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The isolation of natural microorganisms degraders of chlorinated compounds

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Abstract
Bacterial strains isolated from soils contaminated by organochlorine pesticides were screened. Bacterial strains capable of growing on mineral media with organochlorine pesticides - the gamma isomer of hexachlorocyclohexane and dichlorodiphenyltrichloroethane as the only carbon source were identified. The degradation processes of hexachlorocyclohexane by monocultures of isolated bacterial strains were studied. Four strains of them per-formed effective destruction of hexachlorocyclohexane when it was contained in the nutrient medium at a concentration of 100 micrograms/ml. It is shown that monocultures of four bacterial strains almost completely destroyed hexachlorocyclohexane within one month. Based on the study of taxonomic attributes of active bacterial strains, three of them are assigned to the species Bacillus subtilis, one to the species Micrococcus roseus.

Keywords: bacteria, pesticides, hexachlorocyclohexane, dichlorodiphenyltrichloroethane, lindane, destruction

Introduction
One of the major current environmental problems is the contamination of natural objects by pesticides, including organochlorine pesticides, which are highly toxic and persistent. Pesticides deteriorate the quality of soils and have a harmful effect on living organisms, primarily on microorganisms that are involved in soil-forming processes.
Self-cleaning of soil from pesticides occurs as a result of chemical, physical and biological processes [5]. In recent years, in the world literature and practice, microbiological degradation of persistent compounds is recognized as one of the most promising, since it is considered the safest and most cost-effective method of utilization [2; 8; 3; 1]. Many scientists have identified various microorganisms (bacteria, algae, microscopic fungi) that decompose pesticides to non-toxic compounds. The advantage of using microbiological methods of destruction of pesticides is explained by the fact that microorganisms mineralize pesticides and products of their metabolism in the natural cycle of substances. Numerous forms of microorganisms have the ability to use chemicals in the structural and energy metabolism of the cell [11]. As a result of exchange processes, the soil is not only cleared of xenobiotics, but also increases soil fertility and restores its main functions [10].
Many researchers [4; 9] claim that there are no forms of organic natural and artificial compounds that could not be used as food sources for certain forms of microorganisms. Usually, bacteria such as Bacterium, Bacillus, Pseudomonas, Actinomyces, Agrobacterium, Sphingomonas, Desulfotomaculum, Hydromonas and others are used for cleaning [6; 7].
The most common method of soil remediation contaminated by pesticides is to select a culture of a microbial destructor, accumulate biomass and mix it with the soil in places where xenobiotics are present. However, their effectiveness is not always equally high, since many cultures "work" only in a relatively narrow range of conditions. In addition, sometimes there is a degeneration of microorganisms before reaching the required level of purification. Their use can disrupt natural biocenoses.
A large number of banned pesticides remain on the territory of the Republic of arakalpakstan (Republic of Uzbekistan) to this day, despite the fact that their use in the region was discontinued in the 1980s. They are concentrated on the territory of former agricultural aerodromes, warehouses for storing pesticides and on a poison dump. Particularly dangerous organochlorine pesticides-hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT), which are persistent and cumulative, are of great concern. Pesticide residues accumulated in one place in large quantities pose a serious threat to the environment and living organisms, as they are transported outside the territory.

Unfortunately, there are currently no effective technologies for cleaning soil with a high level of pollutants in the Republic. In this regard, the disposal of organochlorine preparations from the most polluted natural objects is an important task of the region.

Equally important is the extreme condition of Karakalpakstan, such as high salinity, insolation, high dryness, and low humus content of soils, which complicates the process of purification by isolated destructive microorganisms. For this purpose, it is advisable to use natural microflora adapted to the conditions of the studied region. As a result, their survival rate in the extreme zone and efficiency in the destruction of pesticides increases.

The purpose of this work is to identify the most promising strains of bacteria that can actively degrade organochlorine pesticides HCH.

Materials and Methods

During of work, we use nutrient media and chemical reagents from High Media (India). A plot of the Kegeyli district of the Republic of Karakalpakstan that has been contaminated by pesticides for long time, including organochlorine, was chosen as the object of investigation. The isolation of destructor strains was performed from soil samples containing 0.1 mg/kg of lindane (the γ-isomer of HCH) by direct exposure to meat-peptone agar with a concentration of technical HCH of 100 mg/l. The toxicity of technical HCH in relation to isolated cultures was determined by the growth of cultures on Petri dishes on meat-peptone agar with the addition of drugs in concentrations from 250 to 1000 mg/l (250, 500, 750 and 1000 mg/l). Nutrient media were autoclaved at 1 ATM in a 30-minute. The growth of culture was taken into account starting from 18 hours of incubation. As the only source of carbon and energy in the environment, lindane was used as a pure chemical compound at a concentration of 20 microgram/ml. Preparations introduced separately into the nutrient medium in Petri dishes before bottling in the form of a hexane solution. The control was the initial environment without lindane. The bacteria were incubated in thermostats at 30 °C for 30 days. The growth of colonies was recorded every 24 hours of culting.

