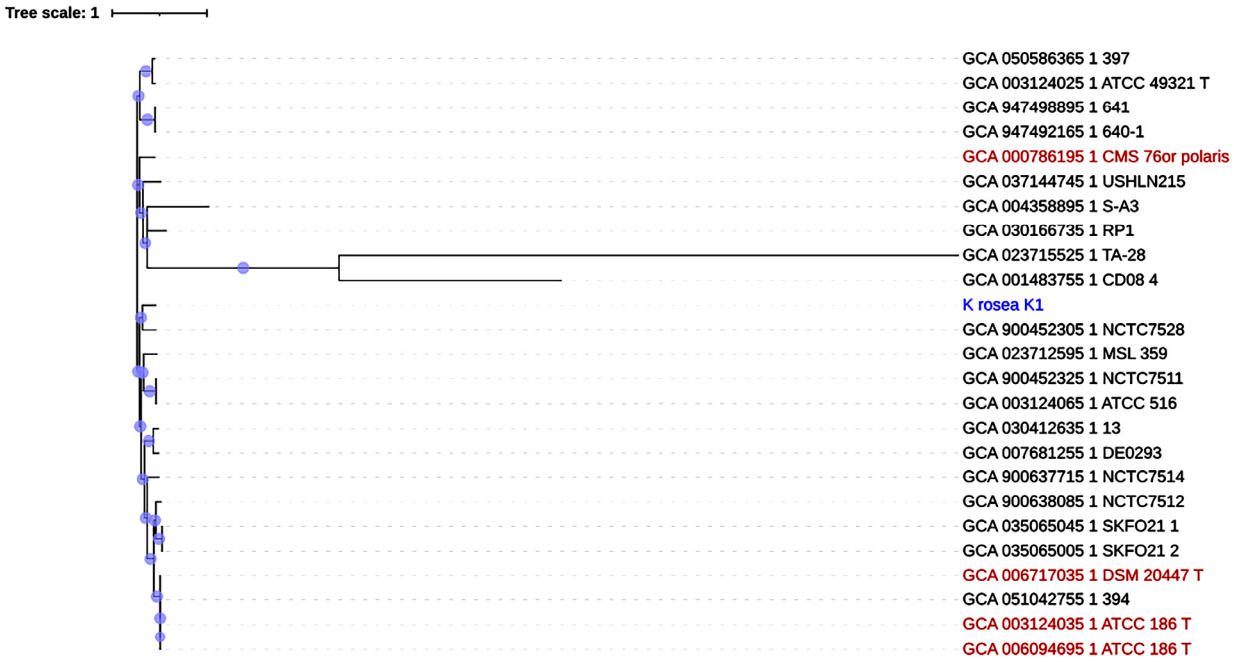


Figure 2. Phylogenomic tree showing the relative positions of *K. rosea* strains. The tree was constructed using the Genome Taxonomy Database [45], release 220, and visualized in iTOL v. 7 [46]. The tree was rooted at the midpoint. The reliability of internal branch points was determined using bootstrap analysis (100 replicates). Branch points with bootstrap values exceeding 80% are indicated by circles. Different genome assemblies of the type strain are highlighted in red. Strain K1, investigated in this study, is highlighted in blue.

The range of ANI values among them is shown in Figure 3.

The ANI value among strain K1 and the type strain *K. rosea* ATCC 186T is 98.20%. The figure clearly shows that strains CD08_4 and TA-28 obviously do not belong to the *K. rosea* species; the ANI values between them and the type strain are 91.2 and 84.22%, respectively.

3.1.2. The Position of the Species *R. kroppenstedtii* Within the Genus *Rhodococcus* and the Current State of Taxonomy of Rhodococci

The taxonomic classification of species within the genus *Rhodococcus* is quite complex. There have been repeated attempts to split the genus, thereby making it paraphyletic.

Proposals to transform the genus *Rhodococcus* into a paraphyletic taxon have involved reclassifying some species into daughter genera. For example, it has been proposed to transfer the species *Rhodococcus equi* and related species (*Rhodococcus agglutinans*, *Rhodococcus defluvii*, *Rhodococcus soli*, and *Rhodococcus subtropicus*) to the new genus *Prescottella* [47]. Sangal et al. [47] supported this proposal with genomic analysis data and average amino acid identity (AAI) calculations. In contrast, in [48], the authors used a number of genomic relatedness indices (AAI, orthologous gene alignment fraction (AF), ANI), and it was noted that, according to the three indicated indices, the species *R. equi* (*Prescotella equi*) formed a monophyletic group with other *Rhodococcus* species at the genus level. In other words, the results of Val-Calvo & Vázquez-Boland [48] did not support the previously proposed reclassification of *R. equi* into the genus *Prescottella*. At the same time, based on the results of calculating the three indices, these authors proposed reclassifying the clade containing

the species *Rhodococcus fascians* into a new genus *Rhodococcoides* [48]. The formation of the new genus involved redefining six species (*R. corynebacterioides*, *R. fascians*, *R. kroppenstedtii*, *R. kyotonensis*, *R. trifolii*, and *R. yunnanensis*) as *Rhodococcoides*.

