

Article

# Stress-Tolerance and Taxonomy of Culturable Bacterial Communities Isolated from a Central Mojave Desert Soil Sample

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Received: 28 February 2019; Accepted: 8 April 2019; Published: 10 April 2019



**Abstract:** The arid Mojave Desert is one of the most significant terrestrial analogue objects for astrobiological research due to its genesis, mineralogy, and climate. However, the knowledge of culturable bacterial communities found in this extreme ecotope's soil is yet insufficient. Therefore, our research has been aimed to fulfil this lack of knowledge and improve the understanding of functioning of edaphic bacterial communities of the Central Mojave Desert soil. We characterized aerobic heterotrophic soil bacterial communities of the central region of the Mojave Desert. A high total number of prokaryotic cells and a high proportion of culturable forms in the soil studied were observed. Prevalence of *Actinobacteria, Proteobacteria,* and *Firmicutes* was discovered. The dominance of pigmented strains in culturable communities and high proportion of thermotolerant and pH-tolerant bacteria were detected. Resistance to a number of salts, including the ones found in Martian regolith, as well as antibiotic resistance, were also estimated.

**Keywords:** physiology of bacteria; extremotolerance; antibiotic resistance; soil; microbial communities; Mars analogue site

#### 1. Introduction

One of the main tasks of modern astrobiology is studying environmental factors of different space bodies and outer space and to determine surviving and functioning limits of terrestrial life forms under extraterrestrial conditions [1,2].

It is known that potentially habitable space objects are exposed to a number of extreme abiotic factors, including high-intensity ionizing radiation, reduced pressure, contrast temperature conditions, low water availability, etc. [3–5]. Up to date, a large amount of data on physicochemical properties of regolith and climatic and cosmic regimes of various extraterrestrial bodies, in particular Mars, have been accumulated [6]. Based on these data, it can be stated that in today's biosphere of the Earth there are ecosystems that possess some environmental parameters similar to those found on extraterrestrial bodies. Hot-arid and cold-arid deserts, permafrost sedimentary rocks and some arid soils are considered as terrestrial analogues of extraterrestrial regolith [7–9] and are used as model objects in astrobiological research.

Microorganisms of arid ecosystems are exposed to a wide spectrum of stress factors such as water and organic matter deficiency, exposure to winds and high-intensity ultraviolet (UV) radiation, and significant temperature fluctuations. However, these areas are not lifeless and are characterized by



significant richness and diversity of prokaryotes [10,11]. Many astrobiological studies consider the Atacama Desert [12–14], the Sonora Desert [15], and the Dry Valleys of Antarctica [16] to be the main objects of research. However, it is important to investigate different ecosystems that are exposed to extreme environmental factors [17]. The Mojave Desert is one of such analogue ecosystems. It is characterized by relief and mineralogy that are close to those found on Mars [18–20]. There are several reasons as to why edaphic (i.e., associated with soil) bacterial communities of hot deserts should be studied, such as the lack of culturable communities' research and the possible biotechnological potential of bacterial strains that inhabit the ecosystems and its highly valuable and significant properties for

further research [21].

The Mojave Desert is located in the southwest of the United States and covers an area of approximately 150,000 km<sup>2</sup>. It is characterized by altitudes of 600–1500 m above sea level and arid-semiarid climate with an average annual precipitation of less than 300 mm (the average annual precipitation is 137 mm/year). Temperatures range from -10 to 50 °C; frequent winds are also observed in this area [22,23]. The average annual temperature is  $20 \pm 0.5$  °C, annual heat-moisture index (AHM) is  $230 \pm 19$  [24].

Ten thousand years ago this territory was characterized by a humid climate and well-formed soil coverage, but the aridization of weather conditions and desertification of the region have led this area to its present state [23]. The region is characterized by volcanogenic geology and topography, a high degree of weathering of surface sedimentary rocks [25] and extremely poor vegetation [19]. The total organic matter content in the soils of this desert is very low (it ranges from 145 to 260 µg of C per gram of soil). Carbon to nitrogen ratio is about 9.5 [26].

All the aforementioned properties of the Mojave Desert make it a suitable terrestrial analogue to Mars, particularly because of the climate changes that had happened here and of the ongoing geological and climatic processes [24]. The soil of this desert has a mineralogical composition close to that of the Martian regolith [27]. As well as that, it also has a similar composition of mineral salts [8,18,28]. Worth noticing is the fact that in the past the territory of modern Mojave Desert had been coated by mature soil coverage, which could contain diverse soil microbial communities. However, as time has been progressing, this region was subjected to long-lasting drought periods and microbial communities have been forced to adapt and alter their metabolism under conditions of moisture and nutrients deficiency and high-stress load. Supposedly the same happened to the Martian regolith [19,28]. The soils of this desert have already been used in model astrobiological studies and in The Mojave Mars simulant (MMS) in particular as a regolith model of Mars [29]. Bacterial isolates from the soils of this desert served as model organisms for studying lithopanspermia processes [15].

Earlier studies revealed a large number and variety of phototrophic organisms in the soil of Mojave confined to hypolith communities and able to carry out photosynthesis under aggressive environmental conditions in situ (i.e., in natural soil conditions) and in vitro (i.e., in laboratory conditions) [8,30].

Presence and metabolic activity of phototrophic and chemotrophic prokaryotes have also been confirmed, as well as presence of nitrogen-fixing bacteria and ability of those organisms to produce primary organic matter (references herein and [10,21]). Thus, it becomes obvious that along with the autotrophic community, there must be a heterotrophic community in the soil of this desert.

In general, previous studies have discovered a similar structure of edaphic bacterial communities at the phylum level in the soils of cold and hot deserts: among aerobic heterotrophic bacteria, representatives of the phyla *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, and *Bacteroidetes* are the most common [10].

In the surface soil of The Mojave Desert, 48 different taxonomic units of bacteria were found by molecular cloning [31].

Biological soil crusts (BSC) confined to the surface layers of soil are common in the Mojave Desert. The next generation sequencing of 16S rRNA genes from the soil under BSC revealed the following phylogenetic structure of the bacterial community: *Proteobacteria* ( $23 \pm 5\%$ ), *Actinobacteria* ( $20 \pm 5\%$ ), and *Chloroflexi* ( $18 \pm 3\%$ ), representatives of the genus *Rubrobacter* were the most dominant

among actinobacteria. *Acidobacteria* ( $10 \pm 2\%$ ), *Bacteroidetes* ( $6.5 \pm 2.0\%$ ), and *Cyanobacteria* ( $11 \pm 10\%$ ); *Gemmatimonadetes, Armatimonadetes, Planctomycetes, Nitrospirae, Chlorobi,* and *Verrucomicrobia* (<5% altogether) representatives have also been identified [32].

*Proteobacteria* (~45% operational taxonomic units (OTU)), *Actinobacteria* (~12% OTU), and *Firmicutes* (~10% OTU) phyla have been found as the most abundant ones by metagenomic analysis of the soils of the desert. The authors have concluded that there was a high number and high taxonomic diversity of bacteria inhabiting the arid soils of the Mojave Desert. They have also concluded that the desert soils were rich with unidentified bacteria species, supporting this with molecular-genetical data obtained [21].

Based on the high-throughput DNA sequencing and shotgun-metagenomic study of the Mojave Desert soils performed by the several research groups, a high relative abundance *Proteobacteria* and *Cyanobacteria* phyla and presence of representatives of the phyla *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Acidobacteria*, *Firmicutes*, and *Crenarcheaota* were also detected [24,33,34]. A high abundance of genes associated with spore formation processes, encoding stress proteins and amino acid metabolism, associated with osmoregulation was also discovered. Based on these results, the authors concluded that there was a significant functional diversity of desert bacterial communities [33].

Using the methods of native fluorescence and fatty acid analysis of phospholipids, the number of bacterial cells was found to be  $10^7$  bacteria per gram of soil [35,36]. Studies of culturable bacteria of this desert are less common, compared to culture-independent methods. A high number of cultured bacteria was shown up to  $10^6$  colony-forming units per gram (CFU/g) [37].

A study of the number and taxonomic composition of cultured actinomycetes in the soils of this desert revealed the dominance of *Actinoplanes*, *Pilimelia*, and *Streptomyces* genera representatives. The authors noted a high diversity of isolated strains, and the fatty acid methyl ester analysis of isolates indicated a significant proportion of previously undescribed actinomycete species. Extreme effects of external factors were proposed to be the driving force of speciation in desert soils, which leads to the morpho-physiological adaptations of prokaryotes inhibiting them [38].

Community level physiological profile analysis of the microbial complexes (using BIOLOG 96-well Eco-Microplates assimilation test) of soils confined to plants revealed the absence of correlation between the structure and the diversity of bacteria in communities under plant coverage and in soils with no vegetation. A positive correlation between the number of substrates consumed by the bacterial community and the presence and diversity of higher plants growing on that soil was proved. However, bacterial communities confined to areas of the desert lacking vegetation showed active assimilation of amino acids, amines, amides, and various polymers. In addition, representatives of *Sinorhizobium*, *Bacillus*, and *Sphingomonas* genera were isolated and identified in culture grown on selective medium for nitrogen-fixing bacteria [39].

In dry conditions, microbial communities are exposed to different extreme conditions, such as temperature and pH fluctuations, soil moisture shortage, high level of ultraviolet radiation. All of them have a significant impact on microbial communities [17].

Generally, little is known about bacterial communities of arid soils, and previous studies revealed significant taxonomic and functional differences between the bacterial communities of extreme soils and those of temperate zones [24,40]. A high diversity and a high number of cells in bacterial communities of the Mojave Desert soils in situ (i.e., in natural conditions), predominated by *Proteobacteria* and *Actinobacteria* phyla were found. The abundance and diversity of photo-and chemotrophic microorganisms were described predominantly by culture-independent methods. However, heterotrophic community studies are surprisingly rare: only individual groups of culturable bacteria, such as, for example, nitrogen-fixing bacteria and actinomycetes, have been investigated previously. Still, little is known about the bacterial communities of arid soil even though bacterial stress resistance research plays an important role when it comes to extraterrestrial analogue ecosystems in astrobiological context.

In this paper, we studied the aerobic heterotrophic bacterial community and the physiological characteristics of cultured bacteria, isolated from the surface soil sample collected in the central part of the Mojave Desert.

#### 2. Materials and Methods

#### 2.1. Soil Sampling

A single soil sample was collected in the central part of the Mojave Desert in the summer of 2015 in a flat area devoid of vegetation (34°55′39.0″ N 115°45′33.7″ W). The surface soil was taken from 0–3 cm depth with no signs of vegetation and biological soil crust and put into a sterile polypropylene container. The sample was transported and stored at room temperature in an air-dry (natural) condition. Microbiological analyses were performed within one month after the sample collection.

The central region where the sample was taken from is characterized by the most typical climate of this desert: the temperature during the year varies from -10 to 50 °C, the average annual rainfall varies from 30 to 300 mm (arid–semi-arid climate type) [22]. It has been shown that the soil of this desert can be heated up to a temperature of 67 °C [29].

The Mojave tectonic block is a part of the Basin and Range tectonic province, where many asymmetrical basins have been formed during regional extension processes. The central Mojave region was formed during localized extension which occurred between 24 and 18.5 million years ago and produced 40–60 km of extended terrain space [41]. The central Mojave Desert is characterized by a variety of geologic surfaces that includes mountain ranges of diverse rock types, playas, ancient lake deposits, lava flows, sand-dune fields, alluvial fans, and riverbeds. The region of sample studied is underlain by middle Tertiary volcanic rocks, ranging mainly from andesitic to rhyolitic compositions [42].

