ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (Ph.D.) on the specialty "8D05105 – Biotechnology"

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on the thesis: "Development of technologies for the use of destructors - microorganisms for bioremediation of soils contaminated with persistent organic pollutants"

General description of work. The dissertation work is devoted to the microbial diversity of soil samples contaminated with persistent organic pollutants and the screening of microorganisms-destructors in settlements of the Almaty region, Talgar region, as well as the creation of a consortium based on destructor strains effective for the bioremediation of contaminated soils.

Relevance of the research topic. Currently, large-scale pollution of the environment with persistent organic compounds is one of the most important environmental problems. Over the past 30 years, more than 700 pesticides belonging to various classes of chemical compounds have been used in Kazakhstan and countries adjacent to the CIS. During 2013-2022, about 1021 types of pesticides were registered and approved for use on the territory of the Republic of Kazakhstan, and the list of pesticides is updated annually with 15-20 new drugs. Organochlorine pesticides are often used as insecticides, fungicides and herbicides in agriculture.

Within the framework of the United Nations Development Program/Global Environment Facility (GEF) project “Providing initial assistance to the Republic of Kazakhstan to fulfill obligations under the Stockholm Convention on Persistent Organic Pollutants”, based on the results of a preliminary inventory of persistent organic pollutants in Kazakhstan, 727 warehouses were identified and 15 pesticide disposal sites.

Persistent organic pollutants are xenobiotics that persist in the environment for a long time and, due to the mechanism of semi-decomposition, have a negative impact on human health through the food chain. Currently, more than 20 organochlorine pesticides are included in the list of persistent organic compounds. Based on the results of studying the effects of pesticides on the human and animal body, it has been shown that reproductive disorders, congenital anomalies and pathological changes, dysfunction of the immune system, neurological diseases and malignant tumors develop. In recent years, international attention has focused on the widespread occurrence and persistence of persistent organic pollutants in the environment, their high potential for bioaccumulation and biologically harmful effects.

The bioremediation method, based on the destructive properties of microorganisms, is of great importance when cleaning environmental objects from persistent organic pollutants. In the bioremediation of contaminated soils, microbiological destruction based on the diversity of microorganisms is one of the environmentally friendly and cost-effective methods. The metabolic mechanisms of destructive microorganisms are capable of converting stable organic compounds into compounds with relatively low toxicity due to their high sensitivity to environmental factors, resistance to adverse conditions and enzymatic activity.

The relevance of the topic lies in the study of the microbiocenosis of soils contaminated with pesticides, the physiological, biochemical, genetic characteristics of destructive microorganisms and the mechanisms of biotransformation of stable organic compounds.

The aim of the work: Study of the microbial diversity of soils contaminated with persistent pollutants, screening of promising microorganisms-destructors and development of a technological scheme for using a consortium of microorganisms-destructors in the bioremediation of contaminated soils.

To achieve this aim, the following tasks were solved:

1. Chemical analysis of soils contaminated with persistent organic compounds in the Talgar district of the Almaty region;
2. Metagenomic analysis on the MiSeq NGS system from Illumina and microbiological study of soil microbiocenosis, isolation of pure cultures of indigenous microorganisms;
3. Study of morphological, cultural, physiological and biochemical properties of the isolated strains and molecular genetic identification;
4. Study of the mechanisms of destructive activity of promising strains and screening of destructive strains;
5. Creation of a consortium based on the biocompatibility of destructor strains and determining the degree of degradation of organochlorine pesticides;
6. The study the activity of destructor strains during the breakdown of organochlorine pesticides in model experiments. Development of a technological scheme for bioremediation of soil contaminated with persistent organic compounds based on a consortium of strain-destructors.

The object of study. The objects of study were soil samples from the villages of Kyzylkairat, Beskainar, Amangeldi No. 1, Amangeldi No. 2, Belbulak, Basshi of the Almaty region, Talgar region and pure cultures of microorganisms isolated from these samples.

Research methods. During the dissertation work, experiments were carried out in laboratory conditions using the gas chromatographic method of chemical analysis Agilent Technologies 6890N, the modern MiSeq NGS metagenomic analysis system from Illumina, traditional microbiological, molecular genetic, scanning electron microscopy (SEM), fluorescence spectroscopy, IR spectroscopy, methods Fourier spectrometer ALPHA II.

Scientific novelty of the research.

