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Bacterial Community Structure in Petroleum Contaminated and Uncontaminated Soils of Mountain Ecosystem

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Abstract

We compared a microbial diversity in uncontaminated and contaminated with petroleum soils in mountain ecosystems in Kyrgyzstan. Culture-dependent and culture - independent methods were used to analyze microbial diversity. PCR primers that target conserved iron binding motifs in alkane monooxygenase (*alkB*) and cytochrome P450 alkane hydroxylase were used to analyze genes of alkane degrading bacteria in chronically contaminated and uncontaminated sites, using an enrichment culture with dodecane.

Analysis of 16S rRNA showed that in contaminated soils *BetaProteobacteria* phylum populations were abundant. In uncontaminated soil *Gammaproteobacteria* phylum populations were abundant. The Shannon index (*H′*) was used to estimate the microdiversity in soils. Diversity was higher in the uncontaminated (*H′* = 4,234) than in the contaminated (*H′* = 3.632) soil biotopes.

These studies have shown that oil contamination affects the structure of local soil microbiota, undermines biodiversity and increases the activity of bacteria involved in hydrocarbon degradation, for example *Proteobacteria* phylum populations.

Keywords*: mountain ecosystems, contaminated and uncontaminated soils, 16S rRNA bacterial diversity, cytochrome P450 alkane hydroxylase genes.*

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1. INTRODUCTION

Oil spills represent a widespread problem; they are caused by tanker accidents, storage tank ruptures, pipeline leaks and transport accidents [1, 2, 3]. Petroleum hydrocarbons are a complex mixture of alkanes, cycloalkanes, aromatic hydrocarbons and asphaltic components [4].

Hydrocarbons are one of the most dangerous, quickly extending and slowly degrading pollutants in natural conditions. Easy fractions of oil, alkane possess a high migratory ability and easily move ahead into a soil structure in subsoil waters.

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in soil structure formation, decomposition of organic matter, in degradation of pollutants and toxin removal [5]. The ability of soil microbes to degrade organic contaminants into harmless constituents has been explored as a means of biological treatment of contaminated environments.

Characterization of oil-utilizing microorganisms has become crucial to our understanding of biological processes responsible for oil biodegradation in natural and polluted environments. Many microbiological and ecological studies have revealed the hydrocarbon oxidizing bacteria in soil, water and oil spills [6, 7, 8, 9, 10, 11, and 12]. Several species of heterotrophic bacteria play an important role in the biological treatment of polluted habitats [13, 14, and 15].These bacteria produced a wide range of enzymes, which degrade xenobiotic compounds and thus may be applicable in the bioremediation of polluted environments.

Alkane monooxygenases function in nature as the initial enzyme in biodegradative pathways for carbon chains of 5 to over 20 carbons. Examples of these types of substrates are octane (8 carbons), dodecane (12 carbons), and hexadecane (16 carbons). At least three different pathways have been characterized for the bacterial degradation of alkanes under aerobic conditions: a diiron terminal monooxygenase initiated pathway, a subterminal (n-1) oxidation pathway, and a cytochrome P450 initiated pathway. This catabolic pathway is initiated either by a membrane spanning alkane monooxygenase (AlkB) or a soluble cytochrome P450 alkane hydroxylase. AlkB and P450 alkane monooxygenases catalyze the addition of one atom of molecular oxygen into the terminal carbon, forming the corresponding alcohol [15].

While classical microbiological methods based on culturing procedures have provided important, but limited information on the microbial diversity of natural samples, novel molecular techniques have been extremely valuable in unraveling the diversity of microbiota involved in the alkane biodegradation.

Kyrgyzstan represents a region of the world with varied habitats ranging from high mountain deserts, permanently snow-capped mountains, heavily saline and alkaline soils, petroleum and heavy metal polluted sites left over from the soviet era. These environments have not yet been systematically examined for the diversity of native microbiota capable for hydrocarbons metabolizing.

In this study, culture-dependent and culture-independent approaches (DNA sequences of the 16S rRNA) were used to determine the taxonomic composition of bacterial communities with AlkB and P450 alkane monooxygenases from soil contaminated with petroleum and to compare it with the bacterial communities in a non-contaminated soil.

2. MATERIALS and METHODS.

2.1 Environmental samples. Environmental samples (5-10 cm deep from the surface) were collected from natural mountain ecosystems and sites around Bishkek city that were long exposed to petroleum spills (table 1).

2.2 Establish and Characterize Enrichment Cultures

Sediment and soil samples from uncontaminated and contaminated sites served as an inoculum source for the enrichment cultures in subsequent experiments. Mineral medium enriched with glucose was prepared:

KNO_3	- 0, 4%
KH_2PO_4	- 0, 06%
Na_2HPO_4	- 0, 06%
$Na_2HPO_4 \cdot 12H_2O$	- 0, 14 %
$MgSO_4 \cdot 7H_2O$	- 0, 08%
Water	- 1000 ml.