The sample pH was 7.2. Literature data on the soils of this region indicate the content of organic matter in the range of 0.04–0.1%, total nitrogen content varies from 0.03% to 0.09% [22]. Soil sample texture is found to be sand. Dominance of silica and iron oxide-containing minerals was visually detected and confirmed by polarized light microscopy.

#### 2.2. Epifluorescent Microscopy

The total number of prokaryotes in the samples was determined with epifluorescence microscopy (EFM) with acridine orange dye. Cell desorption was carried out by vortexing using Heidolph Multi Reax vortex for 30 min at 2000 rpm. The preparations were produced in six replicates, fixed by heating, then stained with water solution of acridine orange (1:10,000) for 3 min, washed in still distilled water for 20 min, dried at room temperature, and viewed under the Primo Star (Zeiss, Germany) microscope with fluorescence epi-illumination system (AmScope, Irvine, CA, USA) at 700 × magnification for 20 fields of vision for each replication. Cells with green fluorescence were counted. The water used for the dilution preparation was simultaneously examined as control.

The prokaryotic cells number was calculated using the equation  $N = (S_1 \times a \times n)/(V \times S_2 \times c)$ , where N is the number of cells per gram of soil;  $S_1$  is the area of the preparation ( $\mu m^2$ ); a is the number of cells in the field of view; n is the dilution index; V is the volume of the soil suspension drop placed on the glass (mL);  $S_2$  is the field of view of the microscope ( $\mu m^2$ ); and c is the sample weight (g) [43].

#### 2.3. Bacteria Culturing and Isolation

Peptone Yeast Glucose (PYG) medium rich in various carbon sources and nutrients (glucose—1 g/L, peptone—2 g/L, yeast extract—1 g/L, tryptone—1 g/L, agar—20 g/L) and modified Czapek (CM) medium (glucose—2 g/L, sucrose—2 g/L, starch—2 g/L, NaNO<sub>3</sub>—2 g/L, KH<sub>2</sub>PO<sub>4</sub>—1 g/L, MgSO<sub>4</sub>—0.5 g/L KCl—0.2 g/L, agar—20 g/L) [44] were used for bacteria culturing and isolation. Dilutions of the soil sample in sterile 0.01 M phosphate-saline buffer solution (pH 7.4,

0.137 M NaCl, 0.0027M KCl) in tenfold dilution step were plated on the solid nutrient media in three replicates [45].

For the more complete characterization of the cultured bacterial community, culturing was performed at temperatures of 10, 25, and 50 °C (characteristic optima of psychrophilic, mesophilic, and thermophilic bacteria [17]) until the formation of visible macrocolonies was completed (typically for 14 days at 25 and 50 °C and 30 days at 10 °C). Colonies' morphotypes were distinguished by colour and morphology of the colonies as well as cell morphology. Isolated bacterial strains (Table A1) were deposited to the Astrobiological Collection of Microorganisms of the National Depository Bank of Live Systems "Noah's Ark" (Available at [46]).

#### 2.4. Amplification and Sequencing of 16S rRNA Genes

For amplification of the 16S rRNA gene, biomass of pure bacterial culture that has been cultured for 2–3 days was subjected to boiling in Tris-EDTA buffer (pH 8.0) which contained 5% Triton X-100, and homogenization by mechanical destruction with sterile glass beads (250–300  $\mu$ m in diameter) using a Homogenizer Minilys (Bertin Instruments, Montigny-le-Bretonneux, France) at 5000 rpm for 30 s. The resulting homogenate was centrifuged and the resulting supernatant was used as a DNA template for PCR [47].

For amplification of the gene of interest, the primer systems 27 f + Un1492 r [48,49], 63 f + 1387 r [47], 341 f + 805 r [50], and 27 f + 537 r [48,50] were used in order of priority. Amplification process was performed with cell lysate, prepared as described above. If the procedure failed to obtain the PCR product by the first primer system, the next primer system with the same DNA matrix was used and so on until a product that can be used for further sequencing was acquired [45].

The PCR products were purified and sequenced by the Research and Production Company "Evrogen" (Moscow, Russia) using the 1100 r [50], 805 r, or 537 r [51] primers (Table A2). The editing of the nucleotide sequences was carried out using Chromas Lite 2.01 [52]. For alignment, comparison and identification of nucleotide sequences, the Clustal Omega [53] and the BLAST algorithm from the GenBank database [54] were used. The sequences were deposited in GenBank under the accession numbers MK403768—MK403884.

The phylogenetic trees were constructed using MEGA 7 software [55]. Sequences were then aligned using the Clustal Omega online alignment tool [53]. The type material sequences from GenBank were predominantly used for phylogenetic trees construction. Strain identification was based upon phylogenetic analysis and similarity of the sequences with GenBank data.

#### 2.5. Physiological Assays

The resistance spectra of all isolated cultures (121 strains) to various factor influences were obtained by culturing isolates on the same liquid medium that was used to isolate each particular strain from a natural sample; for each of the investigated factors the experiments were conducted in three replicates. To determine resistance to temperature, cultures were incubated in thermostats at temperatures of 2, 4, 10, 25, 37, 45, and 50 °C. Phosphate (KH<sub>2</sub>PO<sub>4</sub> + H<sub>3</sub>PO<sub>4</sub> final concentration of 100 mM, pH 2–6) and tris-alkaline (Tris + HCl + NaOH final concentration of 100 mM, pH 7–12) buffer systems were used to determine resistance to various pH levels. Bacterial strains' resistance to presence of salts was determined by aseptic preparation and subsequent dilution of 20% (w/v) stock solutions of NaCl, KCl, NaHCO<sub>3</sub>, MgSO<sub>4</sub> to final concentrations of 2, 5, 10, 15, and 20%; the series of magnesium perchlorate concentrations were prepared similarly by diluting a 20% (w/v) sterile stock solution to final concentrations of 0.5, 1, 2, 5, 10, and 15%. Antibiotic resistance was determined on nutrient media containing ampicillin (Amp), cephalexin (Ceph), chloramphenicol (Chl), tetracycline (Tet), doxycycline (Dox), kanamycin (Kan), or rifampicin (Rif) (Belmed, Russia) with a final concentration of 100  $\mu$ g/mL. All tests (except for resistance to temperature) were carried out at a temperature of 25 °C [44]. The growth was registered on the tenth day after inoculation for the cultures cultivated at temperatures of 10, 25, 37, 45, and 50 °C; for cultures growing at temperatures of 2 and 4 °C the incubation period was 60 and 30 days, respectively. Growth registering was performed using a Sunrise (Tecan, Menendov, Switzerland) plate photometer at a wavelength  $\lambda$  = 620 nm: The plates were measured immediately after inoculation and after incubation period. Detailed protocol of physiological assays is described in [44].

#### 2.6. Multisubstrate Testing

To assess the potential metabolic activity of a microbial community, multisubstrate testing (MST) of the soil microbial community was performed [56,57]. The testing was carried out with 47 substrates including sugars, alcohols, amino acids, salts of organic acids, and polymers (all substrates are listed in [43], all tests were performed in two replicates). The soil sample was diluted (1:100) in sterile phosphate saline buffer (pH 7.4) and vortexed for 15 min at 2500 rpm using Heidolph Multi Reax vortex. The mineral particles were precipitated by centrifugation (2000 rpm, 2 min). The hydrogenase activity indicator (triphenyltetrazolium bromide) was added to the supernatant, and after mixing 200  $\mu$ L aliquot was added to each well of a 96-well plate containing a set of 47 test substrates in two replicates. The plates were incubated at +28 °C for 96 h. After the incubation, the optical density of the solutions was measured photometrically at wavelength  $\lambda$  = 510 nm using a Sunrise (Tecan, Menendov, Switzerland) plate photometer.

#### 2.7. Data Analysis

Statistical data processing was performed using the Microsoft Office Excel 2011 software package (Redmond, Washington, D.C., USA). For data on cell numbers, confidence intervals of the mean were calculated using Student's test at p < 0.05. When determining the physiological spectra, the results obtained in three replicates was considered a positive reaction. Indices of similarity and biodiversity of microbial communities were calculated according to Chernov and Lysak [58] at genera level of taxonomic affiliation.

### 3. Results

#### 3.1. Cell Numbers

The total number of prokaryotic cells in the soil sample, obtained by epifluorescence microscopy method, was  $(5.8 \pm 1.7) \times 10^8$  cells per gram of soil. Cultured bacteria on PYG medium were detected in the amount of  $(8.0 \pm 0.5) \times 10^6$  colony-forming units (CFU) per gram and  $(5.5 \pm 0.2) \times 10^6$  CFU/g at 25 °C and 10 °C, respectively. On the CM medium  $(8.8 \pm 0.4) \times 10^5$  and  $(5.5 \pm 0.2) \times 10^5$  CFU/g were found at 25 °C and 10 °C, respectively (Figure 1). Colony-forming units were not detected after incubation at 50 °C.



**Figure 1.** Total prokaryotic cells count obtained by epifluorescent microscopy (EFM) and culturable bacteria count on peptone-yeast-glucose (PYG) and Czapek modified (CM) media in the examined soil sample. Error bars are within the standard deviation, p < 0.05.

#### 3.2. Culturable Communities' Structure

Pigmented forms of microorganisms dominated in all the cultured communities isolated from the sample. In the PYG medium plated communities, which were characterized by a higher cell counts, red-pigmented colonies were predominant and yellow-pigmented colonies were dominant on CM medium (Figure 2). Maximum diversity of colony morphotypes was observed on PYG medium: 61 morphotypes were described in the course of culturing at mesophilic optimum; on CM medium 28 morphotypes were isolated under similar temperature conditions. At low temperature (10  $^{\circ}$ C), diversity decreased: 22 and 10 colony morphotypes were isolated on PYG and CM media, respectively (Figure S1). In total 121 strains of aerobic heterotrophic bacteria were isolated from the investigated soil sample and subjected to further analysis.



Figure 2. Proportion of pigmented colonies in the communities studied.

The greatest taxonomic diversity of cultured bacteria was recorded in the microbial community isolated on PYG medium under mesophilic conditions (25 °C). Representatives of 24 genera of bacteria were identified: *Agrococcus, Arthrobacter, Bacillus, Brachybacterium, Brevibacterium, Cellulomonas, Georgenia, Janthinobacterium, Kocuria, Labedella, Leucobacter, Massilia, Microbacterium, Micrococcus, Mycetocola, Paracoccus, Planomicrobium, Pseudarthrobacter, Rufibacter, Salinibacterium, and Streptomyces.* Bacteria of the species *Arthrobacter* sp. were dominant and the subdominant positions were occupied by *Microbacterium* sp., *Pseudoarthrobacter* sp., and *Agrococcus* sp. representatives (Figure 3).

Under similar conditions on CM medium, representatives of the genera *Agrococcus Arthrobacter*, *Bacillus, Burkholderia, Cellulomonas, Massilia, Microbacterium, Paracoccus, Pontibacter, Pseudoarthrobacter, Salinibacterium, Sphingomonas*, and *Streptomyces* were identified (listed in descending order of representation degree in the community, Figure 4).