For the first time, modern metagenomic analysis MiSeq NGS Illumina was carried out on soil samples contaminated with persistent organic compounds in the villages of Kyzylkairat, Beskainar, Amangeldi No. 1, Amangeldi No. 2, Belbulak, Basshi in the Republic of Kazakhstan, Almaty region, Talgar region, the microbial diversity was dominated by the following bacterial phyla: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria, Planctomycetes, Chloroflexi, Gemmatimonadates, Saccharibacteria, Firmicutes, Verrucomicrobia,* and 68-80% members of the family *Bacillus, Pseudomonas* were found.

During the research work, 40 strains were isolated from soil samples contaminated with persistent organic compounds, their morphological, cultural, physiological, biochemical and destructive properties were studied, and 10 active strains were selected.

A phylogenetic analysis of promising destructive strains was carried out and identified to species: *Pseudomonas plecoglossicida K2, Bacillus aryabhattai K3, Solibacillus isronensis KS1, Pseudomonas sp. KS2, Bacillus pumilus B1, Bacillus amyloliquefaciens B2, Bacillus subtilis AK5, Pseudomonas koreensis AK1, Bacillus megaterium AS1, Bacillus paramycoides SA1*.

The mechanisms of destructive activity of promising strains are based on their enzymatic activity: it has been established that destructive strains produce enzymes protease, laccase, catalase, cellulase, dehydrogenase, capable of breaking down organochlorine pesticides.

To increase the efficiency of the breakdown of persistent organic compounds, consortia based on promising strains were constructed. The consortium *Pseudomonas plecoglossicida K2 + Bacillus aryabhattai K3* was found to degrade organochlorine pesticides by 55%, and *Bacillus pumilus B1 + Bacillus amyloliquefaciens B2* by 64%. Based on research results, it has been established that promising strains can break down stable organic compounds into alicyclic compounds, cycloalkanes (-CH_2), aromatic heterocyclic compounds (C=C, COO-) and part of the aliphatic compounds into alkyl groups (-CH_3 and -CH_2).

Based on the results obtained in the research work, a technological scheme for the use of consortia based on destructor strains in the bioremediation of soils contaminated with persistent organic pollutants has been proposed.

Provisions submitted for protection:

Destructor strains of *Pseudomonas plecoglossicida K2 (OK217230), Bacillus aryabhattai K3 (MW866565), Solibacillus isronensis KS1 (OK236011), Pseudomonas sp. KS2 (OL348382), Bacillus pumilus B1 (OL348383), Bacillus amyloliquefaciens B2 (OL348394), Bacillus subtilis AK5 (MW866566), Pseudomonas koreensis AK1 (OL348403), Bacillus megaterium AS1 (OL348404), Bacillus paramycoides SA1 (OL34 8439)* have been identified to species and are registered in the Genbank database of the National Center for Biotechnology Information (NCBI). New strains were included in the collection of the laboratory of applied microbiology of Kazakh National University. al-Farabi for use in research work.

In model experiments, the destructive activity of consortia based on destructor strains obtained in the work was studied, and it was found that consortium strains *Pseudomonas plecoglossicida K2 + Bacillus aryabhattai K3* 83%, *Bacillus pumilus B1 + Bacillus amyloliquefaciens B2* decompose 86% of organochlorine pesticides. The germination of wheat seeds in soils contaminated with pesticides in the mono strain *Bacillus aryabhattai K3* is 67–83%, in variants with a consortium of strains *Pseudomonas plecoglossicida K2 + Bacillus aryabhattai K3* 82–88%, *Bacillus pumilus + B1 Bacillus amyloliquefaciens B2* 87–94%.

Promising destructive microorganisms were selected from soil samples contaminated with persistent organic compounds, their destructive properties were studied and patents were obtained: “Method of microbiological destruction of pesticides”, (No. 34115, 01/09/2020), “Method for microbiological decomposition of organochlorine pesticides (No. 7731, 13/01/2023). For the bioremediation of soils contaminated with persistent organic compounds, consortia based on destructor strains have been proposed.

The results of research work can be used as material for theoretical and practical courses for students and masters, doctoral students of higher educational institutions.

Provisions for defense:

1. High amounts of organochlorine pesticides 4,4-DDE, 4,4-DDT, α-HCH, β-HCH and γ-HCH were found in soils contaminated with persistent organic pollutants in the Talgar district of the Almaty region. In soil samples, the concentration of organochlorine pesticides was Kyzylkairat – 121,054, Beskainar - 47,334, Amangeldi No.1-5382, Amangeldi No.2-1032, Belbulak - 1025, Basshi - 146 micrograms/kg^{-1}, respectively.