The ability of cultures to utilize lindane and DDT as the only source of nutrition was studied by sowing of pure cultures on a solid synthetic medium M-9 of the following composition (g /l): $Na_2HPO_4{}^xH_2O$–17; KN_2RO_4–3.0; NaCl–0.5; NH_4Cl–1.0, agar–20; pH 7.0.

The destruction of lindane was determined by growing bacterial strains in meat-peptone broth in a thermostat at a temperature of 28 °C, bringing the culture titer to 10^8 CFU/ml. Then, lindane was added to the broth at the rate of 100 mcg/ml and incubated in a thermostat for 1 month at a temperature of 280 °C. The concentration of lindane was determined using a gas chromatograph AT 6890 from Agilent Technologies (USA) using a calibration curve in the ChemStation software. Chromatography conditions: initial temperature: 100 °C, then the temperature rose to 250 °C at a speed of 10 deg/min and was kept at this temperature for 15 min. The total analysis time is 30 minutes. The evaporator operated in the mode without dividing the flow at a temperature of 28 °C. The device used a carrier gas-nitrogen at a speed of 1.5 ml/min. Separation was performed on a quartz capillary column of the Scienti DB-608 model with a cross-linked methylsilicon fixed liquid phase. The column was 30.0 m in length and 0.53 mm in diameter. The micro ECD operated at a temperature of 30 °C with a constant blowout of 45 (ml/min).

Results and Discussion

As a result of direct sowing of soil suspensions, about 130 native cultures of microorganisms were isolated, which differed from each other in a complex of morphological and cultural characteristics. Further, 10 bacterial strains were selected by transferring to meat-peptone agar containing technical HCH in concentrations of 250 500, 750 and 1000 mg/l, which were conventionally designated by an ordinal number from 1 to 10.

Chromatographic analysis of the study of the content of pesticides in water, soil, dust and bottom sediments of the model site revealed the predominance of organochlorine compounds, which were base on HCH and DDT and their derivatives. In this regard, the ability of selected pure bacterial cultures to assimilate compounds similar in chemical structure, namely HCH and DDT, was determined. For this purpose, experimental bacterial cultures were sowed on a solid mineral medium M-9 and as the only source of carbon in one version, HCH was added and in the other, DDT at a concentration of 20 micrograms/ml.

The results of the experiment showed that on the synthetic medium with HCH on the third day after sowing, the growth of strains № 2, 7 and 9 was observed, and in the case of DDT - strains № 5, 6 and 7. On the fifth day, the growth of strains was observed № 3, 4, 5, 6, 8 and 10 on a medium with HCH and strains № 3 and 4 on a medium with DDT. On the seventh day of cultivation, the growth of strains 2 and 10 was detected. During the experiment, no growth of strains № 1, 8 and 9 was recorded on the synthetic medium with DDT and strain 1 on the medium with HCH. Thus, seven of ten selected strains are able to absorb both HCH and DDT in a synthetic medium without additional organic substrate.

In the next series of experiments, we studied the destructive activity of bacteria isolated in pure culture growing on the medium in the presence of lindane. Lindane is the gamma-isomer of HCH, which determines its toxicity, which is actually effective in agriculture as an insecticide. The ability of the monoculture of bacteria to degrade lindane was performed on a gas chromatograph. The results of the chromatogram showed that within 30 days the process of destruction by bacterial cultures proceeded at different rates. Within a month, only four tested cultures from ten caused active destruction of lindane in the nutrient medium. By the end of first month, complete destruction of the pesticide was observed by culture № 4, 5, 7 and 10 (Fig.1).

Destructive activity of other cultures (№ 1, 2, 3, 6, 8 and 9) was low, since there was a very slight decreas in the concentration of lindane (5-10%) during the given period.

Thus, the chromatographic analysis gives a basis to believe that the cultures of soil bacteria № 4, 5, 7 and 10 have a high gamma-HCH-destructive activity and are able to degrade lindane in the nutrient medium within one month.