Smaller diversity was observed in the community incubated under low temperature conditions. Bacteria of the genus *Planomicrobium* were dominant on PYG medium, subdominant positions were represented by species *Arthrobacter agilis*, and *Planomicrobium glaciei*, and representatives of *Arthrobacter*, *Pseudarthrobacter*, *Leucobacter*, *Microbacterium*, *Massilia*, *Micrococcus*, *Pseudarthrobacter*, *Rhodococcus*, and *Sphingomonas* genera were minor components of the culturable bacterial community (Figure 5).

On CM medium at low temperature, representatives of the *Arthrobacter* and *Pseudarthrobacter* genera accounted for 85% of all colonies cultured under these conditions; representatives of *Cellulomonas* and *Streptomyces* were identified as minor components (Figure 6).



**Figure 3.** Structure of the culturable bacterial community isolated on PYG medium at 25 °C. Hidden percentage corresponds to 1%; the "others" group includes *Bacillus* sp., *Brachybacterium* sp., *Massilia varians*, *Microbacterium aurantiacum*, and *Pseudarthrobacter oxydans* represented by 0.5% each.



Figure 4. Structure of the culturable bacterial community isolated on CM medium at 25 °C.



Figure 5. Structure of the culturable bacterial community isolated on PYG medium at 10 °C.



Figure 6. Structure of the culturable bacterial community isolated on CM medium at 10 °C.

The coefficients of biological diversity, evenness, and similarity of bacterial communities, isolated from the examined site, are presented in Tables 1 and 2. The summarized communities' diversity data obtained are presented in Table A3. The phylogenetic trees of isolated strains are presented in Supplementary materials Figures S2–S4.

 Table 1. Ecological indices characterised of cultured communities.

Index	PYG 25 °C	PYG 10 °C	CM 25 °C	CM 10 °C
Shannon index	3.87	2.93	3.56	1.44
Evenness index	0.87	0.88	0.91	0.72

Index	PYG 25 °C–PYG 10 °C	CM 25 °C–CM 10 °C	PYG 25 °C–CM 25 °C	PYG 10 °C-CM 10 °C
Common genera number	8	4	12	3
Jaccard index	0.33	0.27	0.48	0.27
Chekanovskiy–Sørensen index	0.50	0.42	0.65	0.43
Sørensen modified index	0.55	0.42	0.57	0.34

Table 2. Ecological indices of cultured communities' similarity.

#### 3.3. Multisubstrate Testing

Multisubstrate testing revealed a wide range of substrates suitable for development of the investigated microbial community. The most active substrate assimilation was observed on simple sugars, easily accessible polysaccharides (pullulan, starch, sucrose), lactate, Tween 80, and some amino acids (alanine, glutamine, and proline) (Table 3).

		Alanine			Arginine
	Amino acids	Glutamine	rate	Amino acids	Asparagine
es		Proline	lbsti		Serine
strat	Lipid-like substance	Tween 80	ated su		Threonine
sqns		Pullulan	late		Phenylalanine
Carbohydrates		Soluble starch	imi	Amino acid polymer	Peptone
	Sucrose	y as	Salt of carbon acid	Propionate	
Issin		Arabinose	akl	Carbohydrates	Dextran 500
ost a		Glucose	Me	Curbonyulutes	Xylose
M		Maltose			
	Alcohol	Sorbitol	-		
	Salt of carbon acid	Lactate	-		

Table 3. Substrate assimilation obtained by multisubstrate testing.

#### 3.4. Physiological Assays

Physiological assays were performed for all isolated 121 strains. Strains that were able to metabolize at low temperatures (up to 2 °C) were found to be more common than those that were able to grow at high temperatures. Thermotolerant strains that were able to metabolize at a temperature of 45 °C were found in communities isolated on both media at 25 °C. These strains made up 39% and 10% of the total number of strains in communities cultured on PYG and CM media, respectively. The strains capable to grow at 50 °C were only found in the community isolated on a rich medium at 25°C, and made up 22% of the total number of isolates under these conditions (Figure 7a,b). Twenty percent of the strains isolated on PYG at 25 °C were able to metabolize at 2 °C and the same percentage was able to grow at 50 °C. At the same time, less than 20% of the community isolated on CM medium under similar conditions were metabolicaly active at 2 °C and only 10% of the community were able to grow at 45 °C. Higher levels of resistance to low temperatures were detected for strains isolated on CM medium at 10 °C. Amongst all investigated bacteria 10 strains were able to metabolize in extremely wide temperature range (2–4 – 45–50 °C). Detailed characteristics of strain resistance are presented in Table S1.

All of the isolated strains were able to metabolize in the pH range of 6–8. The greatest resistance to pH of the medium was observed in the community isolated on CM medium at 25 °C: more than a half of the isolates were able to metabolize in a medium with pH of 3 and the same percentage was observed for pH of 12. Among communities isolated at 10 °C, the community isolated on CM medium (Figure 7c,d) was also characterized by a higher (by 20% on average) proportion of pH-tolerant strains. The pH range of strains' metabolic activity was shifted to the alkaline region and ranged from 3–12 pH



units. Among all investigated bacteria 44 strains were able to metabolize in extremely wide pH range (3–4 – 12 units). Detailed characteristics of strains' resistance are presented in Table S1.

**Figure 7.** Isolated strains resistance to: (**a**,**b**)—cultivation temperature; (**c**,**d**)—pH of the media; (**e**,**f**)—sodium chloride, (**g**,**h**)—potassium chloride. Left column: strains isolated on PYG medium; right column—strains isolated on CM medium.

Tests on media with addition of sodium chloride or potassium chloride revealed a moderate halotolerance of all the studied communities. Less than 50% of the strains retained metabolic activity in the presence of 5% NaCl. At the same time, 14% of the community isolated on PYG medium at 25 °C were capable to grow in the presence of 10% sodium chloride and 6% of the same community were able to metabolize on media containing 15% sodium chloride. In addition, almost the same level of resistance was revealed for the communities isolated on CM medium at 10 °C and 25 °C (Figure 7e,f).

Potassium chloride had a smaller inhibitory effect (on average two times smaller) on the examined communities: 14% of the strains isolated on PYG at 25 °C metabolized in a medium containing 15% of this salt; similarly to sodium chloride resistance, 80% of the strains isolated on CM at 25 °C were resistant to the presence of 2% KCl, and 10% were resistant in 10% solution (Figure 7g,h).

The presence of magnesium sulphate in the medium had a low inhibitory effect on all of the studied communities: 39% and 50% of the strains isolated on PYG medium at 25 °C and 10 °C respectively retained metabolic activity in presence of 20% magnesium sulphate. Seventy-three percent and 60% of the strains isolated on CM medium at 25 °C and 10 °C retained metabolic activity in the same conditions (Figure 8a,b).



**Figure 8.** Isolated strains resistance to: (**a**,**b**)—magnesium sulphate; (**c**,**d**)—magnesium perchlorate, (**e**,**f**)—antibiotics. Left column: strains isolated on PYG medium; right column—strains isolated on CM medium. *Amp*—ampicillin; *Chl*—chloramphenicol; *Rif*—rifampicin; *Tetr*—tetracycline; *Kan*—kanamycin; *Dox*—doxycycline; *Ceph*—cephalexin.

Sodium bicarbonate had the highest inhibitory effect among the studied water-soluble salts: few strains resistant to 5% of this salt in the culture medium were detected among the communities isolated on PYG and CM media at 25 °C (9% and 3% of strains, respectively) and among the community isolated on CM at 10 °C (10% of strains) (Figure 9).



Figure 9. NaHCO<sub>3</sub> resistance diagram of strains isolated on PYG medium and CM medium.

Strains of the community isolated on CM medium at 25 °C (33% of strains) were resistant to the presence of 10% magnesium perchlorate. The community isolated on PYG at 25 °C was resistant to concentrations of up to 5% of magnesium perchlorate (28% of strains) (Figure 8c,d). Furthermore, higher (on average by 20%) proportion of the strains isolated on CM medium showed high resistance to the presence of this salt in the medium compared to the strains isolated on PYG medium.

The individual ranges of resistance of the isolated strains to physicochemical stress factors are presented in supplementary materials (Table S1).

In general, the investigated communities showed low resistance to the presence of antibiotics—about 20% of isolates in each of the four communities were capable to grow in the presence of a single clinical antibiotic.

High resistance of strains isolated on CM medium at 25 °C to ampicillin, tetracycline, and cephalexin is noteworthy (Figure 8e,f). In all the communities the strains characterized with multiple antibiotic resistance were found. These strains were related to the genera *Bacillus, Agrococcus, Arthrobacter, Leucobacter, Pseudarthrobacter, Planomicrobium, Massilia, Microbacterium,* and *Pontibacter* (Table S2).

#### 4. Discussion

The total number of prokaryotes and numbers of culturable bacteria revealed in soil studied are in accordance with the previously obtained data for the soils of other regions of the Mojave Desert [32,36,37]. Different cell numbers observed in different regions of the desert could be caused by the high heterogeneity of soil composition and the high heterogeneity of soil resources in this desert, as well as past and current climatic conditions or plant associations which influence bacterial communities [59,60]. At the same time, considering the climatic regime of the studied region, the absence of growth during the isolation of culturable bacteria at high (50 °C) temperature is worth noticing, whereas some strains isolated at lower temperatures (10 °C, 25 °C) were capable of growing at this temperature in axenic culture. This is probably due to the conditions of culturing and intra- and interpopulation interactions between microorganisms, which can complicate the transition from the state of metabolic dormancy to grow under stressful conditions [61–63]. It is notable that the numbers of bacteria cultured at 10 °C and 25 °C were virtually equal. Based on these data, it is possible to assume that there is a high proportion of strains with psychrotolerant properties in the investigated soil, as was previously found for bacteria isolated from the Sahara and the Gibson Desert soils [44].

Lower numbers of CFUs for CM nutrient medium could be explained by lack of vegetation coverage at the sampling site and, as a result, the rareness of plant origin carbon sources (such as starch and sucrose in CM medium) in the soil in situ [39]. In addition, these compounds require preliminary hydrolysis for further assimilation by the cells. However, not all bacteria can produce hydrolytic enzymes for assimilation of these substrates [61].

The predominance of pigmented bacteria in the studied surface soil of the Mojave Desert is not surprising due to the role of pigments in protecting cells from damaging ultraviolet radiation [63,64]. The maximum diversity of the community detected on the rich PYG medium under the conditions of mesophilic temperature optimum indicates a predominantly mesophilic strategy of development for

the majority of the microbial community studied. Diversity decreases along with decreasing culturing temperatures and limiting of easily available carbon sources and growth factors (such as amino acids and vitamins) in the composition of the nutrient medium. Mesophilic development strategy common for the acquired isolates is probably due to the climatic conditions experienced by the soil sample. The development of psychrotolerant properties could be caused by a high contrast of both diurnal and seasonal temperatures and can be considered to be an adaptive feature under these conditions. The higher biodiversity of cultured bacteria on PYG medium is due to the diversity of carbon sources and the presence of growth factors (such as vitamins and amino acids), which can be used by a larger number of bacteria. The presence of growth factors may also contribute to the reactivation of cells that were in a state of metabolic rest [62].

Representatives of the *Actinobacteria* phylum were predominant in all of the cultured communities, representatives of the *Proteobacteria* phylum were the second most common ones, *Firmicutes* and *Bacteriodetes* were identified as minor components. The dominance of representatives of *Proteobacteria* phylum in the edaphic bacterial communities of the Mojave Desert has been shown based on the molecular-genetic tests [21,24]. Simultaneously, the results we obtained reveal the similar structure of bacterial communities in the studied soil of this desert, consistent with culture-independent data published earlier [10,32,33]. The predominance of *Actinobacteria* in the community is quite natural, considering the fact that many species among them are resistant to desiccation, UV radiation, and other stress effects [22]. Low abundance of representatives of the *Firmicutes* phylum was previously detected in the Mojave Desert [24]. Among all isolated strains low relative abundance of spore-forming bacteria was observed (seven isolates only), while a high abundance of genes associated with spore formation processes was found in situ by culture-independent methods [33].

