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Environmental Assessment of Contaminated Permafrost Soils and Development of Bioremediation Methods

M P Neustroev[1], MMNeustroev[2], N P Tarabukina[3], and A M Stepanova[3]
[1]Laboratory of Veterinary Biotechnology, Yakut Scientific Research Institute of Agriculture, Yakutsk, 677001, Bestuzheva-Marlinskovo Str., 23/1, Russia
[2]Republican Information Analytical Center of Ecological Monitoring of the Ministry of Protection, Yakutsk, 677000, 202 microdistrict, 18/2, Russia
[3]Laboratory of Microbial Preparations Development, Yakut Scientific Research Institute of Agriculture, Yakutsk, 677001, Bestuzheva-Marlinskovo Str., 23/1, Russia

Abstract. The article presents the ecological assessment of contaminated soils. Residual oil pollution significantly affects the structure of microbiocenoses of permafrost sod-steppe, meadow-sod-loamy, sod-humus-bog soils. Strains of indigenous bacteria Bacillus subtilis possess a strong hydrocarbon, emulsifying and antifungal, and moderate growth stimulating properties; can form the basis of biological products for the restoration of oil contaminated permafrost soils. As the data show, emulsifying index is 55 to 85%; therefore the studied liquid cultural strains of bacteria of the genus Bacillus, isolated from permafrost soils and paleomicroflora possess emulsifying properties. As a result of studying the oxidizing properties of the strains of bacteria Bacillus subtilis Kolyma - 7/2k and Bacillus subtilisOimyakon - 6/1 had been certified and deposited in the Russian National Collection of non-pathogenic microorganisms for agricultural purposes as promising for the recovery of oil contaminated permafrost soils under the registration numbers "CIAM 167" and "CIAM 168" in the group of spore-forming microorganisms (All-Russian Research Institute of Agricultural Microbiology reference number 616/12 and number 617/12 from 20.12.2009). For the first time it was offered a biological method of oil contaminated permafrost soils recovering. Scientific novelty of the development is confirmed by Russian patent "Method of cleaning permafrost soils from oil spore-forming bacteria Bacillus subtilis" (№2446900, priority of invention, 13.07.2010, registered in the State Register of Inventions of the Russian Federation 10.04.2012). Presented results of research to find ways of bioremediation of oil contaminated soils can be used as an effective and environmentally friendly method of recovery of northern ecosystems.

1. Introduction

A special role in solving environmental problems acquires the use of living organisms, especially microorganisms. Various methods of bioremediation using microorganisms developed and used in the oil contamination. The works devoted to the study of microorganisms used in the destruction of petroleum and petroleum products during bioremediation in the northern regions, is extremely small[1]. In recent decades it has seen an active search for, and development of highly versatile microbial preparations, designed from

the beneficial endophytic and rhizospheric bacteria. Among them bacteria of the genus Bacillus are of particular interest, which make up 30 to 36% of the microbial rhizospheric and endophytic populations[2]. Microbiocenosis of Yakutian permafrost soils is dominated by bacteria of the genus Bacillus, the most promising group in biotechnology, which have a broad spectrum of expressed antagonistic, enzymatic, interferon inducing, and immunomodulatory properties[3]. However, it is not sufficiently studied the combination of oil oxidizing, antifungal and growth promoting properties in native strains of bacteria of the genus Bacillus, that are relevant to the modern environmental biotechnology. The urgency of the problem is increasing with the development and exploitation of oil fields in the Republic of Sakha (Yakutia), the construction of the pipeline system "Eastern Siberia - Pacific Ocean", and the lack of research on finding effective, ecological, safe methods of biological treatment of permafrost soil from oil pollution.

Based on the above, the objective is to conduct an environmental assessment of soils and oil contamination to find a biological way to restore them.