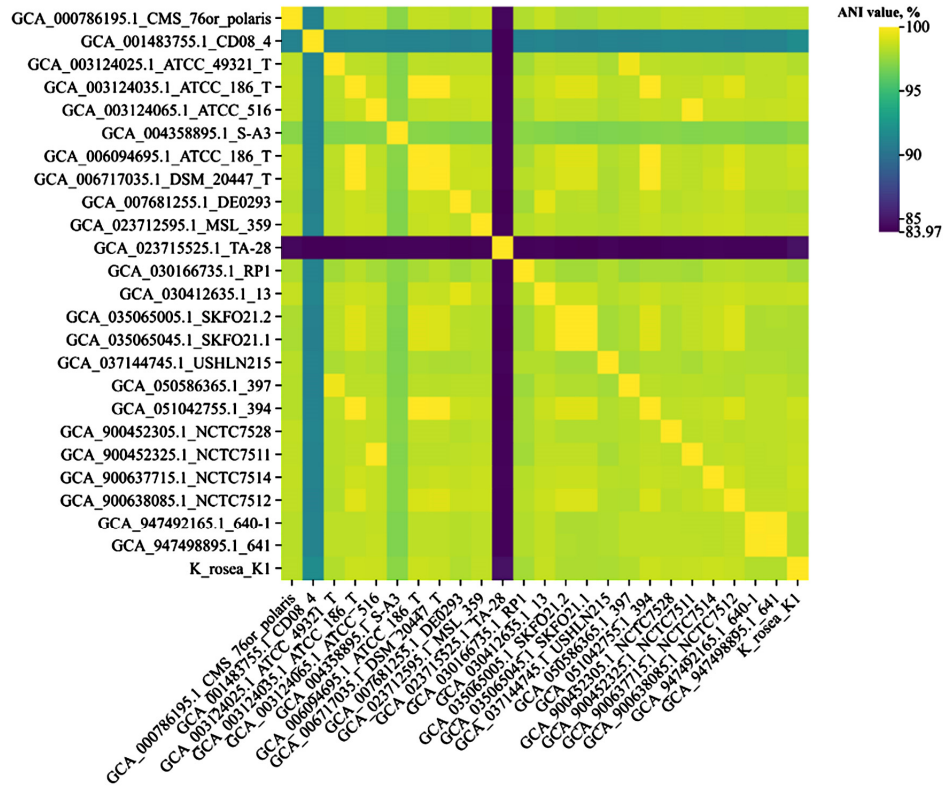


Figure 3. Range of ANI values among *K. rosea* species.

Currently, according to the LPSN [49], the genera *Prescottella* and *Rhodococcoides* are recognized as synonyms of the genus *Rhodococcus*, and all species proposed for redefinition as belonging to *Prescottella* and *Rhodococcoides* continue to be considered part of the genus *Rhodococcus*. Therefore, we use the designation "*Rhodococcus kroppenstedtii*" for strains K18 and MF2.

3.1.3. The Position of Strains K18 and MF2 Within the Species *R. kroppenstedtii*

As of June 2026, 17 genomes of various *R. kroppenstedtii* strains and isolates have been deposited in the Genbank database [44], including the K18 and MF2 strains studied in this study. All of them, except ContRs1_6 and 5jYO1wRl5Z_bin.3.MAG, have a completeness of >98% and a contamination of <1%, allowing them to be considered of sufficient quality for analysis. They are arranged on the phylogenomic tree as follows (Figure 4). The range of ANI values among them is shown in Figure 5.

The ANI values among the studied strains (K18 and MF2) and the type strain *R. kroppenstedtii* DSM 44908T (GCF_900111805.1) are 97.92 and 97.80%, respectively.

3.2. Degradative Potential of K. rosea K1, R. kroppenstedtii K18 and MF2 Strains: Genomic and Phenotypic Analysis

Biodegradative properties of *Rhodococcus* spp. strains have been most frequently described for *R. erythropolis* (including *R. qingshengii*) [50,51], *R. opacus* [52–54], and *R. jostii* [55]. However, there are no data in the available literature reporting that *R. kroppenstedtii* strains can utilize pollutants.

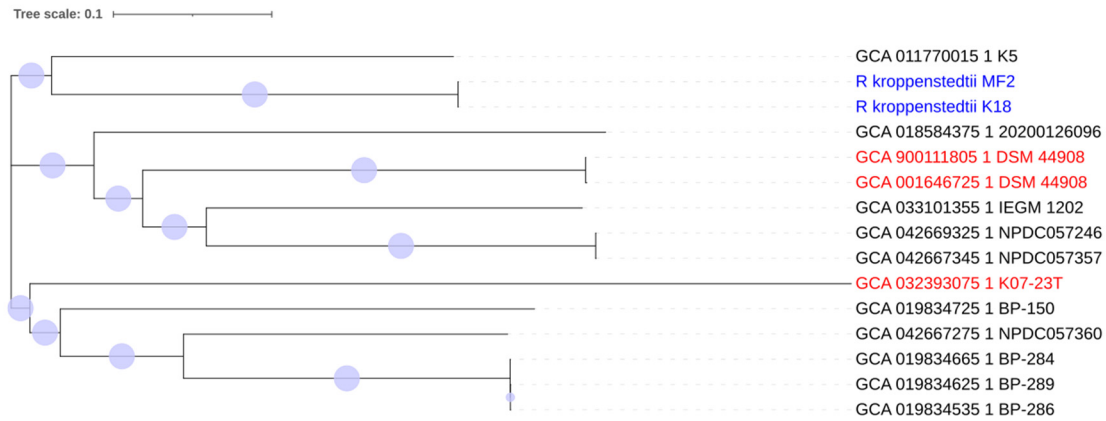


Figure 4. Phylogenomic tree showing the relative positions of *R. kroppenstedtii* strains and isolates. The tree was constructed using the Genome Taxonomy Database [45], release 220, and visualized in iTOL [46]. The tree was rooted at the midpoint. The reliability of internal branch points was determined using bootstrap analysis (100 replicates). Branch points with bootstrap values exceeding 80% are indicated by circles. Different genome assemblies of the type strain are highlighted in red. Strains K18 and MF2, which are investigated in this study, are highlighted in blue.