The bacteria of genera *Arthrobacter*, *Bacillus*, *Kocuria*, *Leucobacter*, *Massilia*, *Mycetocola*, *Microbacterium*, *Paracoccus*, *Planomicrobium*, *Pseudarthrobacter*, *Rufibacter*, *Sphingomonas*, and *Streptomyces* were previously isolated from other hot deserts [44,63–65]. Bacteria of the genus *Agrococcus* were previously cultured from cold deserts [66]. Among the representatives of the genus *Brachybacterium* several species connected to the root systems of halophilic plants were described and characterized by having plant growth-promoting potential [67]. Members of the genus Plantibacter, which have not been previously isolated from deserts, are also considered to be associated with herbaceous plants [68]. Representatives of the genus *Cellulomonas* are often isolated from soil [69] with some of them known to be halotolerant species [70]. Bacteria of the genus *Pontibacter* have been previously isolated from arid soils [71,72].

In this research, bacteria of the genera *Janthinobacterium*, which were found dominant in the meltwaters of glaciers [73], *Salinibacterium*, previously found in marine ecosystems and glaciers [74,75], and *Labedella* were isolated from desert soil for the first time.

Noteworthy is the discovery of the bacteria *Planomicrobium glaciei* in the desert soil. This species was first isolated from glacier, and after that from desert, ecosystems [76]. This allows us to assume that the intracellular mechanisms that determine the resistance and survivability of the species turn out to be adaptive in both cold and hot deserts.

According to community structure diagrams and ecological indices of communities' similarity, all the obtained communities differ from each other significantly. It indicates that culturing conditions (such as medium composition and cultivation temperature) are the main factors influencing the structure of the communities isolated from studied soil. Nevertheless, the genera represented in all cultured communities are quite similar.

It should be considered that transport of microorganisms by winds is possible [15], which is especially important in case of surface soil sampling. According to this fact, the presence of introduced bacteria could not being excluded. Both culture-dependent and culture-independent methods could not separate indigenous and introduced microorganisms. However, the sampling site is located in the central part of the Mojave Desert, thus minimizing the chance to long distant cell transport from non-desert regions. Thus, even if some of the isolates were not indigenous, they probably were

introduced from the other desert sites. Moreover, virtually all representatives of genera isolated were previously detected in different desert soils. The resistance data obtained also testifies to the high adaptation to the desert conditions and confirms predominantly desert-associated strains were isolated.

Substrates on which the most active development of the examined community was observed, could be produced as metabolites of cyanobacteria [8]. This suggests a functional relationship between the previously shown metabolically active cyanobacterial communities in the studied soil of the Mojave Desert and the heterotrophic community studied by us that exists due to the presence of the primary organic substance of cyanobacterial origin. Based on the aforementioned facts and data on the taxonomic and morphological diversity of isolates, it can be presumed that the culturable bacterial community of the studied site in central Mojave Desert, which is considered to be a Mars analogue site, is functionally differentiated, and is highly adapted to harsh physicochemical conditions. These results are consistent with the data obtained previously in the other regions of this desert [32,33].

The analysis of the resistance of isolates to temperature reveals the thermotolerant properties of communities cultured at 25 °C. The expansion of the range of temperatures suitable for growth occurs mainly in the region of low temperatures, which is probably caused by daily and seasonal temperature fluctuations [19]. The highest resistance was recorded for the community isolated on PYG media at 25 °C, which is probably due to the high number of cells and diversity of this community.

The wide pH ranges in which the metabolic activity is maintained can be explained by contrast temperature conditions and precipitation regimes leading to a change in the solubility of the substances in the soil solution [22]. Higher levels of pH resistance of strains isolated on CM could be caused by their hydrolytic activity, often associated with changes in the pH of the solution [61].

The studied region is characterized by a low content of water-soluble salts [19,20,24], which could mean that the abundance of salts present in the studied sample is low. It explains the moderate general resistance of isolated communities to the presence of sodium chloride or potassium chloride. However, strains with high resistance (up to 15%) to sodium chloride were found. It was previously shown that the same physiological mechanism provides resistance for both desiccation and salinization, which could explains the obtained result [77,78].

High resistance to magnesium sulphate was previously shown for bacterial communities isolated from desert soils [44]. It is probably associated with the assimilation of the components of this salt as elements of mineral nutrition. Low resistance to sodium bicarbonate is associated with the hydrolytic alkaline nature of this salt and its local distribution in the desert in the form of soda lakes. The presence of resistant forms is probably caused by a slight aeolian transfer of sodium bicarbonate and bacteria adapted to its presence, as well as by the formation of this salt in situ during weathering processes [42,44].

High resistance to magnesium perchlorate is particularly important in the astrobiology-oriented review of the obtained results. Maintenance of metabolic activity in vitro in the presence of perchlorates, at concentrations significantly exceeding those found in the regolith of Mars [79], testifies their non-limiting role in the regolith. Resistance to oxidizing agents is also likely due to the physiological adaptation of bacteria to arid conditions [78]. The higher level of resistance of the community isolated on a rich medium (PYG) at 10 °C, compared with the one isolated at 25 °C on the same medium, can be explained by the physiological connection of processes that ensure adaptation to low temperatures and the presence of oxidizers [80]. It has previously been shown that bacterial communities of other desert soils are resistant to high perchlorate concentrations [44].

The highest levels of resistance to physicochemical stress factors were found in representatives of the genera *Arthrobacter*, *Bacillus*, *Kocuria*, *Leucobacter*, *Microbacterium*, *Planomicrobium*, and *Pontibacter* (Table S1). Representatives of these genera are widely distributed in desert soils and have previously demonstrated high resistance to various types of stress effects [15,17,22,25,44,59,66].

For the representatives of majority genera isolated from soil studied resistance to ionizing and UV radiation was shown ([22,43,81–89] and references therein). It is in accordance with pigmented colonies' high proportion data and it allows to suggest the presence of high UV- and radioresistance of

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the strains studied. Data on the resistance to physicochemical factors combined with the literature data on UV and ionizing radiation resistance could be considered as additional evidence of potential survivability of this bacterial community in Martian-like conditions. UV- and radioresistance of the isolates should be confirmed in further research.

The low resistance of the studied strains to antibiotics suggests that there is no active antibiotics-dependant competition in the communities. It is possible that the communities are realizing a co-metabolism strategy [90], as indirectly confirmed by the results of culturing on PYG medium and MST analysis: they revealed the most intensive growth of a cultured community on peptone containing various oligopeptides, amino acids, and vitamins. A similar result was obtained earlier in the study of the number of antibiotic resistance genes copies in the soils of the Mojave Desert [33]. At the same time, the discovery of a high number of antibiotic-resistant bacteria in the community isolated on CM medium at 25 °C is probably due to the signal role of antibiotics as quorum sensing agents [91,92] for activation of hydrolytic processes in the community. We have not found any previously published data on antibiotic resistance of representatives of *Planomicrobium, Salinibacterium*, and *Labedella* genera.

#### 5. Conclusions

Stress effects, such as low water availability, nutrients deficiencies, high levels of solar radiation, and wide temperature fluctuations in the surface soil of the Mojave Desert lead to the formation of physiological survival strategies for prokaryotes existing under these conditions [2,3]. Our study revealed a high diversity (both taxonomic and morphophysiological) of culturable bacteria in the investigated soil. Species typical for various soils, including arid soils, and species that have not been previously isolated from hot arid soils were found in isolated bacterial communities. This suggests that the mechanisms of adaptation to cryo-arid and xero-arid conditions include similar physiological processes and adaptations. The obtained spectra of metabolic activity in the gradients of physicochemical factors indicate predominantly mesophilic and neutrophilic optima of all the studied strains, mostly capable of maintaining metabolic activity in a wide range of conditions, up to extreme ones for individual strains. Extreme resistance levels were identified in bacteria of *Arthrobacter*, *Microbacterium*, *Bacillus*, *Planomicrobium*, *Kocuria*, *Leucobacter*, and *Pontibacter* genera.

The obtained results, considered together with the data of previous works [25,41,64], testify in favour of viability, functionality, and taxonomical diversity of soil bacterial communities in the central part of the Mojave Desert studied, which is considered as a terrestrial analogue of Martian regolith. The isolated strains require further characterization and description for potential biotechnological applications. Due to the low similarity of the nucleotide sequences of some individual strains to those found in the database, these isolates could be the representatives of undescribed species of culturable bacteria. The most resistant strains that have been found could be used as model objects in further astrobiological studies.

As mentioned earlier, the Mojave Desert has undergone processes similar to those that took place on Mars and formed its current state. The properties of culturable bacterial communities revealed in this study indicate that they have adapted to survive in aggressive changing environmental conditions. Consequently, such communities could be considered as a model for further research of the Martian regolith habitability and the ability of terrestrial bacteria to adapt to extraterrestrial conditions.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3263/9/4/166/s1; Figure S1. Number of colonies' morphotypes in the communities studied; Figure S2a. Phylogenetic analysis tree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 1100r primer; Figure S2b. Phylogenetic analysis subtree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 1100 r primer; Figure S2c. Phylogenetic analysis subtree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 1100 r primer; Figure S2c. Phylogenetic analysis subtree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 1100 r primer; Figure S3. Phylogenetic analysis tree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 805r primer; Figure S4. Phylogenetic analysis tree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 805r primer; Figure S4. Phylogenetic analysis tree of 16S rRNA genes of the strains isolated from the Mojave Desert soil, which were sequenced using 537r primer; Table S1. Physiological characteristics of isolated bacteria, Table S2. Antibiotic resistance spectra of isolated strains.

**Author Contributions:** A.A.B. and V.S.C. designed the study, all authors performed the study, A.A.B. and V.S.C. prepared draft manuscript, all authors edited and commented on the manuscript.

**Funding:** This research was funded by Russian Foundation for Basic Research according to the research project No 18-34-00331, and partially by PRAS no. 17 (Evolution of organic world. Role and influence of planetary processes), subprogram no. 1 (in the part of study of bacterial resistance to perchlorates).

**Acknowledgments:** The authors express their respect to T. Yu. Ulyanova, who provided the soil sample from the Mojave Desert. The authors thank anonymous reviewers for the help in improving the article. The authors thank George A. Abashin for the help in language improving the article.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing the manuscript, and in the decision to publish the results.