To the 25 ml of this medium in Erlenmeyer flasks (150 ml) was added 1g of soil or sediment samples, then the flasks were shaken during 2-4 day, at 250 rev/min, at temperature 25-30 ^{0}C. After one cycle of a non-alkane carbon source, we used an enrichment on dodecane (500 µl / per 1000 ml of medium). We expected that enrichment cycles would cause different diversity of alkane degrading bacteria and alkB, P450 and 16S rRNA gene populations.

Table 1. Environmental sites for collecting of soil samples

Uncontaminated soils of high-mountainous ecosystem		
Geographic Position, Elevation, locality	Zone/Ecosystem, Soil Source Type	Physical and chemical characteristics of soils
N-42 , 22’, E –73^0, 47, 2860 (m), Naryn Province, Tuy-Ashu Pass	**High-mountain: Meadow sub-alpine soils** The climate of this zone is cold, with negative average annual air temperature. They are formed under sub alpine meadow vegetation (timothy, bluegrass, fescue, sedge).	They are leached of carbonates, and contain significant amount of humus (8-15 %). Organic total carbon - 3.93 %; total nitrogen -0.36% ; C: N - 10.5; humic acid-18. 0%, fulvic-16, 4, and carbon balance of soil - 65.6,% . The texture of meadow sub-alpine soils has a high content of sand, gravel and crushed stone. The mechanical structure contains mainly clay particles in the horizon. The amount of aggregate size of < 10 mm and > 1 mm ranges from 40 to 50%, whereas the amount of particles < 1 and > 0.25% - 20-32%. Soil pH - 7.0.
N-42^0,19’, E –73^0 ,49’, 2900 (m) Naryn Province, Descent after of Tuy Ashu Pass	**High-mountain: mountain alpine soils** The climate of this zone is cold, with negative average annual air temperature, and a short warm period, which is broken off by night frost.	These soils morphologically are characterized by the availability of turf horizon of dark-gray or brownish and black color, and granular structure. The upper turf horizon contains 10-15 % of humus and horizons fertilized with peat contain up to 20 % of humus. Fulvo acids prevail in the humus structure. Soils are distinguished by high content of common nitrogen (0.6-0.8 %), general phosphorus (0.25-0.40 %), and potassium (2.6-4.0%). These soils are lixiviated and have sub acidic reaction. The amount of aggregate size of > 5 mm – 5.0%; 5-1mm- 28.60%; 1- 0.25mm – 24.20%; <0.25mm – 42.20%, > 0.25mm - 57.80%.
N- 41º54.666', E-73º37.616' 2500m, Jalal-Abad Province, Kabylan place of Toluk district	**Mountain valley: light-chestnut soils** The vegetation cover is represented mainly by fescue-wormwood fine sod associations. Climate is characterized by high dry summer and high continentally.	Mechanical structure of these soils is easy and medium loam. Upper humus horizon contains humus between 2.5 and 3.5%, which gradually decreases down the profile. The number of total nitrogen is 0.2 – 0. 25%. The ratio of carbon to nitrogen is equal to 6-9. The volume of absorption is low and makes up 15-20 mg-equip per 100 g, pH- 7.42. The amount of aggregate size of 1.0-0.25mm- 13.0%; 0.25-0.05mm- 16.70%; 0.05-0.01mm- 30.66%; 0.01-0.005mm-33.84%; 0.005-0.001mm- 6.30 %; <0.001mm-4.20%; <0.01mm- 3.30%.

N –41º54.666', E-73º37.616' 2700m,Jalal-Abad Province, Jamanbay, Toluk district,	**Mountain black soils, medium and fertile** They are usually formed under the serried grass-forbs meadow steppes, where the annual precipitation is 500-600 mm more rainfall in the spring-summer maximum. There are cold and snow winters, relatively warm summers with moderate temperatures in July (17-19^0 C).	A1 horizon, firm sod, brownish-black, powdery-granular structure. Its power is in the range of 10-15 cm. Mountain black soils in most cases, heavy and medium-loamy. Mechanical fractions of medium and coarse particles dominate among them and they are distributed in the soil profile rather evenly. And moderate fertile black soil contains up to 10-15% of humus, the amount of which decreases gradually with depth. Reaction of upper horizons is neutral or slightly acid. Soils have a high absorption capacity - 40-55 meq per 100 grams. Soil absorbing complex is saturated with calcium and magnesium
Contaminated soils and silt of moderate climatic ecosystem		
850-1200m, Chy, province, Bishkek city outskirts	Silt from polluted river Alamedin with a total length of 78 km; it flows through Bishkek settlements. River takes its origin from the glaciers in the northern slope of the Kyrgyz Ala-Too. River water is widely used for irrigation. Runoff of agricultural industry, parking lots and gas stations fall into the river.	Silt pH -7.5
850-1200m, Chy Province, Bishkek city outskirts	**Light serozem soils** They are formed by vegetation of ephemeral desert steppes. Parent rocks are loess-like loams. The climate of this zone is continental. In summer air temperature can reach up to 43^{0}C, and in winter up to - 25^{0}C.	Mechanical composition is silt-loam, sandy loam and loamy less. The humus content – 0.7-1.7%; total nitrogen-0.1-0.14%. They are calcareous to the surface: the CO_2 in the upper layer is about 2-3%. C: N - 6.3. Mechanical fraction %: 1,0-0.25mm- 5.34; 0.25-0.05mm- 17.5; 0.05-0.01- 47.73; 0.01-0.005mm- 13.73; 0.005-0.001mm- 12.97; < 0.001-5.32; 0.001- 32.02. Soil pH- 8.0
760 -980 m, Chy Province, Bishkek city outskirts	Sand–soil from country road	pH -7.6