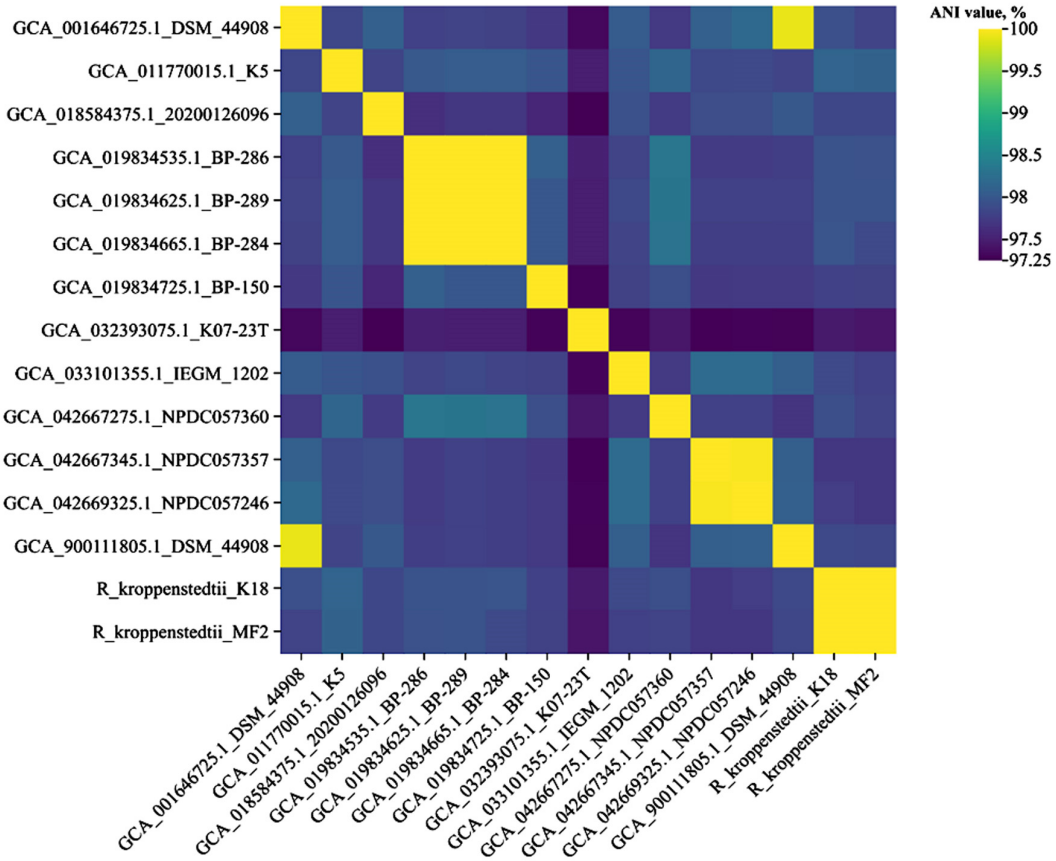


Figure 5. Range of ANI values among representatives of the species *R. kroppenstedtii*.

Previously, the isolation of representatives of the genus *Kocuria* (including *K. rosea*) from oil-contaminated soils of the Balgimbaev, Dossor, and Zaburunye oil fields in Kazakhstan was reported [56]. The ability of *Kocuria* spp. strains to utilize PAHs has been repeatedly confirmed [57–59]. For example, the *Kocuria flava* strain CMG2028 growing on naphthalene and phenanthrene, and *K. rosea* CMG2042 growing on naphthalene, phenanthrene, and fluoranthene have been described [58]. Ezima et al. [59] also isolated a *K. rosea* strain that efficiently utilizes naphthalene. Furthermore, treatment with corn seed culture

fluid increased germination from 13.3% to 46.7%. Thus, members of the genus *Kocuria* hold promise for bioremediation; however, their degradation potential for various pollutants remains poorly understood.

As shown in our previous experiments [20], isolated indigenous actinobacteria strains K1, K18, and MF2 exhibit weak crude oil degradation capacity (oil loss was 12–22% after 10 days of cultivation in liquid MSM). All strains are capable of utilizing hexadecane and benzoate as the sole sources of carbon and energy. Furthermore, *Rhodococcus* K18 and MF2 were found to utilize the n-alkanes dodecane and eicosane, as well as salicylate. *K. rosea* K1 did not utilize these substrates but could grow on phenol and gentisate (Table 3). The strains did not utilize naphthalene, phthalate, protocatechoate, benzene, or toluene.

Table 3. The ability of the studied strains to grow on various substrates.

Substrate	*K. rosea* K1	*R. kroppenstedtii* K18	*R. kroppenstedtii* MF2
Crude oil	+ [1]	+	++
Diesel fuel	++	+	+
Dodecane (C_{12})	−	+	+
Hexadecane (C_{16})	+	++	++
Eicosane (C_{20})	−	+	+
Benzoate	+	+	+
Gentisate	+	−	−
Salicylate	−	±	±
Phenol	±	−	−
Catechol	−	+	−

[1] The growth of the strains was assessed on the 7th day of cultivation using a scale where (++)—good growth (final cell titer $\geq 1 \times 10^8$ CFU/mL broth), (+)—medium growth (1×10^7–1×10^8 CFU/mL broth), (±)—weak growth (5×10^6–1×10^7 CFU/mL broth), and (−)—no growth ($\leq 5 \times 10^6$ CFU/mL broth). The initial cell concentration during inoculation was about 5×10^6 CFU/mL broth.

Whole-genome sequencing revealed the typical set of ring-cleavage dioxygenase genes for the phylum *Actinomycetota* in the genomes of the studied strains (the list of enzymes showing the degradative potential is given in Table S1). In *R. kroppenstedtii* strains K18 and MF2, typical actinomycete genes for alkane catabolism (including alkane monooxygenase *alkB* genes) were detected, along with individual genes for the degradation of aromatic compounds (Figure 6). The organization of the corresponding genomic regions is identical in both strains.

In the genomes of bacteria of the genus *Rhodococcus*, complete or partial duplication of metabolic pathways performing the same or similar functions is common [60]. The *alkB* genes are a striking example of genetic redundancy in rhodococci. The genomes of strains of different species of the genus *Rhodococcus* may contain approximately 4–6 copies of these genes [61,62]. There is no unambiguous classification of the *alkB* genes; the clearest ideas about the *alkB* genes of rhodococci have been formulated only for *alkB1–alkB4*; the designations of subsequent copies may differ among different researchers [62–65].

Species of the genus *Rhodococcus* with one or two copies of *alkB* are also known [66]. For example, among representatives of *R. opacus*, strains that have a single or multiple *alkB* cluster are known. Thus, in the genome of the *R. opacus* R7 strain [67], one copy of the *alkB* cluster was found. In the *R. opacus* B4 strain, two *alkB* clusters are present—*alkB1* and *alkB2* [66]. Unlike the *R. opacus* B4 strain, whose two *alkB* clusters provided the ability to utilize C_5–C_{16} alkanes [66], the *R. opacus* R7 strain [67] (one *alkB* cluster) utilized C_{12}–C_{24} alkanes, that is, the heavier fraction.