2. Materials and methods

Soil samples were collected from different horizons of the soil profile (0-20; 20-50 cm) in accordance with GOST 17.4.3.01-83 and GOST 17.4.4.02-83 "The Nature Conservancy. Soils.General requirements for sampling", "Methods of preparing soil samples for agrochemical research" (GOST 2642-85). Agrochemical research carried out on IR analyzer NIR SCANNER model 4250, in the Laboratory of Biochemistry and Mass Analysis of Yakut Scientific Research Institute of Agriculture. Selection and preparation of soil samples for microbiological research was according to methods of soil microbiology and biochemistry. Meat-peptone agar (MPA), the mineral form of nitrogen - starch-ammonia agar (SAA), microscopic fungi – Chapek medium, hydrocarbon-oxidizing microorganisms - McClung medium were used to determine the total number of microorganisms that use organic forms of nitrogen. The number of microorganisms was determined in colony forming units (CFU) by 1g. Identification of microorganisms was carried out according to "Bergey bacteria determinant" (1997). Chemical analyses of the soil for the content of petroleum products were carried out in the accredited laboratory of State budgetary institution Republican Information and Analytical Center for Environmental Monitoring" of the Ministry of Nature Protection of the Republic of Sakha (Yakutia) using the methods of fluorimetry (PDNF 16.1.21-98) and chloroform extraction. Phytoxic activity of microorganisms was determined by seed germination (GOST 19449-93), emulsifying activity - by the method of D.L. Gutnik (1987), described in the work of E.V. Karpenko (2006) [6]. In the study of emulsifying activity, bacterial strains Bac. subtilisOymyakon 6/1, Bac. subtilis Kolyma 7/2k, Bac. were used as test cultures. mycoideas, Bac. subtilis TNP-3, Bac. subtilis TNP-5. Agrochemical studies were performed on an IR analyzer NIR SCANNER model 4250.

The mathematical processing of the data was performed using Applied Snedecor program, Microsoft Excel. The research results were subjected to statistical analysis by the Student's method.

3. Results and discussions

During the period 2008-2014 inYakutia was 182 accidental oil spills. By the number of incoming contaminants in the natural environment it can be characterized as small and medium sized (up to 3-10 t). The most significant contamination has occurred as a result of road traffic accidents at Mirninsky, Lensky and Tomponsky districts, and Khangalassky - in case of fire, as well as the leakage of oil from stationary object storage in Bulunsky district (Republic of Sakha(Yakutia), Russia).

Studying the impact of oil pollution on the microbiological and agrochemical characteristics of permafrost soil after the restoration work carried out in the village Chapchylgan of Amginsky district, where was spilled about 5 tons of diesel fuel in the diesel power plant on May 7, 2009. Soils were tainted with an area of 522.9 m^2 and the lake Haly-Baly. In the samples of soils contaminated with diesel fuel, with the background value of 0.02 g/kg, the excess of oil content ranged from 71.5 to 3750 times. It was produced mechanical cleaning of contaminated areas, excavation and removal of contaminated soil and clean soil delivery, collection and disposal of contaminated water.

Remediation works has not been evaluated in subsequent years. Our study was carried out in July 2012, i.e. 3 years after the spill of diesel fuel and recovery operations.

For 3 years after the spill of diesel fuel in Amginsky diesel power plant, the oil content in the soils decreased in comparison with the initial values of more than 1000 times (almost to background levels), but it marked a deep breach in the structure of soil microbiocenoses. Microbiological studies have fixed implications of diesel fuel even in those cases when the analytical flowmetry methods of oil content is (0.03-0.02 mg/g) just 2-3 times higher than the background level (0.01 mg/g).

Obtained research results are consistent with reports of Zvolinsky V.P., and allow[4] to confirm that the oil getting into the soil causes profound disturbances in the functioning of the microbiota. The shift taking place in the composition of the soil microbiota, may serve as a basis for the diagnosis of the degree of contamination and the development of methods of rehabilitation of the affected soil.

Microorganisms possessing psychotrophic and halophilic properties are extremely important and necessary, especially in Siberia and the North for the bioremediation of contaminated soils and water bodies. Therefore, one of the goals of our research was the finding of hydrocarbon oxidizing bacteria of the genus Bacillus, isolated from paleomicroflora and permafrost soils.

In the study and selection of microorganisms – the destructors of oil and petroleum products allocated from various regions of Russia, preference is given to the most active psychotrophic microorganisms capable of oil destruction at low positive temperatures and in the presence of an elevated concentration of salt.

Due to the above noted, we have studied 23 strains of bacteria of the genus Bacillus capable of oil destruction, having both halo tolerant and psychotrophic properties, including 18 strains isolated from paleomicroflora and 5 strains out of the permafrost soils.

Research results have shown that from the 23 studied strains of bacteria of the genus Bacillus 10 strains have the ability to oxidize paraffin at + 28 °C, and 7 strains at 4 °C. 5 strains of these bacteria: Bacillus mycoideas; Bacillus subtilis TNP-3; Bacillus subtilis TNP-5

isolated from permafrost soils, and Bacillus subtilis Kolyma 7/2k, Bacillus subtilisOimyakon 6/1 from paleomicroflora are able to grow in the liquid mineralized medium with 4% oil and gasoline.