2.3 Extraction of total DNA

DNA was extracted during the active phase of microbial growth from enrichment cultures using Ultra Clean ™ Soil DNA Isolation Kit and alternative protocol of MO BIO Company.

2.4 DNA extraction from pure cultures

Purified bacteria were incubated in MPM medium for 2 days at 25^{0}C. Cells were harvested at the early exponential growth phase, and their DNA was then extracted by alternative protocol of MO BIO Company.

2.5 PCR amplification

Amplification was performed with a Multigene Thermal Cycler (TC9600-G/TC, Labnet International), using a (25 µl) mixture containing: 15 µl of PCR Master Mix (Taq DNA polymerase, $MgCl_2$, deoxyribonucleotide triphosphate and reaction buffer), 2µl of each primer, 1µl of template DNA and 1 µl of H_2O. The amplification program was used as following: 94°C for 5 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s, and 72°C for 7 min. PCR products were electrophoresed in a 1,0% agarose gel and visualized by BioDoc-It™ Imaging Systems (Ultra-Violet Products

Ltd) after ethidium bromide staining. To control a contamination a negative control reaction was used and a sterile water was added as a matrix.

2.6 Primers

Almost – full –length fragments of 16Sp RNA gene were amplified using the primers: 16S-27Fand 16S-907R. Fragments of genes encoding the subunits of alkane monooxygenases were amplified using specific sets of primers. The primer set of alkB-F and alkB-R were used to amplify the alkane hydroxylase and P450R were used to amplify the cytochrome P450 alkane hydroxylase (table.2).

Table.2. PCR primers used for amplifying the fragments of genes

Primer pair	Sequence of primers	Gene recognized	Product
27f 907R	5'-AGA GTT TGA TCCTGG CTC AG 5'- CCG TCA ATT CCT TTG AGT TT	16S rRNA	900 bp
alk B_F alk B_R	5'-AAT ACH GSV CAY GAG CTC RGY CAY AAR 5'-GCR TGR TGA TCA GAR TGH CGY TG	alkane hydroxylase	550 bp
P450fw1 P450 rv3	5'-GTS GGC AAC GAC ACS AC 5'- GCA SCG GTG GAT GCC GAA GCC RAA	cytochrome P450 alkane hydroxylase	339

2.7 Sequence analysis was performed by Macrogen Company (10F World Meridian Center, # 60-24 Gasan-dong, Geumchun-gu Seoul, Korea, 153-023) and sequences were edited with Applied Biosystems 3730XL sequencers. Only sequences with > 700 nucleotides were used for diversity analyses. The phylogenetic relatedness among different sites was determined using the Cluster environment. The 16S rRNA gene sequences were deposited in the Gen Bank and DB of NCBI nucleotide sequence databases. The sequences were aligned Using Cluster W and checked manually using the software MEGA4 [16].

2.8 Characterization of isolated bacteria.

Representative morphotypes were selected in order to perform the taxonomic characterization. Isolated bacteria were grouped on the basis of their morphological, biochemical and physiological characteristics. Catalase activity , NO_3 reduction, insole production from tryptophan, fermentation of 12 different organic carbon compounds, urease activity, hydrolysis of gelatin, starch were determined. Fluorescent, diffusible pigments, growth at 4 ºC**,** 27ºC and 37 ºC were determined.

29.Evaluation of statistical analyses of bacterial diversity.

Soil bacterial diversity was estimated via the Shannon index ($H' = -\sum p_i \ln p_i$). Species richness was assessed by using rarefaction method [17]. This method was selected instead of other richness indices because it allows comparison between communities based on unequal sample numbers from contaminated and uncontaminated sites.

A measurement of taxa abundant and very abundant was calculated by Hill's [18, 19] modified Shannon's and Simpson's index.
Chao1 [20], non-parametric estimator for species richness that takes the form: $S^*_1 = S_{obs} + (a^2/2b)$

Where S_{obs} - is the number of species observed
a - is the number of species observed just once
b - is the number of species observed just twice

3. RESULTS and DISCUSSION.

3.1. Biodiversity of uncultured bacteria isolated from contaminated soils by PCR analyze

We used three sets of primers to analyze genes of alkane degrading bacteria in contaminated soils and silts that had a long time of exposure to oil spills pollution. DNA was extracted directly from silt and soil, also from culture enriched with dodecane.

The PCR primers successfully amplified the fragments of alkane degrading genes of DNA extracted from soil and silt. Most of the DNA samples extracted from the soil were amplified with P450fw1 and P450 rv3 primers showing by the presence of cytochrome P450 genes (Figure 1).