Two orthologs of the *alkB* genes were detected in the genomes of strains K18 and MF2. They are not accompanied by rubredoxins and reductase and do not have the characteristic genomic environment detected for the *alkB1–alkB5* genes in other representative species of

the genus *Rhodococcus*, such as the genes for D-cysteine desulfhydrase, the AraC family regulator, the glutamate tRNA ligase gltX, and two tRNAs (tRNA-Gln and tRNA-Glu). It can be concluded that the regions containing the alkB genes in representatives of the species *R. kroppenstedtii* are not related to those in other rhodococci species. All genes involved in alkane catabolism in strains K18 and MF2 are localized on chromosomes.



Figure 6. Heat map illustrating the representation of genetic determinants of major metabolic pathways in the genomes of strains K1, K18, MF2.

Whole-genome sequencing revealed the presence of benzoate-1,2-dioxygenase and catechol-1,2-dioxygenase genes. Homogentisate-1,2-dioxygenase genes were also detected, although the studied rhodococci were not capable of growth on this substrate. Genes encoding enzymes involved in the initial stages of naphthalene oxidation (naphthalene

dioxygenase and salicylate hydroxylase) are absent from the genomes of *R. kroppenstedtii* K1 and MF2 bacteria.

Despite the ability of the *K. rosea* K1 strain to utilize petroleum hydrocarbons, its genome lacks the alkane monooxygenase complex (*alkB*) genes characteristic of the genus *Kocuria* and other standard determinants of alkane utilization.

While the *alkB* operon is most widely studied, it is not the only system involved in alkane catabolism by members of the phylum Actinomycetota. In some strains genes encoding CYP153 monooxygenases will participate in alkane degradation. This has been demonstrated in both *Rhodococcus* spp. [68] and *Gordonia* spp., but not *Kocuria* spp. These systems can also coexist [64,69].

According to Nie et al. [70], genes encoding CYP153 monooxygenases can be either stand alone or accompanied by genes encoding ferredoxin and ferredoxin reductase.

During genome annotation of the K1 strain, the CYP153 monooxygenase genes were not found. It has been hypothesized that they have not been properly identified due to limited data from public databases. To refine the search for these genes, we used the CYP153 gene sequences of *Rhodococcus* spp. strains as references: *R. opacus* (AJ843243.1), *R. erythropolis* (AJ833998.1), and *Rhodococcus* sp. (FJ435356.1). The search was performed against the nr/nt and WGS databases for the entire genus *Kocuria*. No sequences that were orthologs of CYP153 P450 monooxygenases were found for either the *K. rosea* K1 strain or other representatives of the genus *Kocuria* whose genomes have been sequenced and deposited in GenBank. In all matching results, query cover was no more than 15%, with a percent identity of no more than 50%. Therefore, we conclude that the classical alkane monooxygenase genes that determine the specific process of alkane catabolism in representatives of the phylum Actinomycetota are absent from the genome of the *K. rosea* K1 strain.

However, various hydroxylase and oxidoreductase genes have been identified, which could potentially mediate the nonspecific oxidation of these substrates. The strain's weak growth is likely due to the nonspecific nature of this process. The *K. rosea* K1 genome contains genes encoding protocatechuate 3,4-dioxygenase (α- and β-subunits), catechol 1,2-dioxygenase, homogentisate 1,2-dioxygenase, and benzoate 1,2-dioxygenase (α- and β-subunits) (Table S1).

3.3. Functional Annotation of the Genomes of K. rosea K1 and R. kroppenstedtii K18 and MF2 Strains and Analysis of Gene Determinants Involved in the Production of Secondary Metabolites

Protein-coding elements of the strain genomes were functionally annotated (Table 4).

Table 4. Results of functional annotation of strains.

Strain	CDS Total	CDS Annotated	CDS Annotated, % of Total
K. rosea K1	3706	1772	47.8
R. kroppenstedtii K18	4122	1900	46.1
R. kroppenstedtii MF2	4025	1925	47.8

CAZyme analysis showed that all three strains possess a similar set of genes determining carbohydrate metabolism processes. Differences lie in the copy number of some elements (Table S2). All modules of central carbohydrate metabolism in all strains are complete.

Analysis performed using the antiSMASH service demonstrates that all strains have some ability to produce secondary metabolites, including those with antibiotic properties (Figure S1).

The spectrum of biosynthetic gene clusters (BGCs) of strain K1 relative to strains K18 and MF2 is unique in that they include clusters producing kocurin and branched-chain fatty acids, which showed high and medium similarity confidence, respectively, with the reference clusters. Kocurin is a secondary metabolite of bacterial origin (by different *Kocuria* spp.), a potent antibiotic from the thiopeptide (or thiazolyl peptide) class [71]. The cluster discovered in strain K1 is identical to the reference one (in *K. rosea* strain NCTC7514) and localized on the chromosome.

A cluster similar to the lymphostin/neolymphostinol B/lymphostinol/neolymphostin B (terpene, NRPS) production cluster in the reference strain *Rhodococcus* sp. PBTS 1, localized on the chromosome was detected in the K18 strain genome. This cluster is absent in the MF2 strain, but a hexose-palythine-serine/hexose-shinorine production cluster was detected. This demonstrates that, despite their close relationship, the K18 and MF2 strains possess distinct gene pools.

3.4. Reduction in Surface Tension of the Culture Medium by the Studied Strains

One of the mechanisms that ensures the high efficiency of hydrocarbon biodegradation by bacteria is the production of biosurfactants. Under moisture-deficient conditions, when oil forms viscous or asphalt-like films, biosurfactants significantly increase the bioavailability of hydrophobic substrates [5].

The ability of actinomycetes isolated from arid soils to synthesize surfactants has been repeatedly described in the literature. *S. ginkgonis* strain KM-1-2 [12] exhibited emulsifying properties when grown on motor oil (EI_{24}) exceeding 80%, while *Streptomyces* sp. MB9 [13] demonstrated this ability on cyclohexane and diesel fuel. Active secretion of surfactants by the *Nocardia mangyaensis* Y48T strain was also demonstrated by the presence of hemolysis zones on blood-agar plates [14]. The *K. rosea* ABR6 strain, recommended by the authors of [72] for oil sludge recovery, was shown to produce biosurfactants.

In our study, when cultured in liquid MSM supplemented with 1.0% hexadecane, rhodococci K18 and MF2 demonstrated a significant decrease in surface tension (31.3 ± 0.4 mN/m), indicating their production of biosurfactants (Table 5). No significant decrease in surface tension was observed for strain K1.