### Appendix A

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation	
KBP 4S 17-PYC 25 °C-MK403789	Pseudarthrobacter siccitolerans [MF682005]—99.0% Pseudarthrobacter oxydans	Pseudarthrobacter oxudans	
KDI.AS.I7-1 10 25 C-WIX405705	[MF681862]—99.0% Pseudarthrobacter oxydans [MF681851]—99.0%	1 seuurinioouerer oxyuuns	
KBP.AS.62-PYG 25 °C-MK403790	Arthrobacter sp. [KX928444]—99.5% Arthrobacter sp. [KT944733]—99.5%	Arthrobacter sp.	
	<i>Arthrobacter</i> sp. [KM507593]—99.5%		
	Brachybacterium sp. [MH518254]—99.3%		
KBP.AS.75–PYG 25 °C–MK403794	[KY622837]—99.3%	Brachybacterium sp.	
	[LN995481]—99.3%		
KBP.AS.105–PYG 25 °C–MK403787	[KT715033]—98.7% Janthinobacterium sp. [FJ605429]—98.7% Janthinobacterium sp.	Janthinobacterium sp.	
	[FJ605404]—98.7% Arthrobacter sp.		
KBP.AS.160–PYG 25 °C–MK403788	[HM216918]—99.2% Uncultured <i>Arthrobacter</i> sp. [JN129287]—99.0%	Arthrobacter sp.	
	Arthrobacter agilis [JN009621]—99.0% Microbacterium aurantiacum		
KBP.AS.747–PYG 25 °C–MK403791	[MH734538]—99.2% Microbacterium aurantiacum [MH715198]—99.2% Microbacterium sp.	Microbacterium aurantiacum	

#### **Table A1.** Taxonomic affiliation of isolated strains.

Table A1. Cont.			
Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation	
KBP.AS.748–PYG 25 °C–MK403792	Pseudarthrobacter siccitolerans [MF682005]—99.22% Pseudarthrobacter oxydans [MF681862]—99.2% Pseudarthrobacter oxydans [MF681851]—99.2%	Pseudarthrobacter sp.	
KBP.AS.749–PYG 25 °C–MK403793	Brevibacterium frigoritolerans [MK318239]—99.9% Brevibacterium frigoritolerans [MK318233]—99.9% Brevibacterium frigoritolerans [MK318218]—99.9%	Brevibacterium frigoritolerans	
KBP.AS.750–PYG 25 °C–MK403795	Planomicrobium okeanokoites [KF749386]—99.3% Planomicrobium okeanokoites [KF749394]—99.3% Planomicrobium okeanokoites [KF724965]—99.3%	Planomicrobium okeanokoites	
KBP.AS.751–PYG 25 °C–MK403796	Agrococcus lahaulensis [KF318382]—100% Agrococcus sp. [JX949729]—100% Agrococcus jenensis [FF672044]—100%	Agrococcus sp.	
KBP.AS.752–PYG 25 °C–MK403797	Agrococcus sp. [KC702716]—99.5% Agrococcus sp. [JX949729]—99.5% Agrococcus jenensis [EF672044]—99.4%	Agrococcus sp.	
KBP.AS.753–PYG 25 °C–MK403798	Agrococcus sp. [MG232346]—99.5% Agrococcus jenensis [AJ717350]—99.5%	Agrococcus sp.	
KBP.AS.754–PYG 25 °C–MK403799	Agrococcus sp. [HF954438]—99.5% Kocuria sp. [DQ192212]—99.3% Kocuria sp. [EF612289]—99.2% Kocuria sp. [DQ180950]—99.0% Planomicrobium glaciei	Kocuria sp.	
KRP 45 755 PVC 25 °C MK403800	[KF387709]—100% Planomicrobium sp.	Dlanomicrobium alaciei	

[JX949538]—100<sup>°</sup>/<sub>0</sub> Planomicrobium glaciei HQ232419]-100% *Leucobacter* sp. [KY623368]—99.6% Leucobacter aridicollis

[KR827428]—99.6% *Leucobacter* sp. [KP152582]—99.6% Planomicrobium okeanokoites [KF724965]—99.5% Planomicrobium okeanokoites

[KF749394]—99.5% Planomicrobium okeanokoites [KF749386]—99.5% Microbacterium esteraromaticum [GU111572]—98.9% Microbacterium sp.

[KU221074]—98.6% *Microbacterium* sp. [KU221073]—98.6%

KBP.AS.756-PYG 25 °C-MK403801

KBP.AS.755-PYG 25 °C-MK403800

KBP.AS.757-PYG 25 °C-MK403802

KBP.AS.758-PYG 25 °C-MK403803

Microbacterium sp.

Planomicrobium glaciei

Leucobacter aridicollis

Planomicrobium okeanokoites

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.759–PYG 25 °C–MK403804	Bacillus pumilus [MH910167]—99.9% Bacillus pumilus [MH910156]—99.9% Bacillus pumilus	Bacillus pumilus
KBP.AS.760–PYG 25 °C–MK403805	[MH908782]—99.9% Microbacterium esteraromaticum [GU111572]—98.9% Microbacterium sp. MH231521]—98.2% Microbacterium sp. [MH231520]—98.2%	Microbacterium sp.
KBP.AS.761–PYG 25 °C–MK403806	Rufibacter sp. [MH050957]—97.6% Rufibacter immobilis [MH050949]—97.6% Uncultured bacterium clone [JQ376150]—98.9%	<i>Rufibacter</i> sp.
KBP.AS.762–PYG 25 °C–MK403807	Microbacterium esteraromaticum [GU111572]—99.2% Microbacterium sp. [MH231521]—98.5% Microbacterium sp. [MH231520]—98.5%	Microbacterium sp.
KBP.AS.763–PYG 25 °C–MK403808	Massilia niabensis [NR_044571]—98.9% Oxalobacter sp. [DQ196473]—99.2% Massilia suwonensis [LN774622]—98.8%	<i>Massilia</i> sp.
KBP.AS.764–PYG 25 °C–MK403809	Bacillus sp. [MF990764]—98.2% Bacillus pumilus [JX988407]—97.8% Bacillus pumilus [EU795022]—97.8% Macilia elleditelemus	<i>Bacillus</i> sp.
KBP.AS.765–PYG 25 °C–MK403810	[KY010279]—99.7% Uncultured bacterium clone [KU515079]—99.7% Massilia alkalitolerans [KP282807]—99.7%	Massilia alkalitolerans
KBP.AS.766–PYG 25 °C–MK403811	Pseudarthrobacter siccitolerans [MF682005]—98.1% Pseudarthrobacter oxydans [MF681862]—98.1% Pseudarthrobacter oxydans [MF681851]—98.1%	Pseudarthrobacter sp.
KBP.AS.767–PYG 25 °C–MK403812	Massilia varians [MF077216]—98.4% Massilia varians [MF077215]—98.4% Massilia varians [MF077214]—98.5%	Massilia varians
KBP.AS.768–PYG 25 °C–MK403813	Planococcus donghaensis [LN774491]—100% Planomicrobium okeanokoites [KF749394]—100% Planomicrobium okeanokoites [KF749386]—100%	Planomicrobium sp.

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.769–PYG 25 °C–MK403814	Microbacterium pseudoresistens [LN890047]—98.9% Microbacterium pseudoresistens [KF687021]—98.9% Microbacterium sp. [MH671545]—98.2%	Microbacterium pseudoresistens
KBP.AS.770–PYG 25 °C–MK403815	Arthrobacter agilis [KF924209]—99.3% Arthrobacter sp. [JX949695]—99.3% Arthrobacter sp. [JX949646]—99.3%	Arthrobacter sp.
KBP.AS.771-PYG 25 °C-MK403816	Arthrobacter sp. [MH714643]—99.5% Arthrobacter sp. [MG757950]—99.5%	Arthrobacter sp.
KBP.AS.772–PYG 25 °C–MK403817	Arthrobacter sp. [JX949321]—99.5% Planomicrobium glaciei [MG025800]—100% Planomicrobium glaciei [KF387709]—100% Planomicrobium sp. [JX949538]—100% Uncultured bacterium	Planomicrobium glaciei
KBP.AS.773–PYG 25 °C–MK403818	[LC026838]—98.9% <i>Cellulomonas</i> sp. [KP126821]—98.4% <i>Cellulomonas cellasea</i> [KR922256]—98.4%	Cellulomonas sp.
KBP.AS.774-PYG 25 °C-MK403819	Planomicrobium okeanokoites [KF749394]—99.8% Planomicrobium okeanokoites [KF749386]—99.8% Planomicrobium okeanokoites [KF724965]—99.8%	Planomicrobium okeanokoites
KBP.AS.775–PYG 25 °C–MK403820	Planomicrobium okeanokoites [KF724965]—99.9% Planomicrobium okeanokoites [KF749394]—99.9% Planomicrobium okeanokoites [KF749386]—99.9%	Planomicrobium okeanokoites
KBP.AS.776–PYG 25 °C–MK403821	Georgenia sp. [EF512742]—99.6% Georgenia sp. [AY880044]—99.5% Georgenia satyanarayanai [NR_117051]—99.5% Microbacterium barkeri	Georgenia sp.
KBP.AS.777–PYG 25 °C–MK403822	[MG706019]—98.4% Microbacterium sp. [MG203882]—98.4% Microbacterium barkeri [KY928100]—98.4%	Microbacterium barkeri
KBP.AS.778–PYG 25 °C–MK403823	Salinibacterium sp. [MH714650]—99.0% Salinibacterium sp. [MH790149]—99.0% Salinibacterium sp. [MH299814]—99.0%	Salinibacterium sp.

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
	Brevibacterium frigoritolerans [MK318239]—100%	
KBP.AS.779-PYG 25 °C-MK403824	Brevibacterium frigoritolerans [MK318233]—100%	Brevibacterium frigoritolerans
	Brevibacterium frigoritolerans [MK318218]—100% Micrococcus sp. [IO2296941—99.3%	
KBP.AS.780-PYG 25 °C-MK403825	Micrococcus sp. [JQ229094]—99.3% Micrococcus sp. [JQ229689]—99.3% Micrococcus sp. [KM613156]—99.2%	Micrococcus sp.
KBP.AS.781-PYG 25 °C-MK403826	Kocuria sp. [KT900591]—98.9% Kocuria sediminis [MH178354]—98.85% Kocuria turfanensis [ME7602221] 08.8%	<i>Kocuria</i> sp.
KBP.AS.782-PYG 25 °C-MK403827	Labedella sp. [KM253033]—98.8% Labedella gwakjiensis [LK021191]—98.2% Labedella sp. [JX273669]—98.2%	Labedella sp.
KBP.AS.783–PYG 25 °C–MK403828	Plantibacter flavus [CP019402]—100% Plantibacter sp. [HE662660]—100% Plantibacter sp. [HE662693]—100%	Plantibacter sp.
KBP.AS.784–PYG 25 °C–MK403829	Arthrobacter agilis [KT804924]—99.9% Arthrobacter agilis [MF767263]—99.8% Arthrobacter agilis	Arthrobacter agilis
KBP.AS.785–PYG 25 °C–MK403830	[KR811202]—99.8% Microbacterium esteraromaticum [GU111572]—99.1% Microbacterium aureliae [NR152652]—98.6% Microbacterium aureliae	Microbacterium sp.
KBP.AS.786–PYG 25 °C–MK403831	[KF793922]—98.6% Pseudarthrobacter siccitolerans [MF682005]—99.0% Pseudarthrobacter oxydans [MF681862]—99.0% Pseudarthrobacter oxydans [MF681851]—99.0%	Pseudarthrobacter sp.
KBP.AS.787-CM 25 °C-MK403832	Uncultured bacterium [KX507906]—98.2% <i>Massilia niabensis</i> [JF496256]—98.0% <i>Massilia alkalitolerans</i>	<i>Massilia</i> sp.
KBP.AS.788-CM 25 °C-None	[KY010279]—97.8% Was identified by morphology Burkholderia sp. [KU060146]	<i>Bacillus</i> sp.
KBP.AS.789-CM 25 °C-MK403833	—95.7% Burkholderia sp. [KU060136]—95.7% Burkholderia sp. [KU060135]—95.7%	Burkholderia sp.

