SCREENING OF SALT-TOLERANT MICROORGANISMS FROM THE SOIL OF A FORMER PESTICIDE STORAGE IN MIRZABAD DISTRICT

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Abstract. *More than 20 strains of persistent cultures of microorganisms were isolated from the soil of a former pesticide warehouse in the Mirzabad district of the Syrdarya region. The prevailing concentrations were distinguished by 3 -bacterial cultures: Bacillus megaterium, Bacillus subtilus, Pseudomonas aeruginosa and 2 -actinomycete cultures: Streptomyces violaceorubidus, Streptomyces rochae. The isolated cultures were resistant to high salt concentrations, such as NaCl and* Na_2SO_4*. It was found that at concentrations of 10-15%, added to the nutrient medium of NaCl and* Na_2SO_4 *salts, the selected cultures had the ability to grow with the formation of a sufficient concentration of biomass in the culture medium.*

Keywords*: contaminated soils, microbial landscape, bacteria, actinomycetes, enzymes, heavy salts, adaptation, growth and development.*

Introduction

Salt excess is one of the abiotic stressors that can significantly affect the microbial communities of “living” soil and their stability, the biodiversity of soil microorganisms and biological creatures, therefore, primarily affect soil fertility, the productivity of cultivated crops, crop quality and the biological safety of food due to a decrease in plant growth and productivity [1]. Irrigated soils in arid and semi-arid areas, naturally, may have increased types of primary and secondary salinization.

However, an even more serious problem is soils that are saline by nature, as well as those generated as a result of unsustainable methods of managing soil resources using “salt leaching” methods, causing increased salinity [2]. This unsustainable practice includes the use of contaminated irrigation water, which may be of poor quality (for example, containing harmful sediments, ions of various metals, including heavy ones, soil erosion, chemical pollution as a result of excessive use of mineral fertilizers, various pesticides, etc.). Improper irrigation methods, poor drainage, and removal of deep-rooted plants have a direct impact on soil fertility. Therefore, the FAO organization (Food and Agriculture Organization of the United Nations) recognizes salinity as one of the most pressing problems at the global level, not only in the field of agriculture, food security, but also the sustainability of their development in arid and semi-arid regions of the world and the economy [2].

Therefore, to solve these problems, the use of natural (organic) metabolites of microbial origin is the most technological approach, which is currently gaining increasing popularity among researchers, allowing to solve key problems associated with the use of microorganisms (cells, associations, endophytes, biospheres, symbionts. mycorrhiza-forming, etc.) to enhance the joint, synergistic growth of plants, especially agricultural crops under conditions of salt stress. This new, organic biotechnology represents a promising, highly effective approach to mitigating the effects of soil salinity on crops, given that these compounds (microbial metabolites) may be less susceptible to salt stress and are required in smaller quantities. For example, some scientists note that compounds derived from microorganisms, such as thuricin-17, lipochitooligosaccharides, phytohormones, and volatile organic compounds, mitigate the effects of salt stress in crops such as soybeans and wheat [3].

The FAO organization in 2020 reported that out of 230 million hectares of irrigated land in the world, 45 million hectares were affected by salinity, and the economic damage and consequences of salinity worldwide are estimated at approximately 12 billion US dollars/year [4]. Researchers report that salinity affects approximately 1 billion hectares of land worldwide, which is about 7% of the planet's total surface area [5].

Thus, soil salinity is a global problem in agricultural production, especially in arid and semi-arid regions where crop production is highly dependent on irrigation [6].

In agricultural practice, soil salinization is understood as the accumulation of excess concentrations of water-soluble salt cations (Na+, K+, Mg2+ and Ca2+) and anions (Cl-, SO2-4, HCO3-, NO-3, NO3- and CO2-3) especially in the root soil. zone of plants, harmful not only to the development of the rhizosphere, but also to rhizosphere microorganisms [7].

In saline soils, microbial communities associated with the rhizosphere, phyllosphere and endosphere of halophytes include members of the domains Archaea and Bacteria and the kingdom Fungi, which are directly or indirectly involved in the osmoregulation of halophytes, allowing them to survive under salt stress [10]. Endophytic bacteria and fungi, the life cycle of which partially or completely takes place inside the plant, can live in the intercellular spaces of various plant tissues and organs [8], but without causing visible external signs of infection or negative effects from the endophytic host [9].