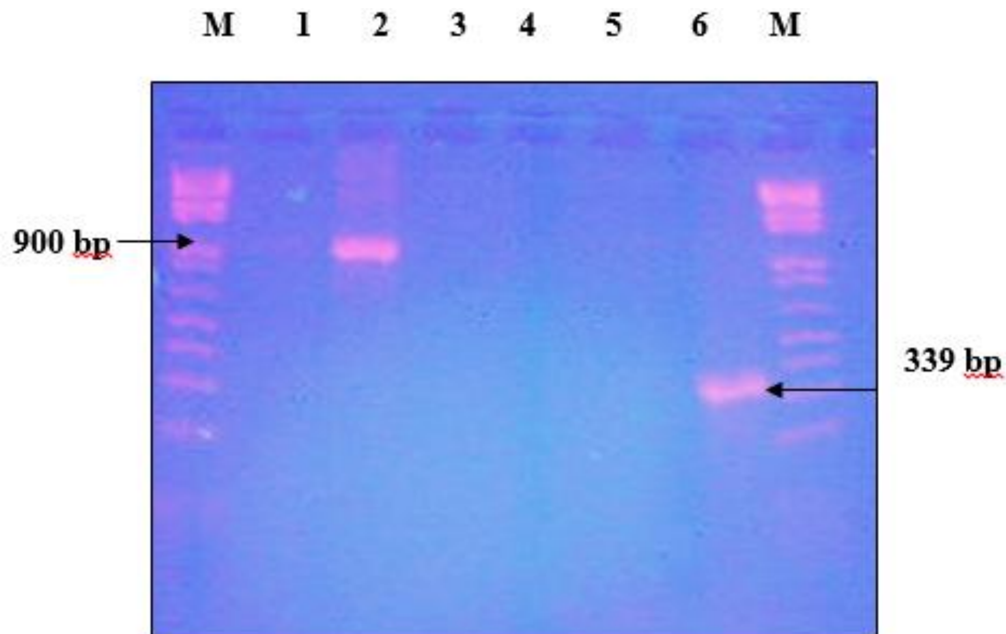

Fig 1.Agarose gel (1.0 %) electrophoresis of some PCR products of DNA samples extracted from enrichment culture obtained with universal and specific primers : lanes M- molecular weight markers ; lanes 1,3 and 5 – negative controls; lane 2 – DNA sample (A-3) amplified with 16SpRNA gene; lane 4 - DNA sample (A-3) no amplified with alkane hydroxylase genes (alkB-F and alkB-R); lane 6 - DNA sample (A-3) amplified with cytochrome P450 alkane hydroxylase genes (P450F and P450R **).**

A total of 5 phyla were delineated based on the 40 high-quality partial 16SrRNA gene sequences from twelve locations. *Proteobacteria* and Unclassified *Bacteria* were the most abundant phyla, corresponding to 70% of the sequences.
A contribution of *BetaProteobacteria* phylum was highest varying between 29-40% in the silt of Alamedin River and in light sierhozem soils around Bishkek city and in sand-soil from country road. Sequences assigned to *BetaProteobacteria* were present in six locations. Second most frequently retrieved groups in allocations were *Firmicutes, Gammaproteobacteria and Alhaproteobacteria* phyla corresponding to 28%. Several other phyla, e.g., *Actinobacteria, Bacteroidetes, Deltaproteobacteria* were found in a relatively lower frequency, often below 10% (Figure 2).

16S rRNA gene sequences of uncultured soil samples detected the most of the bacteria were belong to classes of *Beta, Alpha and Gamma Proteobacteria*. The representatives of *BetaProteobacteria* phylum were predominated in contaminated soils.Uncultured bacteria were abundant in this phylum: uncultured betaproteobacterium (*Bacteria*); uncultured *Ralstonia* sp. (*Burkholderiales; Burkholderiaceae; Ralstonia*); uncultured *Ralstonia* sp. clone F3Bjun.58 (*Burkholderiales; Burkholderiaceae; Ralstonia*) (table 3).