Table 5. Surface tension values during cultivation of the studied strains in liquid MSM supplemented with 1.0% hexadecane.

Strain	Surface Tension, mN/m
K1	56.1 ± 0.5 [1]
K18	31.4 ± 0.3
MF2	31.3 ± 0.4
sterile control with hexadecane	64.7 ± 0.4

[1] The table shows the mean values ± standard deviations. The results were obtained from three independent experiments.

Thus, our data showing that *R. kroppenstedtii* K18 and MF2 reduce surface tension are consistent with previous findings that related actinomycetes from similar habitats exhibit surface-active properties with respect to hydrocarbons.

3.5. Antibiotic Resistance and Resistance to Heavy Metals in the Studied Strains

Along with petroleum hydrocarbons, soil often contains trace and sometimes significant amounts of heavy metals (e.g., vanadium, nickel, iron, cadmium, zinc, copper, lead, chromium, etc.). This is due to several facts: (1) oil and petroleum products themselves contain metals (e.g., vanadium, nickel, iron, cadmium, zinc, copper and sometimes lead, chromium, etc.) in trace amounts; (2) oil pollution changes the chemical conditions of the

soil (pH, complexation), which can release metals from the mineral phase and increase their mobilization; transport and spills of petroleum products can be accompanied by other pollutants (the mechanism of metal transfer from equipment, waste, etc.), which adds to the load of heavy metals [73,74].

HM resistance is an important property for oil-degrading bacteria during in situ bioremediation. Even sublethal concentrations of HMs reduce the activity of hydrocarbon degradation enzymes (alkane hydroxylases, catechol dioxygenases), slowing biodegradation. Metal resistance preserves the catalytic activity of bacteria.

The studied strains were sensitive to 1.5 mM Co, Ni, Cu, Zn, and Cd, but resistant to 3 mM Pb. Moderate lead resistance may be useful for the remediation of soils contaminated with this metal.

According to existing data, the ability of bacteria to degrade petroleum products and aromatic hydrocarbons is often associated with antibiotic resistance [75,76]. To prevent the spread of antibiotic-resistant bacteria and resistance genes in the environment, careful selection of candidate strains for bioremediation with minimal levels of antibiotic resistance is recommended.

The actinobacteria studied were isolated from a single site (soil from the Daulet Asia site) and generally exhibited similar antibiotic resistance profiles (Table 6). All strains were resistant to trimethoprim and ceftazidime. *K. rosea* strain K1 was also resistant to chloramphenicol; *R. kroppenstedtii* strain K18 was resistant to chloramphenicol and meropenem; and MF2 was resistant only to meropenem.

Table 6. Resistance of the studied strains to antibiotics.

Strain	Antibiotics Group				
	Chloramphenicol (Amphenicols)		Trimethoprim (Diaminopyrimidines)	Ceftazidime (β-Lactam Antibiotics, Cephalosporins)	Meropenem (β-Lactam Antibiotics, Carbapenems)
	50 [2]	100	20	20	20
K1	+ [1]	+	+	+	−
K18	+	+	+	+	+
MF2	−	−	+	+	+

[1] Growth was assessed on the 2nd day of cultivation, (+) indicates growth, (−) no growth. [2] Concentration, μg/mL cultivation agar medium.

The strains were sensitive to other antibiotics related to aminoglycosides (streptomycin, kanamycin, gentamicin, neomycin, amikacin), tetracyclines (tetracycline, chlortetracycline), penicillins (penicillin, carbenicillin), cephalosporins (cefotaxime, ceftriaxone), rifamycins (rifampicin) and coumarins (DNA gyrase B inhibitors, novobiocin) (Section 2.5).

Genes conferring resistance to β-lactam antibiotics, including ceftazidime and meropenem, were detected in the genomes of all three strains (Table 7).

However, despite the presence of the Penicillinase repressor blaI genes in strains K18 and MF2 (Table 7), these bacteria were found to be sensitive to penicillin.

All antibiotic resistance genes in the strains are localized on chromosomes. In the genome of the *K. rosea* K1 strain, two prophages, both non-transposable, are predicted on the chromosome: prophage 1 (8728 bp)—*Myoviridae*/*Siphoviridae*, and prophage 2 (19,765 bp)—*Myoviridae*. ARGs were not detected on these prophages or within transposable elements. Sequences corresponding to prophages were not detected in the genomes of *R. kroppenstedtii* strains.

Table 7. Presence of β-lactam antibiotic resistance determinants in the studied strains.

Strain	Gene	Full Product Annotation
K1	blaB4	Lactamase_B, Metallo-beta-lactamase superfamily
	blaB4	Lactamase_B, Metallo-beta-lactamase superfamily
	blaB	Lactamase_B, Metallo-beta-lactamase superfamily
K18	blaI	Penicillinase repressor blaI
	bla	Beta-lactamase
MF2	bla	Beta-lactamase
	blaI	Penicillinase repressor blaI

The resistance of these actinobacterial strains to various groups of antibiotics in the absence of known specific resistance genes is highly likely due to nonspecific efflux pumps [77,78]. This mechanism explains the multiple resistance profiles and correlates with the ability to grow on hydrocarbons, for which these bacteria have already developed systems for the active removal of hydrophobic toxic compounds. Moreover, exposure to hydrocarbons can induce the expression of such pumps. The strains were cultured in laboratory conditions and isolated from soil containing hydrocarbons. Thus, their efflux systems may be constitutively active or easily activated.

The identified phenotypic resistance of strains K18 and MF2 to the clinically important antibiotic meropenem, along with the presence of genes encoding *β*-lactamases, requires control during their large-scale use to avoid the uncontrolled spread of antibiotic resistance genes in the environment.

3.6. Auxin Production by the Studied Strains

The ability to synthesize auxins, the key representative of which is indole-3-acetic acid (IAA), is widespread among rhizosphere microorganisms of various taxonomic groups, including actinobacteria [79,80]. However, there are only limited data in the literature on the ability of oil-degrading actinobacteria to produce IAA [81,82]. Auxin production by the studied strains was determined in a mineral-salt medium containing glycerol, hexadecane, or diesel fuel as the sole carbon and energy source, both with and without the addition of tryptophan.

The diversity of IAA-producing enzymes, the genes of which were found in the genomes of strains K1, K18 and MF2 is presented in Table S3.