Powerful regulator of microbial population activity, including natural, are surface-active agents (surfactants), in particular of microbial origin surfactant [5,6]. In the absence of surfactant spectral analysis method, exactly for evaluating the surface active properties of microorganisms it was used emulsifying activity index, which is based on the property of a surfactant to form an emulsion by shaking a cultural medium or cells of microorganisms with hydrocarbon or oil [7].

As the data show, emulsifying index is from 55 to 85%, therefore, the studied liquid cultural strains of bacteria of the genus Bacillus, isolated from permafrost soils and paleomicroflora possess emulsifying properties.

Thus, our results are consistent with literature data that strains of bacteria of the genus Bacillus produce effective organic surfactants and can be used for cleaning oil pollution from ecosystems [7,8,9,10].

High efficiency is achieved in the field experiments: the application of a suspension of strains of bacteria Bacillus subtilis Kolyma 7/2k at the rate of 250 ml/m^2, destruction of oil reached up to 0.64 ± 0.1 mg/g or was 99.53% for the three summer months. Use of a suspension of strains of bacteria Bacillus subtilisOimyakon 6/1 reduces oil content in soil by 23.8%. Less pronounced efficiency of strains of bacteria Bacillus subtilisOimyakon 6/1 compared with the Bacillus subtilis Kolyma 7/2k in the purification of oil pollution, possibly due to the fact that the experiments were set in the field season: June-July-August are the hottest months in Yakutia (the average temperature is + 28 °C). As the previous studies have shown, the strains of bacteria Bacillus subtilis Kolyma 7/2k more efficiently dispose hydrocarbons at a temperature of 28 °C, and a strain of bacteria Bacillus subtilisOimyakon 6/1 more actively "worked" at low positive temperatures (+4 °C).

Application of a combination of strains of bacteria Bacillus subtilis Kolyma 7/2k and Bacillus subtilisOimyakon 6/1, oil content decreased to 0.84 ± 0.2 mg/g or destruction of oil amounted to 99.38%. This may be due to the fact that microorganisms – destructors are capable to degrade various petroleum fractions, so their destruction efficiency increased when they were used together.

After oil contamination of soil the total bacterial count rises sharply (in 53.6 times), completely disappear spore forms of spore-forming aerobic bacteria. On a nutrient medium used for determining the total microbial count, the colony grew, which is corresponded to spore growth of aerobic bacteria by cultural properties. Microscopic examination of the colonies has detected the Gram-positive movable bacilli, which subsequently formed spores, and possessed the typical physiological and biochemical properties of aerobic spore-forming bacteria of the genus Bacillus.

Bacilli are found in soil at spore state or in the form of vegetative cells. When running out of stocks of easily moving organic matter a significant amount of vegetative bacillus cells form spores. Soil enriched with fresh forms of organic compounds causes transition of spores into vegetative cells [11].

Thus, completely disappeared aerobic spore-forming bacteria in the spore (stationary) form, and simultaneously increased the number of these bacteria in vegetative form. Spore-forming aerobic bacteria actively produce biologically active substances in the vegetative form [12]. Therefore, after the oil pollution in soil sharply increases the number of spore-forming bacteria of the genus Bacillus in an active vegetative form.

4. Conclusions

The obtained research results allow us to conclude that the spore-forming aerobic bacteria of the genus Bacillus, dominating in the microbiocenosis of permafrost soils, have hydrocarbon-oxidizing properties and participate in the process of self-purification of permafrost soils from oil pollution.

Based on the results of field experiments, it can be concluded that the additional introduction of effective, native hydrocarbon-oxidizing bacteria of the genus Bacillus not only strengthened, but also accelerated the natural process of self-purification of the soil from oil.

The identification and certification of bacterial strains of Bacillus subtilis capable of restoring oil-contaminated soils are consistent and complement the work of authors who have noted the possibility of using microorganisms for bioremediation of ecosystems [1, 7-10].

Based on the conducted studies, bacterial strains of Bacillus subtilis "Kolyma 7/2k", and Bacillus subtilis "Oymyakon 6/1" were certified and deposited at the All-Russian Research Institute of Agricultural Microbiology (all-Russian Research Institute of Agricultural Microbiology, St. Petersburg), which can be used by other researchers in improving methods of restoring not only oil-contaminated permafrost soils, but also other lands. The developed method can be successfully used for bioremediation of soils contaminated with oil and petroleum products.

The scientific novelty of the development was confirmed by obtaining patents of the Russian Federation "Method of cleaning permafrost soils from oil by spore-forming bacteria Bacillus Subtilis" (№. 2446900, 2012) and "Method of bioremediation of oil-contaminated permafrost soils" (№. 2538125, 2014).

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