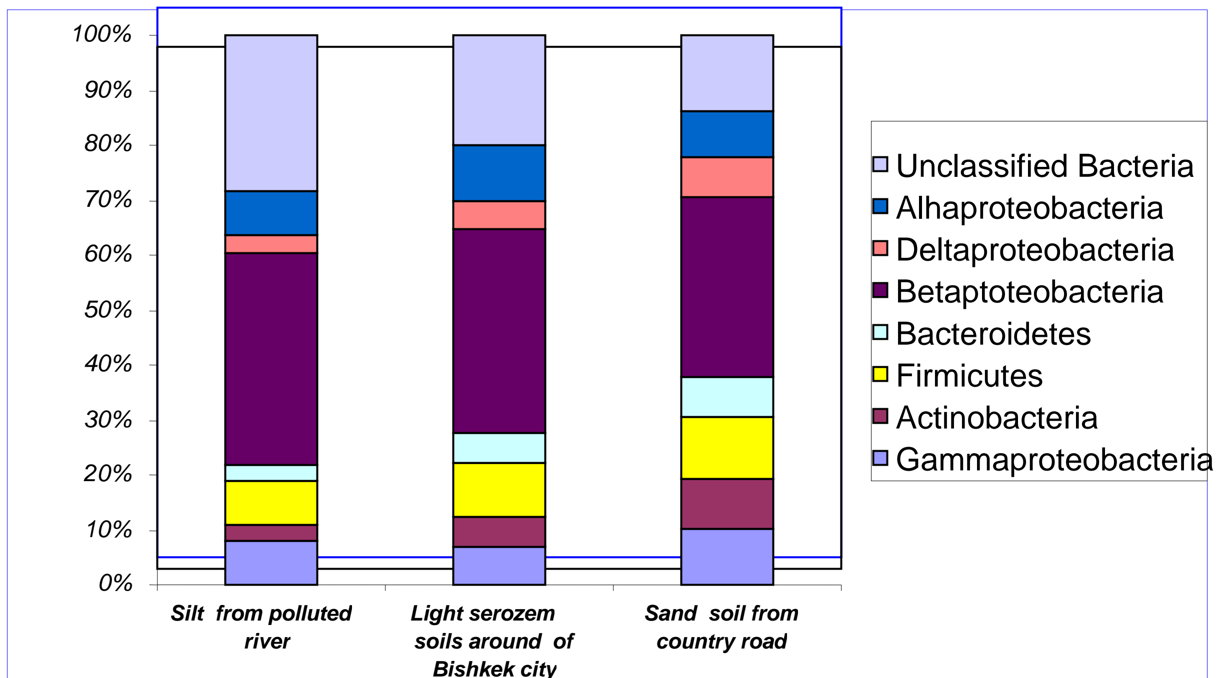

Figure 2. Percentage of bacteria taxa detected in petroleum- contaminated sites.

Oil contamination seemed to encourage the propagation of *Proteobacteria*, as known this bacterial group commonly associated with hydrocarbon degradation processes [21, 22, 23].

Table 3. Bacterial taxa detected in petroleum-contaminated soil samples

Soil samples.	Strain Closest relative in GenBank (accession No.)	Similarity, (%)	Phylogenetic group
A1	AF089858.1 *Aquabacterium* sp. Aqua2 16S ribosomal RNA gene, partial sequence	96	*Betaproteobacteria*
A2	FM201117.1. *Uncultured bacterium partial* 16S rRNA gene, clone MBR-8_LF_AS07	95	*Bacteria*
A3	FJ218234.1.*Parvibaculum sp. S18-4 cytochrome* P450(CYP153A) gene, CYP153A-p6 allele, partial cds	93	*Alphaproteobacteria*
A4	AJ299055.1.*Uncultured beta proteobacterium* Ch5E, partial 16S rRNA gene	90	*Betaproteobacteria*
A5	EU705853.1.*Uncultured Ralstonia sp*. clone 3P-3-2-K04 16S ribosomal RNA gene, partial sequence	89	*Betaproteobacteria*
A6	FJ593135.1 *Uncultured bacterium clone* Port_1_60 16S rib...	88	*Bacteria*
A7	ZP_03667773.1.Hypothetical protein LmonF1_06877 [*Listeria monocytogenes* Finland 1988]	57	*Firmicutes*
A8	FJ786054.1 *Massilia sp*. III-116-18 16S ribosomal RNA gene, partial sequence	98	*Betaproteobacteria*
D1	FJ263917.1.*Acinetobacter johnsonii* strain BA28	98	*Gammaproteobacteria*
D3	GQ417776.1. *Uncultured Ralstonia* sp. clone F3Bjun.58	83	*Betaproteobacteria*
A1- R	EU104018.1. *Uncultured bacterium clone M0111*_14 16S ribosomal RNA gene, partial sequence	89	*Bacteria*
A2-R	EU167476.1.*Uncultured Hydrogenophaga sp*. clone C0_E08_056 16S ribosomal RNA gene, partial sequence	93	*Bacteria*

Phylogenetic analysis has detected two culturable bacteria species: *Aquabacterium* sp. Aqua2 (*Burkholderiales; Aquabacterium*); *Massilia* sp. III-116-18 16S ribosomal RNA gene (*Burkholderiales; Oxalobacteraceae; Massilia*). *Alphaproteobacteria* phylum was presented by *Parvibaculum sp.* (*Rhizobiales; Phyllobacteriaceae; Parvibaculum*) that contains the S18-4 cytochrome P450 (CYP153A) gene. *Gammaproteobacteria* phylum was presented by *Acinetobacter johnsonii (Pseudomonadales; Moraxellaceae; Acinetobacter*) (Figure 3).