The experiments showed that all the studied strains synthesized auxins in a mineral medium containing glycerol (Table 8). The addition of tryptophan, a precursor of IAA, increased the auxin content in the culture fluid by 5–22 times, indicating a tryptophan-dependent pathway for IAA biosynthesis in these cultures.

K. rosea K1 produced the highest amount of auxin in all experimental conditions, including on medium containing hexadecane (4.3 ± 0.2 μg/mL) and diesel fuel (5.8 ± 0.7 μg/mL) without the addition of tryptophan, demonstrating its metabolic flexibility. Adding tryptophan to the culture medium increased IAA synthesis in this strain.

R. kroppenstedtii K18 exhibited moderate auxin production. It did not synthesize IAA when grown on hexadecane, but the addition of tryptophan induced auxin formation, reaching a concentration of 10.4 ± 1.2 μg/mL. On diesel fuel, auxin levels reached 0.6 ± 0.1 μg/mL (without tryptophan) and 6.7 ± 0.3 μg/mL (in its presence).

R. kroppenstedtii MF2 produced the lowest amount of IAA among the strains studied. For this strain, maximum auxin accumulation (2.2 ± 0.2 μg/mL) was observed when grown on a medium containing glycerol and tryptophan. No auxin synthesis was detected on hexadecane (either with or without tryptophan). When grown on diesel fuel with added tryptophan, IAA production was 1.5 ± 0.07 μg/mL, which is comparable to the value ob-

tained on a medium containing glycerol. Genes encoding various IAA-producing enzymes were found in the genomes of all strains. The greatest diversity of these determinants was observed in strain K18 (Table S4), while the pool of genes involved in IAA production varied among strains belonging to the same species (K18, MF2). Several orthologs of amidase genes were found in the genome of strain K18, while their multiplicity was uncommon for the other strains.

Table 8. Auxin production (μg/mL) by the studied strains in a liquid mineral-salt medium containing different carbon sources and tryptophan.

Strain	Glycerol	Glycerol + Tryptophan	Hexadecane	Hexadecane + Tryptophan	Diesel Fuel	Diesel Fuel + Tryptophan
K1	1.1 ± 0.1 [1]	13.7 ± 0.8	4.3 ± 0.2	6.5 ± 0.9	5.8 ± 0.7	24.1 ± 2.4
K18	0.4 ± 0.1	9.1 ± 1.1	0.0	10.4 ± 1.2	0.6 ± 0.1	6.7 ± 0.3
MF2	0.4 ± 0.1	2.2 ± 0.2	0.0	0.0	0.3 ± 0.1	1.5 ± 0.1

[1] The table shows the mean values ± standard deviations. The results were obtained from three independent experiments.

R. fascians D188 (GCF_001620305.1) was used as a reference strain for studying the pool of IAA synthesis genes. Its genome contained the *iaaM* gene, designated Tryptophan 2-monooxygenase (AMY51695.1), and the *iaaH* gene, designated (R)-stereoselective amidase (AMY51696.1), on the chromosome. Genes related to the *iaaM* and *iaaH* genes of this strain were not found in the genomes of the studied strains.

3.7. Solubilization of Calcium Hydroxyapatite by the Studied Strains

The total phosphorus content in soils is known to fluctuate between 0.01 and 0.2%, but the proportion of its bioavailable compounds does not exceed 0.1% [83]. Many bioremediation technologies are based on the application of nitrogen-phosphorus fertilizers to stimulate native microflora [6,84], so assessing the phosphate-solubilizing activity of microorganisms is of significant interest when selecting oil-degrading strains.

We found that rhodococci strains K18 and MF2 possessed the ability to solubilize phosphates, as evidenced by the formation of transparent zones of calcium hydroxyapatite dissolution around the colonies. The *K. rosea* strain K1 demonstrated very weak growth on NBRIP medium; it lacked phosphate-solubilizing activity (Figure 7).



Figure 7. Dissolution of calcium hydroxyapatite by strains *R. kroppenstedtii* MF2 (1), *R. kroppenstedtii* K18 (2), *K. rosea* K1 (3) on NBRIP agar medium.

Genomic analysis revealed that all three strains possess a set of genes responsible for the dissolution of inorganic phosphates and the mineralization of organic phosphates.

Their genomes also contain genes responsible for phosphate transport. Genetic control of phosphate transport is provided by the Phosphate ABC transporter complex, which is represented by the *pst* genes. The complex consists of the following elements: *pstS*, *pstA*, *pstB*, and *pstC* (Table S4). The *pst* gene sets in K18 and MF2 are identical, while *K. rosea* K1 has three copies of *pstS*, one copy each of *pstA* and *pstB*, but no *pstC*.

3.8. Stimulation of Plant Growth and Biocontrol of Phytopathogens by the Studied Strains

In addition to pollutant degradation, key functions of bacteria in phytoremediation include improving plant growth and protecting them from phytopathogens. Studying these properties is essential for implementing phytoremediation approaches based on plant-microorganism interactions.

R. kroppenstedtii K18, *R. kroppenstedtii* MF2, and K. rosea K1 strains were used to colonize the plants. The strains were found to consistently associate with the plants and did not negatively impact rapeseed morphogenesis.

In vitro experiments showed that among the studied strains, only *R. kroppenstedtii* MF2 after three weeks exhibited a significant phytostimulating effect on rapeseed plants (Figure 8).