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.790–CM 25 °C–MK403834	Cellulomonas hominis [KF817811]—100% Cellulomonas hominis [MF928361]—100% Cellulomonas sp. [LC133615]—100% Develoption significations	Cellulomonas hominis
KBP.AS.791–CM 25 °C–MK403835	[MF682005]—99.5% Pseudarthrobacter oxydans [MF681862]—99.5% Pseudarthrobacter oxydans [MF681851]—99.5%	Pseudarthrobacter sp.
KBP.AS.792-CM 25 °C-MK403836	Massilia sp. [JQ511858]—100% Massilia sp. [KY635898]—99.6% Massilia sp. [KY635895]—99.6%	Massilia sp.
KBP.AS.793-CM 25 °C-MK403837	[KC994764]—98.8% Salinibacterium sp. [MH299814]—98.7% Salinibacterium sp. [CP026951]—98.7%	Salinibacterium sp.
KBP.AS.794-CM 25 °C-MK403838	Arthrobacter sp. [LN871743]—99.7% Arthrobacter sp. [KU951449]—99.7% Arthrobacter sp. [KJ191079]—99.7% Arthrobacter sp.	Arthrobacter sp.
KBP.AS.795-CM 25 °C-MK403839	[LN871743]—99.2% <i>Arthrobacter</i> sp. [KU951449]—99.2%	Arthrobacter sp.
KBP.AS.816–PYG 25 °C–MK403840	Arthrobacter sp. [KJ191079]—99.2% Arthrobacter sp. [KJ191079]—99.8% Arthrobacter sp. [KJ191059]—99.8% Arthrobacter sp. [KJ191035]—99.8% Uncultured bacterium clone	Arthrobacter sp.
KBP.AS.842-PYG 25 °C-MK403841	[KX507404]—99.1% Massilia suwonensis [NR116872]—99.1% Massilia sp. [KM187344]—98.8%	Massilia sp.
KBP.AS.843–PYG 25 °C–MK403842	Mycetocola sp. [MH299815]—98.4% Mycetocola sp. [MH298813]—98.4% Mycetocola sp. [CP026949]—98.4% Arthrobacter agilis	<i>Mycetocola</i> sp.
KBP.AS.844–PYG 25 °C–MK403843	[KT804924]—98.9% Arthrobacter agilis [MF767263]—98.8% Arthrobacter agilis [KP811202]—98.8%	Arthrobacter agilis
KBP.AS.845–PYG 25 °C–None	Was identified by morphology	Streptomyces sp.
KBP.AS.846–PYG 25 °C–MK403844	Arthrobacter pascens [KY880876]—100% Pseudarthrobacter defluvii [KY880845]—100% Arthrobacter sp. [LM994042]—100%	Arthrobacter sp.

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Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.847–CM 25 °C–MK403845	Cellulomonas sp. [MH046844]—99.2% Cellulomonas sp. [HM587947]—99.2% Cellulomonas flavigena [KY951360]—99.0%	<i>Cellulomonas</i> sp.
KBP.AS.848–PYG 25 °C–MK403846	Massilia alkalitolerans [KY010279]—99.6% Uncultured bacterium clone [KU515079]—99.6% Massilia alkalitolerans [KP282807]—99.6%	<i>Massilia</i> sp.
KBP.AS.849–PYG 25 °C–MK403847	Microbacterium esteraromaticum [GU111572]—98.8% Microbacterium aureliae [NR152652]—98.2% Microbacterium aureliae	Microbacterium sp.
KBP.AS.850-PYG 25 °C-MK403848	[KF793922]—98.2% Massilia sp. [MH734560]—99.6% Massilia sp. [KY635898]—99.6% Massilia sp. [KY635895]—99.6% Paracoccus marcusii	<i>Massilia</i> sp.
KBP.AS.854-CM 25 °C-MK403849	[MH744726]—99.9% Paracoccus marcusii [MH725412]—99.9% Paracoccus marcusii	Paracoccus marcusii
KBP.AS.855–PYG 25 °C–None	[MH725411]—99.9% Were identified by morphology Arthrobacter crystallopoietes [CP018864]—98.7%	<i>Rufibacter</i> sp.
KBP.AS.858-CM 25 °C-MK403850	Arthrobacter crystallopoietes [CP018863]—98.7% Arthrobacter sp.	Arthrobacter crystallopoietes
KBP.AS.862–PYG 25 °C–MK403851	[KX645729]—98.7% Arthrobacter sulfonivorans [KR233773]—99.9% Uncultured soil bacterium clone [JX490026]—99.8% Uncultured bacterium clone [KC554813]—99.7% Uncultured bacterium clone	Arthrobacter sp.
KBP.AS.866–CM 25 °C–MK403852	[EU647527]—98.8% Sphingomonas xinjiangensis [NR_108386]—98.6% Sphingomonas mucosissima [KM502884]—98.3%	Sphingomonas sp.
KBP.AS.867–PYG 25 °C–MK403853	Microbacterium paraoxydans [MH819720]—99.9% Microbacterium paraoxydans [MH281749]—99.9% Microbacterium sp. [MH558380]—99.9%	Microbacterium paraoxydans

Table A1. Cont.

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.868–PYG 25 °C–MK403854	Pseudarthrobacter siccitolerans [MF682005]—99.2% Pseudarthrobacter oxydans [MF681862]—99.2% Pseudarthrobacter oxydans	Pseudarthrobacter sp.
KBP.AS.869–PYG 25 °C–MK403855	[MF681851]—99.2% Kocuria rosea [KY194315]—98.8% Kocuria sp. [KY194287]—98.8% Kocuria turfanensis [KY194236]—98.8%	Kocuria sp.
KBP.AS.876–PYG 25 °C–MK403856	Arthrobacter agilis [CP024915]—99.1% Arthrobacter agilis [MG279727]—99.1% Arthrobacter sp. [K]191029]—99.1%	Arthrobacter agilis
KBP.AS.877–PYG 25 °C–MK403857	Salinibacterium sp. [MH790149]—99.0% Salinibacterium sp. [MH299814]—99.0% Salinibacterium sp. [CP026951]—99.0%	Salinibacterium sp.
KBP.AS.899–CM 25 °C–MK403858	Streptomyces sp. [MG930074]—100% Streptomyces sp. [MG930073]—100% Streptomyces atrovirens [MF662228]—100%	Streptomyces sp.
KBP.AS.916–PYG 10 °C–MK403859	Planomicrobium glaciei [MG025800]—99.1% Planomicrobium glaciei [KY307898]—99.1% Uncultured bacterium clone [KU514918]—99.1%	Planomicrobium glaciei
KBP.AS.926–PYG 10 °C–MK403860	Sphingomonas xinjiangensis [NR108386]—98.9% Uncultured bacterium clone [EU647527]—98.6% Sphingomonas mucosissima [KM502884]—98.6% Arthrobacter acilis	Sphingomonas sp.
KBP.AS.927–PYG 10 °C–MK403861	[MG279728]—99.4% Arthrobacter agilis [MF767263]—99.4% Arthrobacter agilis	Arthrobacter agilis
KBP.AS.928–PYG 10 °C–MK403862	Uncultured bacterium [KX509715]—99.5% Uncultured bacterium [KX508022]—99.5% <i>Massilia niabensis</i>	<i>Massilia</i> sp.
KBP.AS.929-CM 10 °C-MK403863	Pseudarthrobacter siccitolerans [MF682005]—98.5% Pseudarthrobacter oxydans [MF681862]—98.5% Pseudarthrobacter oxydans [MF681851]—98.5%	Pseudarthrobacter sp.

Table A1. Cont.	
<b>BLAST Search Results</b>	Taxonomic Affiliation
seudarthrobacter siccitolerans [MF682005]—98.7% Pseudarthrobacter oxydans [MF681862]—98.7% Pseudarthrobacter oxydans [MF681851]—98.7%	Pseudarthrobacter sp.
Arthrobacter boritolerans [MH828349]—99.7%	

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.930-CM 10 °C-MK403864	Pseudarthrobacter siccitolerans [MF682005]—98.7% Pseudarthrobacter oxydans [MF681862]—98.7% Pseudarthrobacter oxydans [MF681851]—98.7%	Pseudarthrobacter sp.
KBP.AS.931–CM 10 °C–MK403865	Arthrobacter boritolerans [MH828349]—99.7% Paenarthrobacter sp. [MH734752]—99.7% Arthrobacter sp. [LC416398]—99.7%	Arthrobacter sp.
KBP.AS.932-CM 10 °C-MK403866	Pseudarthrobacter sp. [MG860452]—99.0% Pseudarthrobacter sp. [MG860451]—99.0% Arthrobacter sp. [MG860340]—99.0%	Pseudarthrobacter sp.
KBP.AS.936–PYG 10 °C–MK403867	Pseudarthrobacter phenanthrenivorans [MF077156]—99.8% Pseudarthrobacter phenanthrenivorans [MF077155]—99.8% Arthrobacter phenanthrenivorans	Pseudarthrobacter phenanthrenivorans
KBP.AS.937–PYG 10 °C–MK403868	Arthrobacter agilis [KF876857]—99.9% Arthrobacter agilis [KT763365]—99.8% Uncultured Arthrobacter sp. [JN129287]—99.7%	Arthrobacter agilis
KBP.AS.938-CM 25 °C-MK403869	Streptomyces sp. [KU182931]—99.8% Streptomyces sp. [MG820052]—99.6% Streptomyces sp. [MG930074]—99.6% Un gulturo d bastorium slopp	Streptomyces sp.
KBP.AS.939-CM 25 °C-MK403870	[KU515164]—99.0% Uncultured bacterium clone [KX509026]—98.9% Massilia sp. [MG733567]—98.6% Microbacterium esteraromaticum	<i>Massilia</i> sp.
KBP.AS.940–CM 25 °C–MK403871	[GU111572]—99.2% Microbacterium sp. [MH231521]—98.8% Microbacterium sp. [KU221074]—98.8%	Microbacterium sp.
KBP.AS.941-CM 25 °C-MK403872	[MF682005]—99.4% Pseudarthrobacter oxydans [MF681862]—99.4% Pseudarthrobacter oxydans [MF681851]—99.4%	Pseudarthrobacter sp.

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.943–CM 10 °C–MK403873	Arthrobacter agilis [JQ684255]—99.8% Arthrobacter agilis [CP024915]—99.7% Arthrobacter agilis [MG279727]—99.7%	Arthrobacter agilis
KBP.AS.947-PYG 10 °C-MK403874	Microbacterium esteraromaticumstrain [GU111572]—99.3% Microbacterium sp. [MH231521]—98.5% Microbacterium sp.	Microbacterium sp.
KBP.AS.954–PYG 10 °C–MK403875	[MH231520]—98.5% Uncultured Microbacterium sp. Clone [MH894291]—99.9% Microbacterium sp. [MH813404]—99.9% Microbacterium sp. [MH714660]—99.9%	Microbacterium sp.
KBP.AS.955–PYG 10 °C–MK403876	Pseudarthrobacter siccitolerans [MF682005]—99.5% Pseudarthrobacter oxydans [MF681862]—99.5% Pseudarthrobacter oxydans [MF681851]—99.5%	Pseudarthrobacter sp.
KBP.AS.956–PYG 10°C–MK403877	Planomicrobium glaciei [KF387709]—99.9% Planomicrobium sp. [JX949538]—99.9% Planomicrobium sp. [IO618344]—99.9%	Planomicrobium sp.
KBP.AS.957–CM 10 °C–MK403878	Arthrobacter sp. [KX645729]—99.5% Arthrobacter sp. [KF889389]—99.5% Arthrobacter crystallopoietes [KM257018]—99.5%	Arthrobacter sp.
KBP.AS.958–CM 10 °C–MK403879	Arthrobacter sp. [KY173031]—99.6% Arthrobacter sp. [LN614607]—99.6% Arthrobacter oryzae [KC934820]—99.6%	Arthrobacter sp.
KBP.AS.960–CM 25 °C–MK403880	Microbacterium esteraromaticum [GU111572]—99.3% Microbacterium sp. [MH231521]—98.6% Microbacterium sp. [MH231520]—98.6% Callulomouse hominic	Microbacterium sp.
KBP.AS.961-CM 10 °C-MK403881	Cellulomonas nominis [KT992126]—99.1% Cellulomonas sp. [Y09658]—99.1% Cellulomonas sp. [MF361869]—98.9%	Cellulomonas sp.
KBP.AS.962-CM 10 °C-None	Was identified by morphology	Streptomyces sp.

