

Biodegradation of pesticide complex chlorpyrifos + cypermethrin by microorganisms isolated from the Southern Aral Sea region

Abstract

Microbiological study of soil samples with low pesticide content was conducted. Seven pure cultures were isolated: four related to *Bacillus* and three to *Pseudomonas* genera. Conducted study analyzing growth and biochemical activity of natural cultures on synthetic media revealed ability of two cultures (*Bacillus* sp. 2 -30 and *Bacillus* sp. 1 -17) to degrade complex of pesticides chlorpyrifos+cypermethrin.

Keywords: selective transfer, superficial layer, structural analysis, intensity x-rays, width of diffraction lines, crystalline network constant

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Introduction

Anthropogenic natural disaster of the Aral Sea and decreasing outflow of rivers Amudarya and Syrdarya caused a number of irreversible changes in soil microbial communities in the Southern Aral Sea region. Common features of these soils is their high salinization, extreme instability of humidity level up to total draught, and high content of different xenobiotics.^{1,2} Soil borne microorganisms are one of the main factors controlling degradation of xenobiotics in the natural ecosystem and essential bioindicators reflecting changes in soil, which is related to their enormous importance in soil formatting processes. There is possibility of emergence of strains in microbial populations of such soils that are capable to active degradation of pesticides.^{3,4} Such cultures, undoubtedly, are perspective for rehabilitation of natural environments.⁵ The approach engaging microorganisms-bi destructors for rehabilitation of soils polluted with agrochemicals has certain efficiency and effectiveness. Indigenous strains of soil borne microorganisms possessing both ability to destroy pesticides and natural resistance to the complex of pollutants and sharp changes of climatic conditions stipulate importance and feasibility of this approach. In these regards, the microbial biota of the Southern Aral Sea region, characterized by extreme natural conditions, was studied. Different microorganisms were isolated and their ability to destroy pesticides was determined.

Materials and methods

Soil samples with low pesticides content were used as an object of

study. A mixture of pesticides chlorpyrifos+cypermethrin was used for determination of pesticides degradation degree by microorganisms. Number of microorganisms in 1g (1ml) of substrate was determined by the method of serial dilutions with cultivation of solid and liquid nutrient media.⁶ Preliminary identification of bacteria was conducted according to Bergey's manual.⁷ Protein was determined according to Lowry.⁸ Study of pesticides degradation was conducted on synthetic liquid nutrient medium M9 with addition of pesticides mixture, final concentration of which was 50/5 g/l chlorpyrifos/cypermethrin. Determination of pesticides in samples was conducted by gas-liquid chromatography in the chemical lab of the Center of Hydro meteorological Service at Cabinet of Ministers of the Republic of Uzbekistan.

Results and discussion

Results of conducted study revealed that in salinized soils with residual concentrations of pesticides determined quantity of sporogenous, ammonifying and oligonitrifying microorganisms considerably decreases, denitrifying bacteria and Actinomycetes are practically not isolated. The total number of bacteria isolated on beef extract peptone agar (BPA) is relatively considerable for such soils (Table 1). Bacterial biota is represented both by non-sporogenous bacteria from genus *Pseudomonas* and by bacillary forms of microorganisms. Presence of sporogenous microorganisms practically in all studied samples should be noted separately.

Table 1 Microbiological analysis of soils with residual concentrations of pesticides

Soil sample	Number of microorganisms, CFU/g					
	BEA	Ammonifying	Sporogenous	Oligonitrifying	Denitrifying	Azotobacter
1	7.5x105	2.0x103	3.4x102	3x102	2.5x102	Single cells
2	3.8x101	6.0x102	2.5x10	2.8x102	-	-
3	5.0x105	6.0x102	2.1x102	5.6x102	-	Single cells
4	3.3x105	2.5x103	6.0x102	1.8x103	-	-
5	2.6x105	6.0x103	1.8x102	3.6x102	6.0x101	-

Out of studied soil samples the most often isolated species of bacteria were selected and received in pure cultures (multiple passages and analysis of growth of corresponding nutrient medium). As result, seven pure bacterial cultures were received. On basis of conducted study of morphological and cultural and physiological and biochemical features four of them were identified as *Bacillus* and three as *Pseudomonas* species. Despite of many literature sources, data on pesticides' impact on soil borne microorganisms often contradictory. Different variations of suppression, activation and indifferent relation towards chemicals are determined among potentially possible reactions of soil borne microorganisms to pesticides' presence in environment and in processes involving their participation. In these regards, experiments on impact of pesticides on growth and vital functions of microorganisms participating in their degradation were conducted. Analysis of growth and biochemical activity of sporogenous cultures at cultivation on synthetic medium revealed univocal activity in several times exceeding control indices, which testifies digestion of pesticides as the only source of energy. Besides, considering indices on protein synthesis, it is necessary to note increased protein content on 7th day of cultivation on synthetic medium, while maximal indices of protein biosynthesis are observed on 14th day (Figure 1). Therefore, such indices of biochemical activity testify ability of bacilli cultures to use pesticides as a source of nutrition. Conducted chemical analysis confirms suggested propositions. Degradation of pesticides for strain *Bacillus sp.* 2-30 reached 93.4%, whereas strain *Bacillus sp.* 1-17 degraded mixture of pesticides to 97% by 14th day.

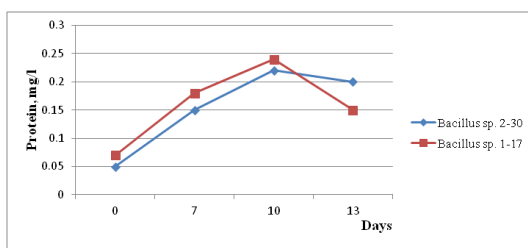


Figure 1 Dynamics of protein production by bacilli microorganisms on synthetic medium.

Conclusion

As result of the conducted research, different bacterial cultures were isolated from mixed populations of soil biota exposed to long-term

impact of anthropogenic and natural factors. Obtained results allow to conclude that microbiota of studied soils is diverse. Detailed analysis of microbial diversity reveals that organizing role in microbiocenoses belongs to bacteria from *Bacillus* and *Pseudomonas* genera. Study of growth and biochemical activity of natural cultures cultivated on synthetic media determined possibility of strains *Bacillus sp.* 2 -30 and *Bacillus sp.* 1 -17 to degradation of pesticide complex.

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Conflict of interest

None.

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