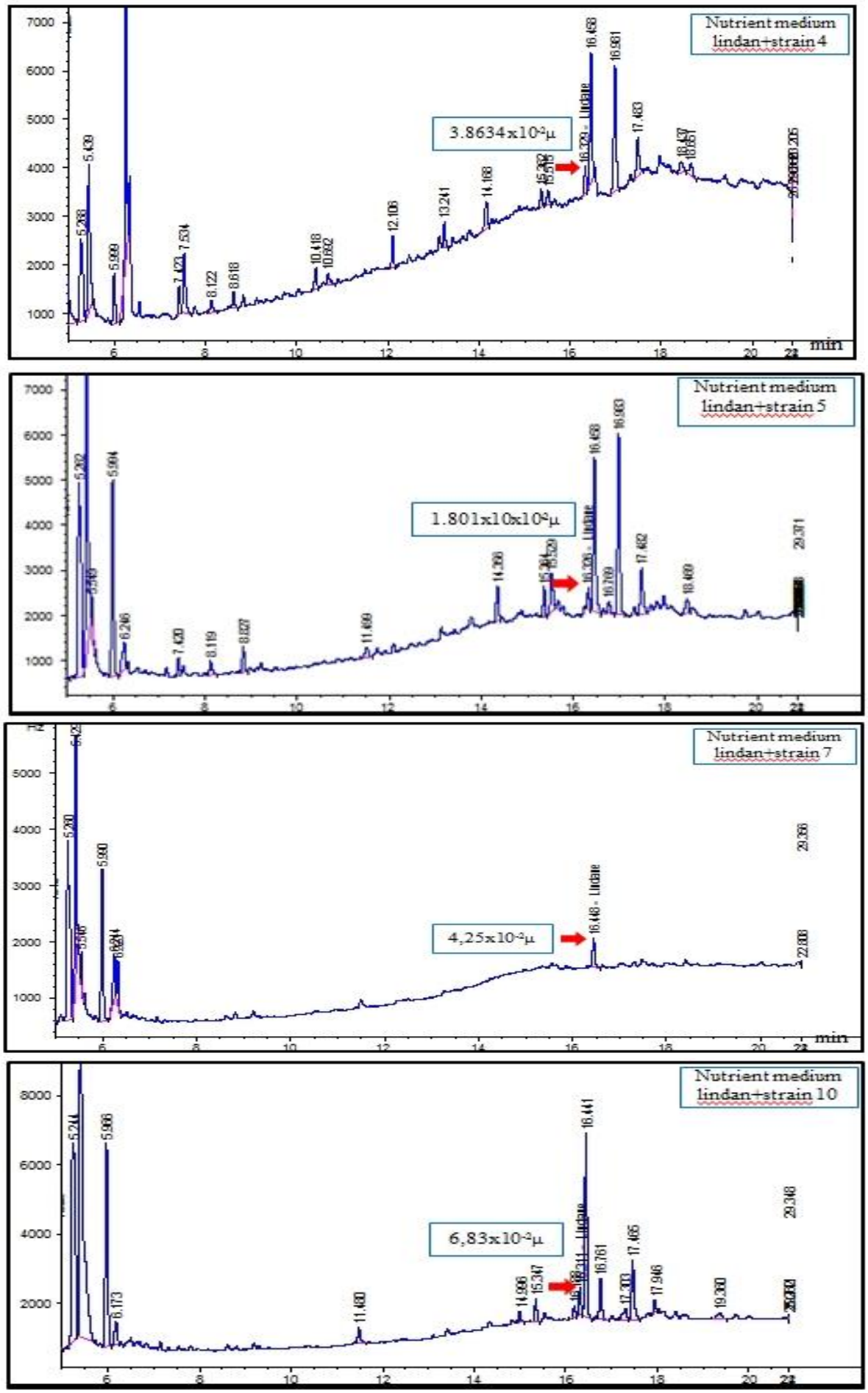

Fig 1: Chromatographic analysis of destruction of lindane in the culture medium of bacteria 4, 5, 7 and 10

The obtained results of research allowed us to select the most active bacterial cultures that are promising for further research. Subsequently, all four active destructor cultures were selected for species identification. The results of determining the taxonomic position of the selected strains showed that cultures № 4, 5 and 7 belong to genus of Bacillus, the species of Bacillus subtilis, culture № 10 – to genus of Micrococcus, the species of Micrococcus roseus.

Conclusion

Based on the results obtained, it can be concluded that bacterial strains capable of using organochlorine pesticides HCH and DDT as the only carbon source and have high destructive activity with respect to the gamma-isomer of HCH have been isolated and identified from the soils polluted and saline by pesticides.

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