Despite the fact that soil is a valuable, stable and non-renewable ecological system, it has always been subject to large-scale degradation due to human activities and, in some cases, natural phenomena. To eliminate pollution, “death” of the soil, and restore biogenicity, increasing efforts are being made to replace environmentally harmful technologies with green (use of microbial biotechnology) and sustainable solutions aimed not only at eliminating or reducing pollution and salinization, but also at minimizing their impact on the environment.

Based on this, the purpose of this work is to study the condition of soil contaminated with pesticides by determining the various classes of soil microorganisms that survive under these conditions, identifying among them the dominant salt-tolerant representatives of bacteria, fungi and actinomycetes to develop methods for bioremediation of saline soils by creating biological products based on them.

MATERIALS AND METHODS

The objects of the study were soil samples taken from various horizons (0-15, 15-30 cm) of the territory of the former pesticide warehouse in the Mirzabad region, as well as the soils of former agricultural aviation airfields in the Sirdarya region. To isolate soil microorganisms and determine the microbial landscape, nutrient media and methods were used, described in the manuals of Egorov A.E. and Netrusova. A.I. [11].

The following nutrient media were used in the work: **MPA** medium for determining the total number of saprotrophic microorganisms, **MPB** for ammonifying bacteria, **endo** and **EE**

Broth for determining the total number of the Enterobacteriaceae bacterial family and produced by Hi Media.

To study fungi, the following media were used: **Czapek-Dox** (for isolating microscopic fungi) (g/l): $NaNO_3$ – 2; KH_2PO_4 - 1; KCl – 0.5; $MgSO_4 * 7 H_2O$ - 0.5; $FeSO_4 * 7 H_2O$ – 0.01; Sucrose – 30; Agar-agar – 20, the rest distilled water – 1000 ml.

The prepared nutrient media were sterilized at 0.7 atm for 60 minutes.

The number of microorganisms in liquid media was determined using the McCready table, and the number of CFU on solid media was calculated using the formula, at a 95% confidence level, as follows:

(P 0.95): (x ±2 σx-) •K• 1/V

where x= Σx / n - the average number of colonies grown when seeded from a given dilution; σx- = ±√ Σx / n - standard deviation; 2 - t – criterion at P 0.95; K - dilution from which seeding was carried out; V is the volume of suspension taken for inoculation, ml; Σx is the total number of colonies counted when sowing a given dilution; n is the repetition number.

The number of microorganisms in liquid media was determined using the McCready table. [11]. The pH of the medium was determined potentiometrically on “Mettler Toledo” pH meter.

Determination of the number of microorganisms in 1.0 g (1 ml) of soil suspension was carried out by the method of serial dilutions with seeding on solid and liquid selective media [12]. The number of microorganisms in liquid media was determined using the McCready table, and the number of CFU on solid media was calculated using the formula, with a confidence level of 95% (P 0.95): (¯x ±2 σx-)•K• 1/V,

Where x= Σx / n is the average number of colonies grown when seeded from a given dilution; σx- = ±√ Σx / n - standard deviation; 2 - t – criterion at P 0.95; K - dilution from which seeding was carried out; V is the volume of suspension taken for inoculation, ml; Σx is the total number of colonies counted when sowing a given dilution; n is the repetition number.

A comprehensive determination of these parameters makes it possible to more accurately determine the direction of changes in the activity of the enzymatic, namely biological pool of soil samples.

The species of isolated soil microorganisms was determined using a Maldi Top analyzer [13].

Results and conviction

While studying microorganisms and their composition found in soil samples taken from the territory of a former pesticide warehouse in the Mirzabad district of the Syrdarya region, the presence of 18 species of microorganisms dominant in number was discovered, among which the prevailing representatives were Streptomyces violaceorubidus, Streptomyces rochae, Kocuria rosea, Pseudoarthrobacter oxydans, Pseudomonas xanthomarina, Klebsiella pneumaniae, Arthrobacter crystallopoietes, Bacillus cereus, Citrobacter braaki, Providencia retgeri, Bacillus megaterium. Bacillus subtilus, Pseudomonas aeruginosa.