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Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.963-CM 10 °C-MK403882	Arthrobacter agilis [CP024915]—99.7% Arthrobacter agilis [MG279727]—99.7% Arthrobacter sp. [KY476520]—99.7%	Arthrobacter agilis
KBP.AS.972–PYG 10 °C–MK403883	Micrococcus sp. [MH202946]—99.5% Micrococcus sp. [AB188213]—99.5% Micrococcus sp. [EF540464] —99.5%	Micrococcus sp.
KBP.AS.973–PYG 10 °C–MK403884	Rhodococcus sp. [MH285872]—100% Rhodococcus sp. [CP034152]—100% Rhodococcus erythropolis [MH251258]—100% Pseudarthropocter	Rhodococcus erythropolis
KBP.AS.1003–PYG 10 °C–MK403768	Pseudarinrobacter phenanthrenivorans [NR_074770]—100% Arthrobacter sp. [LN871743]—100% Arthrobacter sp.	Arthrobacter sp.
KBP.AS.1005–PYG 10 °C–MK403769	[K0931449]—100% Leucobacter sp. [KY623368]—99.9% Leucobacter aridicollis [KR827428]—99.9% Leucobacter sp. [KP152582]—99.9%	Leucobacter aridicollis
KBP.AS.1007–PYG 10 °C–MK403770	Arthrobacter sp. [K1944733]—100% Arthrobacter sp. [KM507593]—100% Arthrobacter pityocampae [MH472818]—100%	Arthrobacter sp.
KBP.AS.1008–PYG 10 °C–MK403771	Uncultured bacterium clone [KU514918]—99.3% <i>Planomicrobium</i> sp. [JX949538]—99.0% <i>Planomicrobium glaciei</i> [JF411320]—99.0%	Planomicrobium glaciei
KBP.AS.1009–PYG 10 °C–MK403772	Pseudarthrobacter siccitolerans [MF682005]—99.8% Pseudarthrobacter oxydans [MF681862]—99.8% Pseudarthrobacter oxydans [MF681851]—99.8%	Pseudarthrobacter sp.
KBP.AS.1010–PYG 10 °C–MK403773	Planomicrobium glaciei [KF387709]—99.7% Planomicrobium sp. [JX949538]—99.7% Planomicrobium glaciei [HQ232419]—99.7%	Planomicrobium glaciei
KBP.AS.1011–PYG 10 °C–MK403774	Arthrobacter phenanthrenivorans [JX840975]—99.3% Uncultured soil bacterium clone [DQ248289]—99.3% Arthrobacter sp. [AY238502]—99.3%	Arthrobacter sp.

Table A1. Cont.

Table A1. Cont.			
Strain–Isolation Conditions–GenBank Accession Number	BLAST Search Results	Taxonomic Affiliation	
KBP.AS.1012–PYG 10 °C–MK403775	Planococcus donghaensis [LN774491]—100% Planomicrobium okeanokoites [KF749394]—100% Planomicrobium okeanokoites [KF724965]—100%	Planomicrobium sp.	
KBP.AS.1021-PYG 10 °C-MK403776	Arthrobacter sp. [KT424968]—100% Arthrobacter agilis [KM036066]—100% Arthrobacter agilis [JN377650]—99.9%	Arthrobacter agilis	
KBP.AS.1024–PYG 10 °C–MK403777	Pseudarthrobacter phenanthrenivorans [NR_074770]—100% Arthrobacter sp. [LN871743]—100% Arthrobacter phenanthrenivorans	Arthrobacter sp.	
KBP.AS.1026-CM 25 °C-MK403778	Uncultured bacterium [AB696407]—98.5% Massilia sp. [JQ511857]—98.0% Massilia sp. [JQ511858]—97.8%	Massilia sp.	
KBP.AS.1028-CM 25 °C-MK403779	Agrococcus unuliensis[KF318382]—99.8%Agrococcus jenensis[EF672044]—99.8%Agrococcus lahaulensis[MF351823]—99.6%	Agrococcus sp.	
KBP.AS.1029–CM 25 °C–MK403780	Uncultured bacterium clone [DQ125573]—99.5% Pseudarthrobacter oxydans [MH304399] 99.3%	Arthrobacter sp.	

Arthrobacter sp.[MH714681]—99.3%Pseudarthrobacter siccitolerans[MF682005]—99.9%KBP.AS.1030-CM 25 °C-MK403781Pseudarthrobacter oxydans[MF681862]—99.9%Pseudarthrobacter oxydans[MF681861]—99.9%MF681851]—99.9%Massilia sp. [MH707216]—99.9%Massilia alkalitolerans	KBP.AS.1029–CM 25 °C–MK403780       Pseudarthrobacter oxydans         [MH304399]—99.3%		Arthrobacter sp.
[MH7/14681]—99.3%         Pseudarthrobacter siccitolerans         [MF682005]—99.9%         Pseudarthrobacter oxydans         [MF681862]—99.9%         Pseudarthrobacter oxydans         [MF681851]—99.9%         Massilia sp. [MH707216]—99.9%         Massilia alkalitolerans		Arthrobacter sp.	
Pseudarthrobacter siccitolerans         [MF682005]—99.9%         Pseudarthrobacter oxydans         [MF681862]—99.9%         Pseudarthrobacter oxydans         [MF681851]—99.9%         Massilia sp. [MH707216]—99.9%         Massilia alkalitolerans		[MH/14681]—99.3%	
KBP.AS.1030-CM 25 °C-MK403781       [MF682005]—99.9%       Pseudarthrobacter oxydans         [MF681862]—99.9%       Pseudarthrobacter oxydans         [MF681851]—99.9%       Massilia sp. [MH707216]—99.9%         Massilia alkalitolerans       Massilia alkalitolerans		Pseudarthrobacter siccitolerans	
KBP.AS.1030–CM 25 °C–MK403781       Pseudarthrobacter oxydans [MF681862]—99.9%       Pseudarthrobacter oxydans [MF681851]—99.9%         Image: State of the state of		[MF682005]—99.9%	
Massilia alkalitolerans [MF681862]—99.9% [MF681851]—99.9% Massilia alkalitolerans	KBP AS 1030-CM 25 °C-MK403781	Pseudarthrobacter oxydans	Pseudarthrobacter sp
Pseudarthrobacter oxydans [MF681851]—99.9% Massilia sp. [MH707216]—99.9% Massilia alkalitolerans	KB1.10.1000 CM 20 C MIR100/01	[MF681862]—99.9%	i seuuurintooueter sp.
[MF681851]—99.9% Massilia sp. [MH707216]—99.9% Massilia alkalitolerans		Pseudarthrobacter oxydans	
Massilia sp. [MH707216]—99.9% Massilia alkalitolerans		[MF681851]—99.9%	
Massilia alkalitolerans		<i>Massilia</i> sp. [MH707216]—99.9%	
		Massilia alkalitolerans	
KBP.AS.1031–CM 25 °C–MK403782 [KY010279]–99.9% Massilia sp.	KBP.AS.1031-CM 25 °C-MK403782	[KY010279]—99.9%	<i>Massilia</i> sp.
Massilia varians		Massilia varians	*
[KX784920]—99.9%		[KX784920]—99.9%	
Arthrobacter agilis		Arthrobacter agilis	
[KF924209]—99.8%		[KF924209]—99.8%	A
KBP.AS.1032–CMI 25 °C–MK403783 Arthrobacter sp. [[X949695]—99.8%	KBP.AS.1032–CM 25 °C–MK403/83	Arthrobacter sp. [[X949695]—99.8%	Arthrobacter sp.
Arthrobacter sp. []X9496461—99.8%		Arthrobacter sp. [IX949646]—99.8%	
Microbacterium sp.		Microbacterium sp.	
[I_T601250]—99.5%		[LT601250]—99.5%	
Uncultured bacterium clone		Uncultured bacterium clone	
KBP.AS.1033–CM 25 °C–MK403784 [MG8050311–99 5%] Microbacterium sp.	KBP.AS.1033–CM 25 °C–MK403784	[MG805031]—99.5%	<i>Microbacterium</i> sp.
Microbacterium sp		Microhacterium sp	
[ME526604]—99 5%		[MF526604]—99 5%	

Strain–Isolation Conditions–GenBank Accession Number	<b>BLAST Search Results</b>	Taxonomic Affiliation
KBP.AS.1037–CM 25 °C–MK403785	Massilia varians [KC429599]—99.7% Massilia alkalitolerans [KY010279]—99.6% Massilia alkalitolerans [KP282807]—99.6%	<i>Massilia</i> sp.
KBP.AS.1044–CM 25 °C–MK403786	Uncultured bacterium clone [KC442649]—87.5% Pontibacter sp. [HM579810]—87.5% Pontibacter diazotrophicus [NR_126288]—87.2%	Pontibacter sp.

Colour highlight indicates strains characterized by 100% sequence similarity.

## Appendix B

Table A2. The primers used for 1	6S rRNA gene amplification	and sequencing.