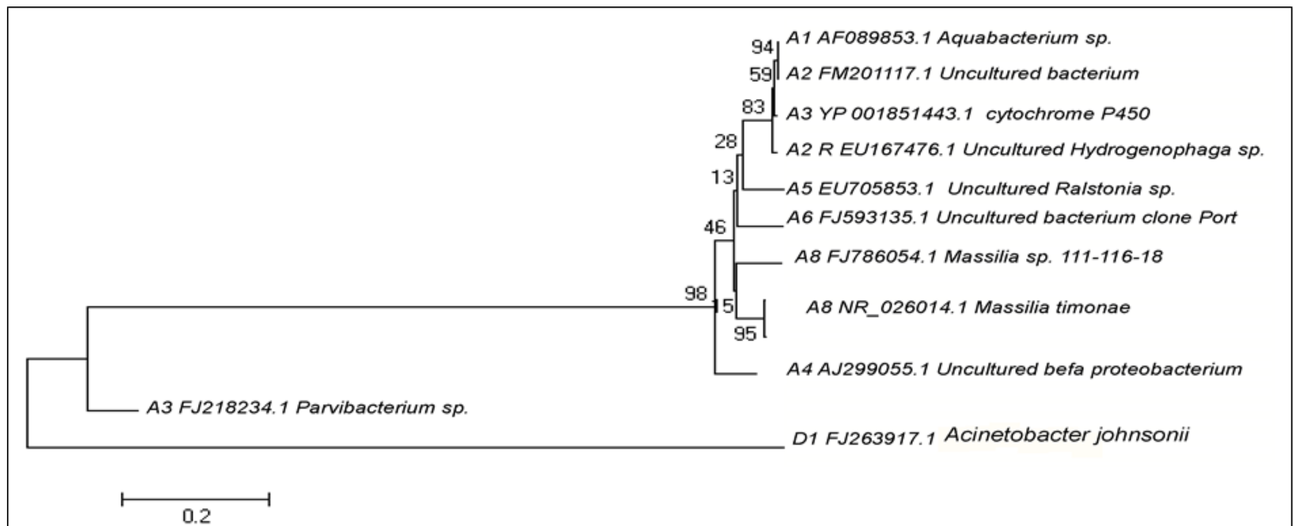

Figure.3. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from contaminated sites. Numbers at branching points represent bootstrap values from 1000 replicates. Bar, 0, 2 substitutions per nucleotide position.

3.2. Bacterial biodiversity isolated from uncontaminated ecosystem

DNA was extracted from enriched culture of soil samples selected from uncontaminated ecosystem both without dodecane and with its addition and PCR-amplified using 16S rRNA bacterial and specific primers for detecting P450 and alkB genes.We detected 8 recognized phyla based on the 28 high-quality partial 16SrRNA gene sequences from the seven locations. Members of the *Gammaproteobacteria* phylum were abundant in uncontaminated mountain soils, corresponding to 45, 0% of the sequences. Second most frequently retrieved groups in allocations were *Actinobacteria*, *BetaProteobacteria and Firmicutes* phyla corresponding to 28, 0%. Other phyla, e.g., *Bacteroidetes, Deltaproteobacteria, Alhaproteobacteria and Unclassified Bacteria* occurred in a relatively lower frequency, often below 2, 0% (Figure 4).

Phylogenetic analysis has detected three groups of bacteria assigned to *Gammaproteobacteria*, two groups assigned to *BetaProteobacteria,* one group assigned to *Actinobacteria* and one group *to Firmicutes (*Figure 5*).* Five groups had more than 90 % 16SrRNA sequence similarity towards sequences of uncultured and unidentified bacteria. Two groups had more than 65 % 16SrRNA sequence similarity (table 4).

Table 4. Bacterial taxa detected in pristine soil samples

Strain	Strain Closest relative in GenBank (accession No.)	Similarity, (%)	Phylogenetic group
B2	AY035996.2 Pseudomonas lini strain CFBP 5737 16S ribosomal RNA gene, partial sequence	92	Gammaproteobacteria
B2	ZP_01772659.1 Collinsella aerofaciens ATCC 25986	68	Actinobacteria
B3	ABE09160.1 Conserved hypothetical protein [Escherichia coli UTI89]	66	Gammaproteobacteria
B4	EF488087.1.Bacillus cereus strain QD232 16S ribosomal RNA gene, partial sequence	99	Firmicutes
B8	AY622234:Uncultured gamma roteobacterium	97	Gammaproteobacteria
B9	GQ416658.1 Uncultured Achromobacter sp. clone F7oct.52 16S ribosomal RNA gene, partial sequence	92	Betaproteobacteria
C9	GQ417846.1 Uncultured Ralstonia sp. clone F3Baug.23 16S ribosomal RNA gene, partial sequence	90	Betaproteobacteria;