Further, isolated crops from soils contaminated with pesticides in the Mirzabad region were subjected to the study of salt tolerance using a nutrient medium containing chlorine and sulfate-containing salts in concentrations from 15% to 25% sodium chloride and sodium sulfate.

It was found that not all subjects from 13 cultures had the ability to grow on salt-containing media at concentrations of 15%, 20% and 25%. For example, cultures of the bacteria Bacillus megaterium, Pseudomonas aeruginosa and Bacillus subtilus developed and showed high survival rates in all concentrations of both salts. Also, the actinomycetes Streptomyces violaceorubidus and Streptomyces rochae showed similar results.

Table– 1.

Growth abilities of surface-grown crops of dominant representative's airfield soil microorganisms on media with some salts

№	**Name of crops**	**NaCl, %**			**Na_2SO_4, %**		
		15	**20**	**25**	**15**	**20**	**25**
1	Streptomyces violaceorubidus	+	+	-	+	-	-
2	Streptomyces rochae	+	+	-	+	-	-
3	Kocuria rosea	-	-	-	+	-	-
4	Pseudoarthrobacter oxydans	-	-	-	-	-	-
5	Pseudomonas xanthomarina	+	-	-	-	-	-
6	Klebsiella pneumaniae	-	-	-	+	-	-
7	Arthrobacter crystallopoietes	+	+	-	-	-	-
8	Bacillus cereus	-	-	-	-	-	-
9	Citrobacter braaki	-	-	-	+	+	-
10	Providencia retgeri	-	-	-	-	-	-
11	Bacillus megaterium	+	+	-	+	+	-
12	Bacillus subtilus	+	+	+	+	+	-
13	Pseudomonas aeruginosa	+	+	+	+	+	-

As it can be seen from the data in table 1, despite the fact that the culture of Citrobacter braaki did not have the ability to grow in all concentrations of **NaCl** saline solutions (15%, 20% and 25%), but showed positive results in survival and growth on media containing 15 % and 20% Na2SO4 salt (table 1).

The results obtained make it possible to use the abilities of stable cultures of these microorganisms to create microbial biotechnologies in the fight against salinity.

Taking into account the above data obtained, we then carried out a quantitative assessment of the growth of the tested salt-tolerant crops based on the accumulation of biomass in liquid media (MPB was used for bacteria, and Czapek-Dox media for actinomycetes) containing NaCl at a concentration of 1% - 2% - 4%. Cultures of actinomycetes Streptomyces violaceorubidus, Streptomyces rochae and bacteria Bacillus megaterium, Pseudomonas aeruginosa and Bacillus subtilus were subjected to analysis.

It turned out that the concentration of 1.0% NaCl salt has a positive effect on the cell number of all tested bacterial cultures. It was found that in Bacillus megaterium the number of growing cells in a liquid medium increased by 30%, Bacillus subtilus - by 48%, while in Pseudomonas aeruginosa - by 24% compared to control media without the addition of salts.

The salt concentration of 2.0% NaCl had a positive effect only on the growth of the Bacillus megaterium strain, in which the cell number increased by 7%. However, the remaining selected cultures at a concentration of 2.0% NaCl showed a reduced cell number by an average of 5.0 - 6.0%. At a concentration of 4.0% NaCl salt, the number of cells in the selected strains decreased, i.e. in Bacillus megaterium by 68.0%, Bacillus subtilus - by 27%, Pseudomonas aeruginosa - by 80% (Table - 2).

The study of salt tolerance in terms of numbers and biomass accumulation in actinomycetes showed that when NaCl was added to the cultivation medium at a concentration of 1.0%, an increase in the amount of biomass was observed: in the culture of Streptomyces violaceorubidus by 27% and Streptomyces rochae by 54.5%.

Table– 2.

Number of cells (CFU/ml) of salt-tolerant bacteria growing in MPB medium with NaCl

Bacteria	NaCl concentration; number of CFU cells/ml			
	1%	2%	4%	*Control
Bacillus megaterium	$52{,}52 \cdot 10^6$	$43{,}4 \cdot 10^6$	$13{,}2 \cdot 10^6$	$40{,}4 \cdot 10^6$
Bacillus subtilus	$61{,}2 \cdot 10^6$	$39{,}3 \cdot 9^6$	$26{,}1 \cdot 10^6$	$41{,}1 \cdot 10^6$
Pseudomonas aeruginosa	$53{,}64 \cdot 10^6$	$37{,}7 \cdot 10^6$	$9{,}1 \cdot 3^6$	$43{,}18 \cdot 10^6$

*Initial medium without adding salt.