GenBank Accession Number	Strain	Taxonomic Affiliation	Primers Used for Amplification	Primers Used for Sequencing
MK403789	KBP.AS.17	Pseudarthrobacter oxydans	27 f + Un1492 r	1100 r
MK403790	KBP.AS.62	Arthrobacter sp.	341 f + 805 r	805 r
MK403794	KBP.AS.75	Brachybacterium sp.	27 f + Un1492 r	1100 r
MK403787	KBP.AS.105	Janthinobacterium sp.	27 f + Un1492 r	1100 r
MK403788	KBP.AS.160	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403791	KBP.AS.747	Microbacterium aurantiacum	27 f + 537 r	537 r
MK403792	KBP.AS.748	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403793	KBP.AS.749	Brevibacterium frigoritolerans	27 f + Un1492 r	1100 r
MK403795	KBP.AS.750	Planomicrobium okeanokoites	341 f + 805 r	805 r
MK403796	KBP.AS.751	Agrococcus sp.	27 f + Un1492 r	1100 r
MK403797	KBP.AS.752	Agrococcus sp.	27 f + Un1492 r	1100 r
MK403798	KBP.AS.753	Agrococcus sp.	27 f + 537 r	537 r
MK403799	KBP.AS.754	Kocuria sp.	27 f + Un1492 r	1100 r
MK403800	KBP.AS.755	Planomicrobium glaciei	27 f + Un1492 r	1100 r
MK403801	KBP.AS.756	Leucobacter aridicollis	27 f + Un1492 r	1100 r
MK403802	KBP.AS.757	Planomicrobium okeanokoites	341 f + 805 r	805 r
MK403803	KBP.AS.758	Microbacterium sp.	27 f + Un1492 r	1100 r
MK403804	KBP.AS.759	Bacillus pumilus	27 f + Un1492 r	1100 r
MK403805	KBP.AS.760	Microbacterium sp.	27 f + Un1492 r	1100 r
MK403806	KBP.AS.761	Rufibacter sp.	27 f + 537 r	537 r
MK403807	KBP.AS.762	Microbacterium sp.	27 f + Un1492 r	1100 r
MK403808	KBP.AS.763	Massilia sp.	27 f + Un1492 r	1100 r
MK403809	KBP.AS.764	Bacillus sp.	341 f + 805 r	805 r
MK403810	KBP.AS.765	Massilia alkalitolerans	27 f + Un1492 r	1100 r
MK403811	KBP.AS.766	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403812	KBP.AS.767	Massilia varians	27 f + Un1492r	1100r
MK403813	KBP.AS.768	Planomicrobium sp.	27 f + Un1492 r	1100r
MK403814	KBP.AS.769	Microbacterium pseudoresistens	27 f + 537 r	537 r
MK403815	KBP.AS.770	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403816	KBP.AS.771	Arthrobacter sp.	27 f+Un1492 r	1100 r
MK403817	KBP.AS.772	Planomicrobium glaciei	27 f + Un1492 r	1100 r
MK403818	KBP.AS.773	Cellulomonas sp.	27 f + Un1492 r	1100 r
MK403819	KBP.AS.774	Planomicrobium okeanokoites	27 f + Un1492 r	1100 r
MK403820	KBP.AS.775	Planomicrobium okeanokoites	27 f + Un1492 r	1100 r
MK403821	KBP.AS.776	Georgenia sp.	27 f + Un1492 r	1100 r
MK403822	KBP.AS.777	Microbacterium barkeri	27 f + Un1492 r	1100 r
MK403823	KBP.AS.778	Salinibacterium sp.	27 f + Un1492 r	1100 r
MK403824	KBP.AS.779	Brevibacterium frigoritolerans	27 f + Un1492 r	1100 r
MK403825	KBP.AS.780	Micrococcus sp.	27 f + Un1492 r	1100 r
MK403826	KBP.AS.781	Kocuria sp.	27 f + Un1492 r	1100 r
MK403827	KBP.AS.782	Labedella sp.	27 f + Un1492 r	1100 r

GenBank Accession Number	Strain	Taxonomic Affiliation	Primers Used for Amplification	Primers Used fo Sequencing
MK403828	KBP.AS.783	Plantibacter sp.	27 f+ 537 r	537 r
MK403829	KBP.AS.784	Arthrobacter agilis	27 f + Un1492 r	1100 r
MK403830	KBP.AS.785	Microbacterium sp.	27 f + Un1492 r	1100 r
MK403831	KBP.AS.786	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403832	KBP.AS.787	<i>Massilia</i> sp.	27 f + Un1492 r	1100 r
None	KBP.AS.788	Bacillus sp.	Was identified	by morphology
MK403833	KBP.AS.789	Burkholderia sp.	341 f + 805 r	805 r
MK403834	KBP.AS.790	Cellulomonas hominis	27 f + Un1492 r	1100 r
MK403835	KBP.AS.791	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403836	KBP.AS.792	<i>Massilia</i> sp.	27 f + Un1492 r	1100 r
MK403837	KBP.AS.793	Salinibacterium sp.	27 f + Un1492 r	1100 r
MK403838	KBP.AS.794	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403839	KBP.AS.795	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403840	KBP.AS.816	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403841	KBP.AS.842	<i>Massilia</i> sp.	27 f + Un1492 r	1100 r
MK403842	KBP.AS.843	<i>Mycetocola</i> sp.	27 f + Un1492 r	1100 r
MK403843	KBP.AS.844	Arthrobacter agilis	27 f + Un1492 r	1100 r
None	KBP.AS.845	Streptomyces sp.	Was identified	by morphology
MK403844	KBP.AS.846	Arthrobacter sp.	27 f+ 537 r	537 r
MK403845	KBP.AS.847	Cellulomonas sp.	27 f + Un1492 r	1100 r
MK403846	KBP.AS.848	Massilia sp.	27 f + Un1492 r	1100 r
MK403847	KBP.AS.849	Microbacterium sp.	27 f + Un1492 r	1100 r
MK403848	KBP.AS.850	Massilia sp.	27 f + Un1492 r	1100 r
MK403849	KBP.AS.854	Paracoccus marcusii	27 f + Un1492 r	1100r
None	KBP.AS.855	Rufibacter sp.	Was identified	by morphology
MK403850	KBP.AS.858	Arthrobacter crystallopoietes	27 f + Un1492 r	1100 r
MK403851	KBP.AS.862	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403852	KBP.AS.866	Sphingomonas sp.	27 f + Un1492 r	1100 r
MK403853	KBP.AS.867	Microbacterium paraoxudans	27 f + Un1492 r	1100 r
MK403854	KBP.AS.868	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403855	KBP.AS.869	Kocuria sp.	341 f + 805 r	805 r
MK403856	KBP.AS.876	Arthrobacter agilis	27 f + Un1492 r	1100 r
MK403857	KBP.AS.877	Salinibacterium sp.	27 f + Un1492 r	1100 r
MK403858	KBP.AS.899	Strentomyces sp.	27 f + Un1492 r	1100 r
MK403859	KBP AS 916	Planomicrohium glaciei	27 f + Un1492 r	1100 r
MK403860	KBP 45 926	Sphingomonas sp	27 f + Un1492 r	1100 r
MK403861	KBP 4S 927	Arthrobacter agilis	27 f + Un1492 r	1100 r
MK403862	KBP 45 928	Massilia sp	27 f + Un1492 r	1100 r
MK403863	KBP 45 929	Pseudarthrohacter sp	27 f + Un1492 r	1100 r
MK403864	KBP 45 930	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403865	KBP 45 931	Arthrohacter sp.	27 f + Un1492 r	1100 r
MK403866	KBP AS 932	Pequdarthrobacter sp.	27 f + Un1492 r	1100 r
MIK403067	KDI.AS.932	Pseudarthrobacter	27 1 + 0111492 1	1100 1
MK403867	KBP.AS.936	phenanthrenivorans	27 f + Un1492 r	1100 r
MK403868	KBP.AS.937	Arthrobacter agilis	27 f + Un1492 r	1100 r
MK403869	KBP.AS.938	Streptomuces sp.	27 f + Un1492 r	1100 r
MK403870	KBP.AS.939	Massilia sp.	27 f + Un1492 r	1100 r
MK403871	KBP.AS.940	Microbacterium sp	27 f + Un1492 r	1100 r
MK403872	KBP.AS.941	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403873	KBP AS 943	Arthrobacter avilis	27 f + Un1492 r	1100 r
MK403874	KBP AS 947	Microhacterium sp	27 f + Un1492 r	1100 r
MK403875	KBP AS 954	Microhacterium sp.	27 f + Un1492 r	1100 r
MK403876	KBP AS 955	Pseudarthrohacter sp	27 f + Un1492 r	1100 r
MK403877	KBP 49 056	Planomicrohium en	$27 f \pm Un1/02 r$	1100 r
MK403878	KBP 4 S 957	Arthrohacter sp	$27 f \pm Un14921$	1100 r
MK403870	KBD VC 026	Arthrohacter on	$271 \pm 01114721$ $97f \pm 11n1402 =$	1100 1
MKA03880	KBD 1 C 040	Microhacterium on	27 + 7 = 0111472 + 7 27 + 1 = 011472 + 7	1100 1
MK403881	KBD 4 C 041	Cellulomonas an	$271 \pm 01114721$ $97f \pm 11n1402 =$	1100 1
None	KBD 1 C 067	Strentomuces on	Was identified	hu mornhologu
MKAN2887	KBD 4 C 042	Arthrobacter acilia	$27 f \pm Un1402 m$	1100 m
MK403002	KBD 1. A3. 703	Micrococcus sp	$27.1 \pm 0111472$ f 27.f + Um1402 r	1100 1
MV/02001	KDI.A0.7/2	Rhodococcus amiliane	$271 \pm 011492 f$ 27 f + Un1492 =	1100 -
IVIN402760	NDT.AJ.9/3	Anthropological arter are	$27.1 \pm 0.01492$ r $27.6 \pm 1.1 \pm 1.402$ r	1100 r 1100 -
IVIN403/08	NDF.A5.1003	Arthrodacter sp.	27 f + Un1492 r	1100 r
IVIN403/09	NDP.AS.1005	Leucobacter ariaicollis	27 f + Un1492 r	1100 r
NIK403//U	KBP.AS.1007	Arthrovacter sp.	2/1 + Un1492 r	1100 r
MIK403//1	KBP.AS.1008	Planomicrobium glaciei	27 t + Un1492 r	1100 r
141/402772	K BP AS 1009	Pseudarthrobacter sp.	27 t + Un1492 r	1100 r
MK403772	KDP 10 1000	D1 · 1· · · ·	07 ( 77 4 ****	
MK403772 MK403773	KBP.AS.1010	Planomicrobium glaciei	27 f + Un1492 r	1100 r
MK403772 MK403773 MK403774	KBP.AS.1010 KBP.AS.1011	Planomicrobium glaciei Arthrobacter sp.	27 f + Un1492 r 27 f + Un1492 r	1100 r 1100 r

GenBank Accession Number	Strain	Taxonomic Affiliation	Primers Used for Amplification	Primers Used for Sequencing
MK403776	KBP.AS.1021	Arthrobacter agilis	27 f + Un1492 r	1100 r
MK403777	KBP.AS.1024	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403778	KBP.AS.1026	Massilia sp.	27 f + Un1492 r	1100 r
MK403779	KBP.AS.1028	Agrococcus sp.	27 f + Un1492 r	1100 r
MK403780	KBP.AS.1029	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403781	KBP.AS.1030	Pseudarthrobacter sp.	27 f + Un1492 r	1100 r
MK403782	KBP.AS.1031	Massilia sp.	27 f + Un1492 r	1100 r
MK403783	KBP.AS.1032	Arthrobacter sp.	27 f + Un1492 r	1100 r
MK403784	KBP.AS.1033	Microbacterium sp.	341 f + 805 r	805 r
MK403785	KBP.AS.1037	Massilia sp.	27 f + Un1492 r	1100 r
MK403786	KBP.AS.1044	Pontibacter sp.	341 f + 805 r	805 r

Table A2. Cont.

Colour highlight indicates strains characterized by 100% sequence similarity.

#### Appendix C

**Table A3.** The bacterial genera cultured from the Mojave Desert soil sample on different media. The table summarizes data of community structure diagrams and allows to compare bacterial diversity in a simpler view.

Brachybacterium			
		Burkholderia	
Cellulomonas		Cellulomonas	Cellulomonas
Georgenia			
Janthinobacterium			
Kocuria			
Labedella			
Leucobacter	Leucobacter		
Massilia	Massilia	Massilia	
Microbacterium	Microbacterium	Microbacterium	
	Micrococcus		
Mycetocola		7	
Paracoccus		Paracoccus	
Planomicrobium	Planomicrobium		
Pluniloucler		Pontihacter	
Pseudarthrohacter	Pseudarthrohacter	Pseudarthrohacter	
1 bennun mitobueten	Rhodococcus	1 ochum mi obacier	
Rufibacter			
Salinibacterium		Salinibacterium	
	Sphingomonas	Sphingomonas	
Streptomyces		Streptomyces	Streptomyces

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