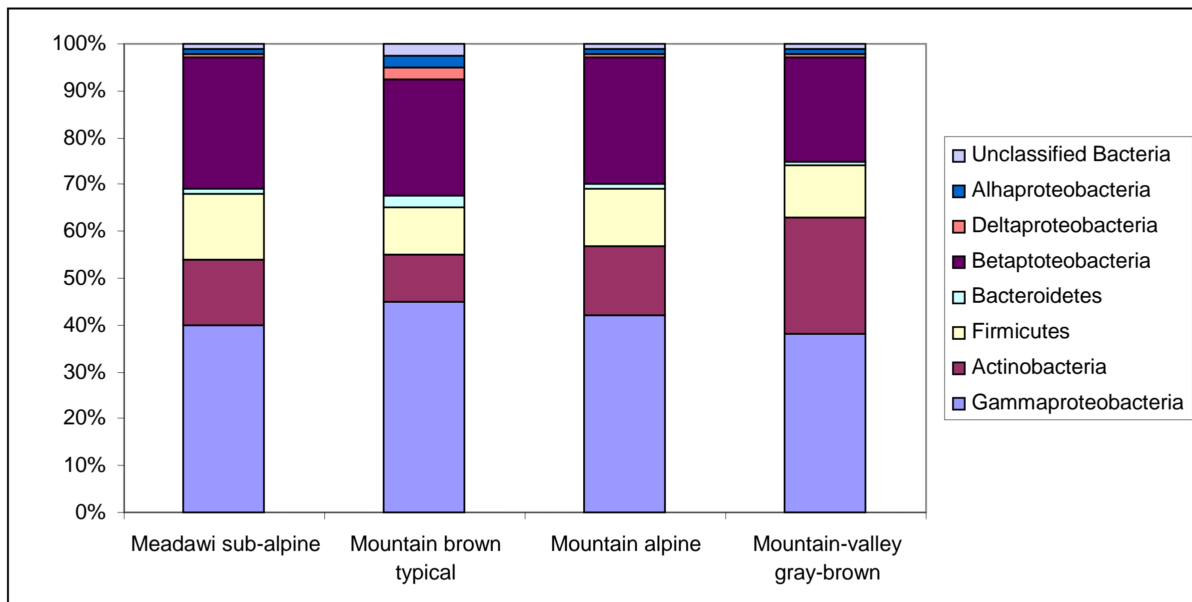

Fıgure 4. Percentage of bacteria taxa detected in uncontaminated sites.

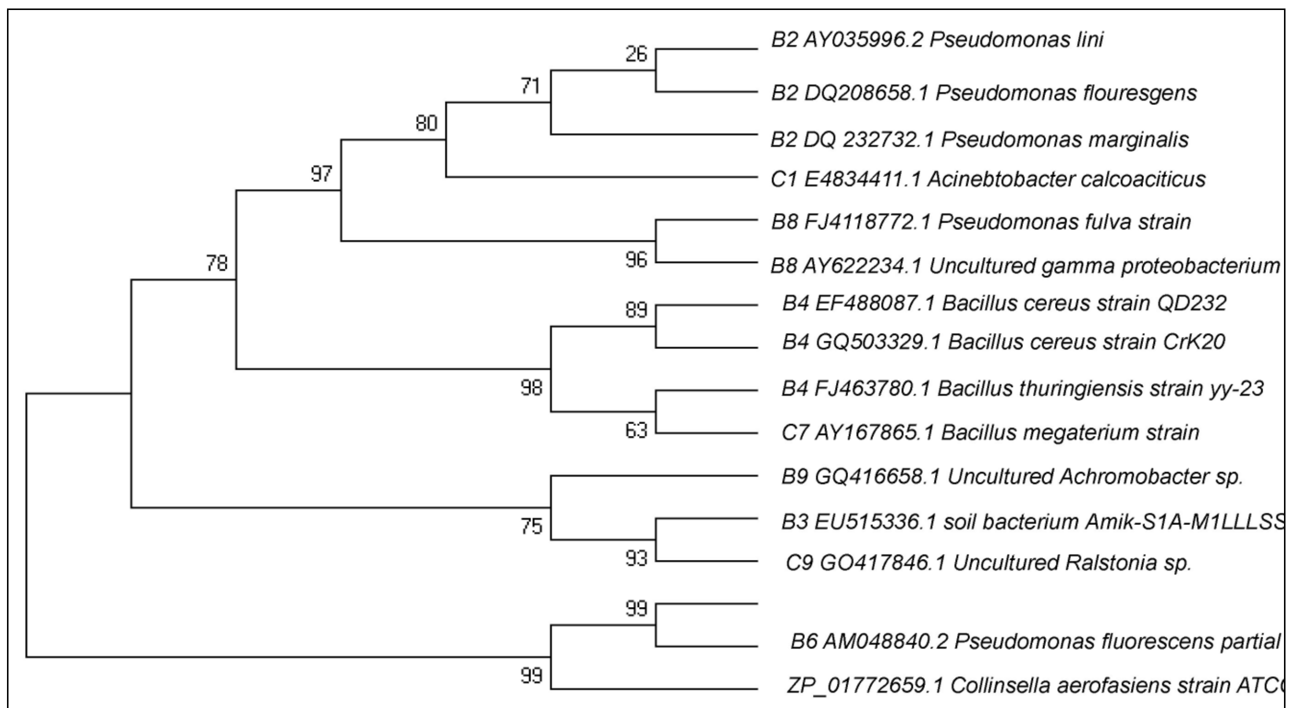

Fig.5. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from uncontaminated sites. Numbers at branching points represent bootstrap values from 1000 replicates. Bar, 0,2 substitutions per nucleotide position.