Table - 3.

Amounts of biomass (g/100 ml) of salt-tolerant actinomycetes on Czapek medium - Dox with NaCl

Actinomycetes	NaCl concentration; amount of biomass g/100 ml			
	1%	2%	4%	Control
Streptomyces violaceorubidus	0,14±0,03	0,09±0,02	0,05±0,02	0,11±0,02
Streptomyces rochae	0,17±0,02	0,15±0,04	0,05±0,03	0,11±0,03

*Cultivation time -48 hours

The data obtained showed that at a concentration of 2.0% NaCl, an increase in biomass was observed only in the Streptomyces rochae strain by 36%, but in the Streptomyces violaceorubidus strain a decrease in biomass was detected by 19.0%. Also, at a concentration of 4.0% NaCl in both strains of Streptomyces violaceorubidus and Streptomyces rochae, the amount of biomass decreased by 55% compared to the control medium without adding salt (table 3).

Thus, more than 18 strains of persistent cultures of microorganisms were isolated from the soil of a former pesticide warehouse in the Mirzabad district of the Syrdarya region. The prevailing concentrations were distinguished by 3 bacterial cultures: Bacillus megaterium, Bacillus subtilus, Pseudomonas aeruginosa and 2 actinomycete cultures: Streptomyces violaceorubidus, Streptomyces rochae. The isolated cultures were resistant to high salt concentrations, such as NaCl and Na2SO4. It was found that at concentrations of 10-15%, the selected cultures added to the nutrient medium with salts NaCl and Na2SO4 had the ability to grow with the formation of a sufficient concentration of biomass in the culture medium.

As a result of experiments carried out to study the microbial landscape, taking into account the larger number, about 20 cultures of bacteria and fungi were isolated from the soil of a former pesticide warehouse, which, according to their properties, belonged to microorganisms adapted to stress conditions. Under conditions of surface cultivation on selective agar nutrient media containing from 15.0% to 25% **NaCl** and **Na2SO4** salts, not all cultures had the properties of growth and development. For example, Pseudomonas aeruginosa and Bacillus subtilus had growth activity at salt concentrations of 15.0% and 20% and turned out to be the most resistant to the tested salts. At a salt concentration of 2.0% NaCl under deep cultivation conditions, an increase in biomass was observed only in the Streptomyces rochae culture by 36%, but in the Streptomyces violaceorubidus strain a decrease in the amount of biomass was detected by 19.0%. Also, at a concentration of 4.0% NaCl salt, the Str. violaceorubidus and St. rochae amount of biomass decreased by 55%, i.e. twice as compared to the control medium without adding salt (table 3).

As noted above, saline soils of the sodic type occupy about 10% of all arable land on the planet, although more recent FAO reports indicate that this figure already reaches 50% of the occupation. It should also be noted that more than 100 countries of the world, including the subsoil of Uzbekistan, suffer from primary or secondary salinization associated with intensive agricultural activities, using excessive amounts of mineral fertilizers of various types and other pollutants, as well as industrial waste.

Based on the fact that more than 800 million hectares of the Earth around the world are subject to primary salinization, and approximately 77 million hectares are subject to secondary salinization [5], of which about 45 million hectares are in irrigated land areas.

Thus, our results indicate the presence in such soils of microorganisms confined to such stress conditions for many years, which dictates the need to study them by isolating them in pure cultures, to enhance their biosynthetic properties, allowing the degradation of pollutants of various types, and to reduce the salt load on the soil and create bioremediation methods using stable, salt-tolerant crops.

In this regard, the experimental data we obtained on the isolation of stable cultures of microorganisms from the contaminated soils of the former pesticide warehouse in the Mirzabad region and the adaptive properties we have proven for cell counting and growth on media containing high concentrations of NaCl and Na2SO4 opens up new opportunities for the creation of biological products for the bioremediation of contaminated soils. only with various salts, but also possibly with pesticides, which is the subject of further research.

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