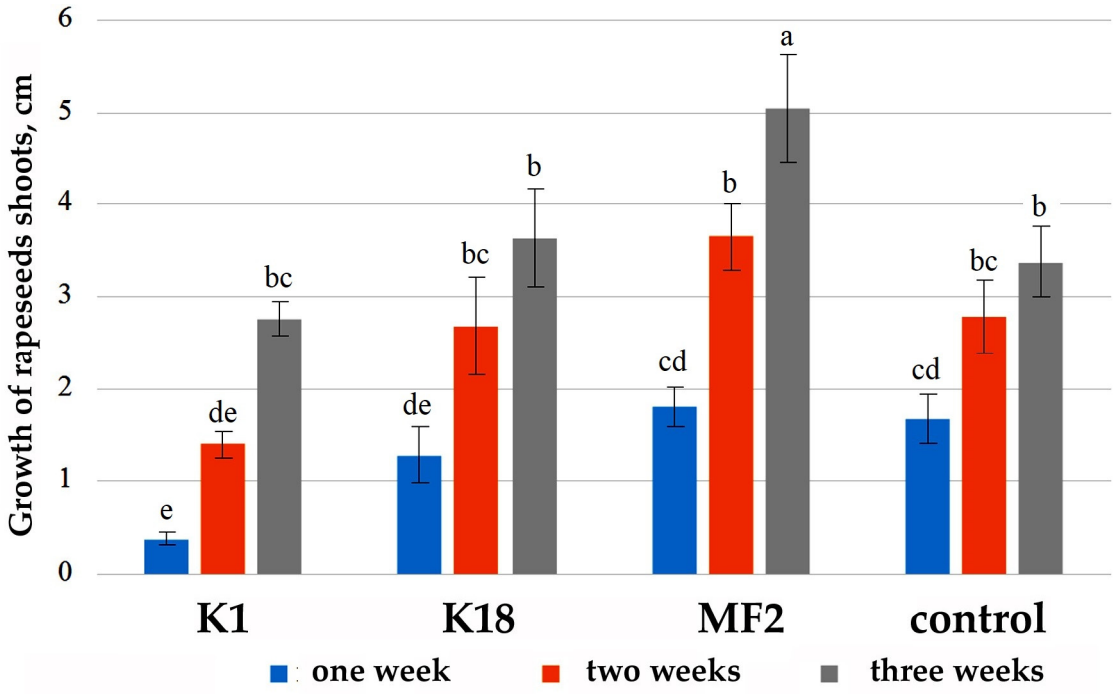


Figure 8. Growth of rapeseed plants inoculated with *K. rosea* K1 and *R. kroppenstedtii* K18 and MF2 strains (control—non-colonized plants). Data are averages of at least three biological replicates consisting of 5–6 plants ± SE. Different letters above the bars indicate a statistically significant difference between the treatments at $p \leq 0.05$, as assessed using the one-way ANOVA test.

Bacteria secrete a variety of low-molecular-weight compounds, typically secondary metabolites, signaling molecules, and phytohormones. Volatile organic compounds, such as 2,3-butanediol, directly stimulate root expansion, increasing the root surface area for water and nutrient uptake, significantly increasing overall plant biomass [85]. It is known that antimicrobial compounds such as bacteriocins, siderophores, and lipid peptide biosurfactants of associative bacteria can displace and destroy pathogenic microorganisms and influence the host immunity [86]. In this work, a test was performed on the resistance of colonized plants to several phytopathogens, namely the fungi *F. graminearum* VKM F-1668, *F. oxysporum* VKM F-140, and *F. culmorum* VKM F-2303 and the bacterium *P. wasabiae* B15.

In microvegetation experiments with an artificial phytopathogenic background, all strains protected rapeseed plants from infection with *F. oxysporum* VKM F-140. In addition, *R. kroppenstedtii* K18 exhibited antagonism towards *P. wasabiae* B15, and *K. rosea* K1 towards *F. culmorum* VKM F-2303 (Figures 9 and S2–S5).

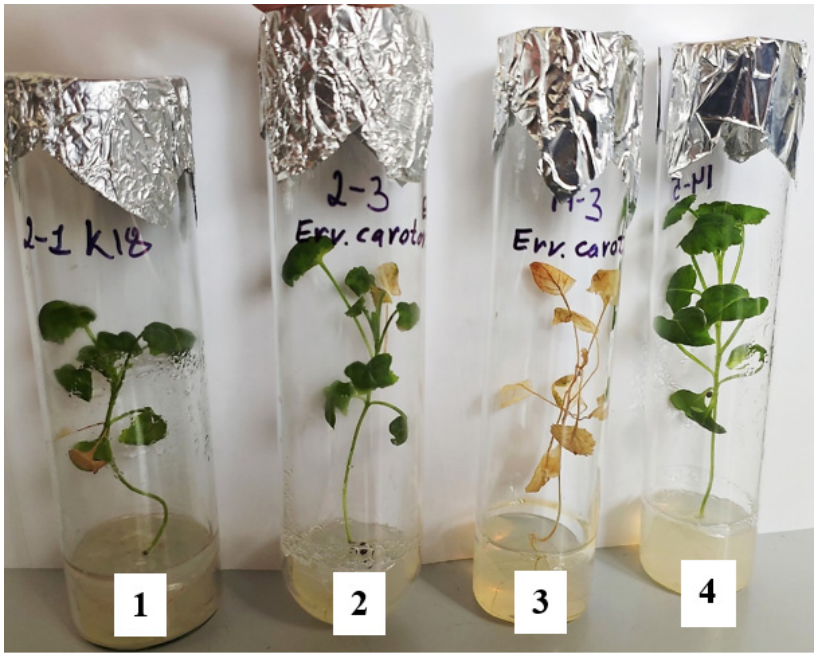


Figure 9. Resistance of rapeseed seedlings colonized with *R. kroppenstedtii* K18 to a bacterial phytopathogen *P. wasabiae* B15: 1—colonization with K18; 2—colonization with K18 followed by infection with *P. wasabiae* B15; 3—infection with *P. wasabiae* B15, 4—control without colonization and infection.