2. In soil samples contaminated with organochlorine pesticides, bacterial phyla (types) *Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Planctomycetes, Chloroflexi, Gemmatimonadates, Saccharibacteria, Firmicutes, Verrucomicrobia* predominate, including representatives of the genus Bacillus, Pseudomonas accounted for 68–80%.

3. Active strain destructors *Pseudomonas plecoglossicida K2 (OK217230), Bacillus aryabhattai K3 (MW866565), Solibacillus isronensis KS1 (OK236011), Pseudomonas sp. KS2 (OL348382), Bacillus pumilus B1 (OL348383), Bacillus amyloliquefaciens B2 (OL348394), Bacillus subtilis AK5 (MW866566), Pseudomonas koreensis AK1 (OL348403), Bacillus megaterium AS1 (OL348404), Bacillus paramycoides SA1 (OL34 8) 439)* were identified to species and registered in the NCBI database.

4. The destructive activity of promising strains against organochlorine pesticides is provided by the enzymes protease, laccase, catalase, cellulase, and dehydrogenase.

5. Based on the biocompatibility of destructor strains, 2 consortia were constructed. The consortium based on strains *Pseudomonas plecoglossicida K2 + Bacillus aryabhattai K3* biodegrades organochlorine pesticides by 84%, and the consortium *Bacillus pumilus B1 + Bacillus amyloliquefaciens B2* by 86%. Consortia based on promising strains for the bioremediation of soils contaminated with persistent organic compounds have been proposed.

The connection of the dissertation work with scientific research. The dissertation work was carried out within the framework of the program of the Institute of General Genetics and Cytology, project IRN BR05236379 “Comprehensive assessment of the impact of non-recycled and prohibited pesticides on the genetic status and health of the population of the Almaty region: Determination of the microbial diversity of the territory adjacent to the burial sites of pesticides, screening, isolation of destructor - microorganisms."

Personal contribution of the dissertation candidate to the creation of the results of the scientific work proposed for defense.

The results of the research work, review of literature data, determination of the goals and objectives of the work, experimental research, statistical processing and analysis of the results were carried out with the personal participation of the author.

Approbation, approval of the results of the work and personal contribution of the author. The main results of the dissertation work were presented and discussed at the following international scientific conferences and symposia:

1. 2nd International Conference on Renewable Energy and Environmental Engineering (30 October 2019);

2. International scientific conference of young scientists “Fundamental research and innovation in molecular biology, biotechnology, biochemistry”, dedicated to the 80th anniversary of academician Murat Aitkhozhin (November 28–29, 2019);

3. VII MATERIALS International Scientific Conference of Students and Young Scientists «FARABI ALEMI» (Almaty, April, 6 – 9, 2020);

4. Italian scientific journal Annali d'Italia, 2020;

5. II International book publication of the Commonwealth of Independent States “Best young scientists - 2020” (Astana, Kazakhstan, September 28, 2020);

6. MATERIALS International Scientific Conference of Students and Young Scientists «FARABI ALEMI» (Almaty, April 6–8, 2021);

7. International scientific and practical conference “Modern problems of biotechnology: from laboratory research to production”, (June 4–5, 2021, Almaty, Kazakhstan);

8. IX MATERIALS International Scientific Conference of Students and Young Scientists «FARABI ALEMI» (Almaty, April 6–8, 2022);

9. MATERIALS International Scientific Conference of Students and Young Scientists «FARABI ALEMI» (Almaty, April 6–8, 2023);

Publications. The materials obtained in the dissertation were published in 27 published scientific papers, including 2 articles in peer-reviewed foreign scientific publications; 1 article in international peer-reviewed scientific journals indexed in the *Web of Science and Scopus* databases, included in the Q2 quartile with a non-zero impact factor; 1 article at an international scientific conference with a CiteScore percentage of at least 25, indexed in the *Web of Science and Scopus* databases; 4 articles in republican scientific publications recommended by the Committee for Control in the Sphere of Education and Science of the Republic of Kazakhstan; 13 theses were published in the proceedings of international conferences. Based on the results of the dissertation work, the invention passed the official examination and 2 patents and 2 copyright certificates of the Republic of Kazakhstan were received.

Structure of the dissertation. The dissertation consists of 173 pages, introduction, literature review, objects of research, materials and methods, research results and their discussion, final sections, 265 bibliographies, 9 tables, 55 figures.