The present study is the first broad survey of bacterial community diversity in the high mountain alpine soils of Kyrgyzstan. This study clearly points out that high mountain alpine ecosystems represent a vast reservoir for bacterial diversity discovery. Using the molecular biology methods we have analyzed chronically polluted soils of moderate climatic zones and poorly polluted soils of cold mountain climatic zones in Kyrgyzstan. The analysis of 16SpRNA genes of chronically polluted soils allowed us to reveal a biodiversity of non-cultured bacteria. Most of the DNA samples extracted from soil and pure cultures were amplified with P450fw1 and P450 rv3 primers, these findings suggest a wild distribution of cytochrome P450 genes in contaminated soils and silts in Bishkek city ecosystem. While the alkB genes have no a functional activity in this sites. As known, cytochrome P-450 monooxygenase hydroxylates a variety of long-chain aliphatic substrates, such as fatty acids, alkanols, and alkylamides at v-1, v-2, and v-3 positions. A wild distribution of cytochrome P450 genes in contaminated soils indicated the activity of indigenous bacteria carrying these genes in transformation of accumulated pollution in environment and their involvement in natural self-soils from petroleum products. On the other hand, these genes may serve as an indicator of environment pollutions by alkane substrates.

The presence of cold-adapted microorganisms was found in alpine soils where temperature above 10^0C is reached only during the period of high solar irradiation and hot summer days by other researchers [24, 25]. An evaluation of 29 uncontaminated and contaminated alpine habitats (soils and glaciers) showed that not only oil- polluted samples but also samples from uncontaminated sites contained a remarkable potential of indigenous microorganisms that degraded diesel oil efficiently at 4 and 10^0C [26].

Our results confirm the hypothesis that alkane degrading bacteria have the spatial and temporal distribution and the soil is an interesting hidden reservoir for them. Our study using the molecular biology tools has obtained the genes of local alkane-degrading bacteria. This microflora can be involved in oil degrading process and being active in all climatic zones at presence of hydrocarbons. Relatively high presence of *Betaproteobacteria* in contaminated ecosystem suggests these bacteria may play a key role in microbial degrading processes whose number increases with the presence of pollution sources. In general, biodiversity of uncultured bacteria, detected by PCR analysis in both soils was higher than of cultured bacteria, it indicates that a molecular technique provides more extensive access than a traditional classic technique.
Soil bacterial diversity was estimated for the 22 samples via the Shannon index, Hulbert's method, which have indicated more rich and abundant diversity of soil bacteria species in uncontaminated sites then contaminated. While

nonparametric richness estimator Chao indicated from 9,2 to 9,5 species in uncontaminated sites and 8,6, 11,2, 13,0 species in contaminated sites. This method revealed that the species observed just once and twice were abundant in polluted sites than in unpolluted (table 6). It is seems that a pollution could activate the targets specific species involved in the recycling process of alkane compounds.

Table 6. Bacterial diversity in soils samples from uncontaminated and contaminated sites

Sequence similarity of 97%			
Diversity Index,	Total	Contaminated soils	Uncontaminated Soils
Shannon, min, max	5.3; 5.2; 5.4	3.6; 3.5; 3.7	4.2; 4.4; 4.7
Hurlber’ts method $\Sigma(_{sn})$		4.5	4.61
Chao1, min, max	30.0; 35.5	8.6; 11.2; 13.0	9. 25; 9.3; 9.5

We have compared a percentage of bacterial taxa detected in petroleum-contaminated and uncontaminated soil samples. Relative abundance of uncultured *Betaproteobacteria group in* polluted soils was two times higher than in unpolluted soils, that again proves this group of bacteria is involved in native oil bioremediation process. The ratio of cultured *Actinobacteria group in* polluted soils was 1,5 times higher than in unpolluted soils, it suggests that this group of bacteria is also actively involved in native oil bioremediation process, and in contrast to *Betaproteobacteria* groups it is well cultivated on mediums. The ratio of uncultured bacteria *Gammaproteobacteria* was more significant in unpolluted ecosystem than in polluted ecosystem (table 7).

Table 7. Percentage of bacterial taxa detected in petroleum-contaminated and uncontaminated soil samples. Taxonomic affiliation of bacteria was determined by BLAST and by neighbor joining analysis

Groups	Contaminated soils		Uncontaminated soils	
	Cultivable isolates, %	16S rRNA Sequence,%	Cultivable isolates,%	16S rRNA sequence,%
Alphaproteobacteria		8.3	5.1	-
Betaproteobacteria	-	41.6	-	28.5
Gammaproteobacteria	40.43	8.3	-	42.8
Deltaproteobacteria	-	0.05	-	0.07
Bacteroidetes	-	-	16.2	-
Actinobacteria	38.08	-	30.8	14.2
Firmicutes	21.63	8.3	49.75	14.2
Unclassified Bacteria	-	33.3	-	-

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