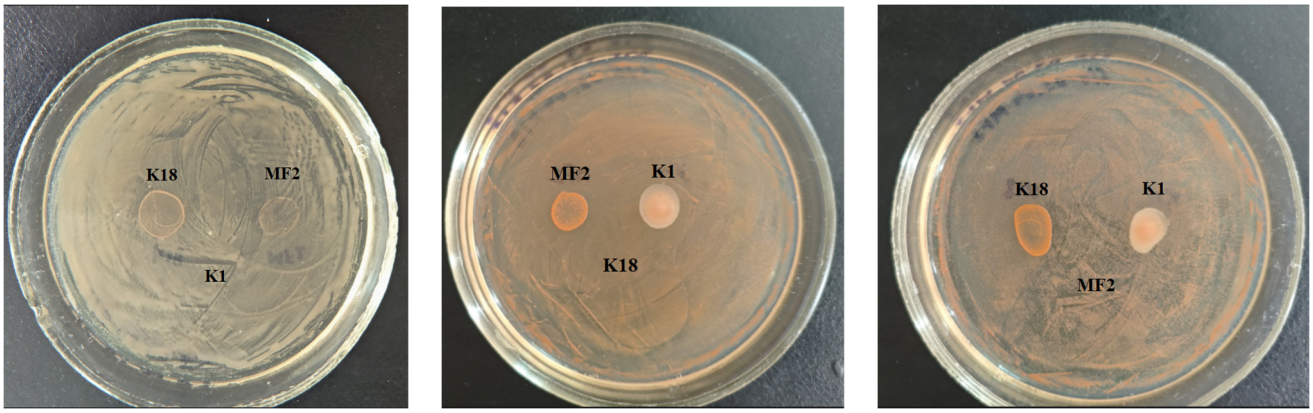
The degree of damage to colonized plants by phytopathogens was assessed as a percentage relative to the control (non-colonized plants) (Table S5). Colonization of plants with the studied bacterial strains did not result in significant protection against the phytopathogen mycromycete *F. graminearum* VKM F-1668. However, it is intriguing whether protection is due to direct antimicrobial activity or induced plant response. Understanding the mechanisms that enable bacteria to interact with plants and positively influence their proliferation will allow for a more complete utilization of the biotechnological potential of these microorganisms.

3.9. Compatibility of the Studied Strains

It is known that biopreparations can be based on both individual strains and consortia of various microorganisms. Complex biopreparations containing microorganisms from different taxonomic groups are believed to exhibit higher efficacy and environmental stability [87].

Furthermore, when developing microbial consortia, it is important to consider the possibility of co-cultivating microorganisms to reduce production costs. Such biopreparations may be more resistant to contamination by foreign microflora during fermentation [88]. Therefore, the design of microbial associations and the selection of strains that do not negatively impact each other is an important step in the development of complex biopreparations.

The experiments revealed that the studied strains did not exhibit antagonism towards each other; they did not exhibit growth inhibition zones (Figure 10). Thus, they can be used as a bacterial association.



(**a**) (**b**) (**c**)

Figure 10. Compatibility of selected native strains: two other strains were applied to a lawn of one strain: (**a**)—*K. rosea* K1 lawn, (**b**)—*R. kroppenstedtii* K18 lawn, (**c**)—*R. kroppenstedtii* MF2 lawn.

4. Conclusions

Our results indicate that two *R. kroppenstedtii* strains, K18 and MF2, are capable of metabolizing not only oil and diesel fuel, but also n-alkanes and salicylate. *K. rosea* strain K1 does not metabolize n-alkanes but can grow on phenol and gentisate. The studied soil microorganisms are antibiotic-resistant. The resistance of these actinobacterial strains to various antibiotics is likely conferred by nonspecific efflux pumps involved in the removal of toxic compounds—both the hydrocarbons themselves and their intermediates.

All three strains contain genes for and are capable of producing the phytohormone IAA, but only strain K18 demonstrated growth stimulation in rapeseed plants. All three strains contain genes for phosphate solubilization, but only the rhodococci strains exhibited this phenotype. A significant decrease in surface tension (31 mN/m) by *R. kroppenstedtii* K18 and MF2 indicates biosurfactant production, a beneficial property due to increased bioavailability of petroleum hydrocarbons for degrading microorganisms. All three strains are compatible within the association.

Although the three isolated native strains have weak oil-oxidizing capacity (oil degradation efficiency of 12–22%), they exhibit a number of interesting properties: IAA production, biosurfactant production, phosphate solubilization (rhodococci strains). This study expands our knowledge of hydrocarbon-oxidizing actinomycetes in desert soils that exhibit phytostimulating properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments13070410/s1, Figure S1: Clusters of secondary metabolite production in the genomes of *K. rosea* K1 (a), *R. kroppenstedtii* K18 (b), and *R. kroppenstedtii* MF2 (c) strains according to the antiSMASH classification; Figure S2: Resistance of rapeseed seedlings colonized with *Kocuria rosea* K1 to a micromycete phytopathogen *Fusarium culmorum* VKM F-2303; Figure S3: Resistance of rapeseed seedlings colonized with *K. rosea* K1 to a micromycete phytopathogen *Fusarium oxysporum* VKM F-140; Figure S4: Resistance of rapeseed seedlings colonized with *Rhodococcus kroppenstedtii* K18 to a micromycete phytopathogen *Fusarium oxysporum* VKM F-140; Figure S5: Resistance of rapeseed seedlings colonized with *Rhodococcus kroppenstedtii* MF2, to a micromycete phytopathogen *Fusarium oxysporum* VKM F-140; Table S1: Diversity of aromatic hydrocarbon catabolism enzymes, the genes of which were found in the genomes of the studied strains K18, MF2 and K1; Table S2. Functional spectrum of carbohydrate catabolism genes; Table S3: Diversity of IAA-producing enzymes, the genes of which were found in the genomes of strains K1, K18 and MF2; Table S4: Diversity of enzymes responsible for phosphate solubilization. Genes encoding these enzymes were found in the genomes of strains K1, K18, and MF2. Table S5: Resistance of rapeseed

seedlings colonized with hydrocarbon-degrading actinobacterial strains to various phytopathogens. The damage caused by pathogens on leaves is indicated as a percentage.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author. DNA full genome sequences for the strains were deposited in an NCBI database: *Kocuria rosea* K1—Accession number NZ_JBVARS000000000.2; *Rhodococcoides kroppenstedtii* strain K18—Accession number NZ_JBVART000000000.1, *Rhodococcoides kroppenstedtii* strain MF2—NZ_JBVARU000000000.1.

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Abbreviations

The following abbreviations are used in this manuscript:

AAI	average amino acid identity
alkB	alpha-ketoglutarate-dependent dioxygenases
dDDH	digital DNA–DNA hybridization parameter
IAA	indolil-acetic acid
bp	nucleotide base pairs
CFU	colony forming units
GBDP	Genome Blast Distance Phylogeny
HM	heavy metals
LB	Louria-Bertany broth
LPSN	List of Prokaryotic Names with Standing in Nomenclature
Mbp	a million of nucleotide base pairs
MSM	mineral salt medium
NBRIP	National Botanical Research Institute's phosphate growth medium
PAH	polycyclic aromatic hydrocarbons
UV	ultraviolet

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