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Plants and Microorganisms for Phytoremediation of Soils Polluted with Organochlorine Pesticides

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***Abstract*—**The goal of presented work is the development phytoremediation method targeted to cleaning environment polluted with organochlorine pesticides, based on joint application of plants and microorganisms. For this aim the selection of plants and microorganisms with corresponding capabilities towards three organochlorine pesticides (Lindane, DDT and PCP) has been carried out.

The tolerance of plants to tested pesticides and induction degree of plant detoxification enzymes by these compounds have been used as main criteria for estimating the applicability of plants in proposed technology. Obtained results show that alfalfa, maize and soybean among tested six plant species have highest tolerance to pesticides.

As a result of screening, more than 30 strains from genera *Pseudomonas* have been selected. As a result of GC analysis of incubation area, 11 active cultures for investigated pesticides are carefully chosen.

***Keywords*—**DDT, Lindane, organochlorine pesticides, PCP, phytoremediation.

I. Introduction

ORGANOCHLORINE pesticides belong to Persistent Organic Pollutants (POPs) as they have high chemical stability and difficultly undergo biotic and abiotic transformations. They easily accumulate in plants and animal tissues and are then incorporated into the food chain that causing a great danger for health.

In the new ecological situation typified by massive emissions of technogenic pollutants in environment, plants and microorganisms exhibited new capabilities for the absorption and metabolic degradation of toxicants. This became the basis for development of phytoremediation technologies, based on unique ability of plants and microorganisms to uptake and degrade of wide spectra of chemical pollutants (among these – organochlorine pesticides) via enzymatic transformation [1].

Concerning to biological methods of remediation that are generally most effective, economically profitable and ecologically friendly technologies for rehabilitation of chemically polluted environment, but in case of organochlorine pesticides, such biotechnologies have low productivity as only a few bacterial strains are able to perform the full mineralization of these pollutants as a result of consequent aerobic and anaerobic conversions [2]. For detoxification of organochlorine pollutants initial dehalogenation of their molecules is necessary. As a result, the residual carbon skeletons become accessible for the oxidizing enzymes that degrade toxic compounds up to cell regular metabolites. It should be noted that data about plants' ability to degrade organochlorine pesticides are very exiguous [2].

The using of plant-microbial tools for intensification of phytoremediation process may be effective in case of pollution with high toxic and persistent organic pollutants such as organochlorine pesticides. According to data, obtained as a results of our previous investigations of the molecular mechanisms of organochlorine toxicants (dichlorodiphenyl-trichloroethane (DDT), 1,2,3,4,5,6-hexachlorocyclohexane (Lindane) and 1,2,3,4,5-pentachlorophenol (PCP) degradation in plants, it has been shown that in vitro experiments detoxification enzymes of some plants have shown high ability of transformation of organochlorine pesticides [3]. In spite of this, in model experiments the using of these plants for clean-up of artificially contaminated soil was not enough effective. We supposed that it is caused by low solubility and consequently low bioavailability of tested pesticides. For solution of this problem, we set a goal to develop a new biotechnological approach based on the targeted use of plants and microorganisms with high detoxification potential and corresponding capabilities for completely removing these pollutants from the environment.

This technology will be based on following concept: initial degradation of pesticides will carry out by specially selected microorganisms. At this time, the radicals that specify toxicityand stability against biotransformation of pesticides are eliminated from molecule of pesticides as a result of the action of microorganisms' enzymes. The forming products become easily available for the plants and their oxidative enzymes that can complete degradation of carbon skeleton of toxicants into standard cellular nontoxic compounds.

II. Materials and Methods

A. Plant Material

Experiments were carried out on the following annual mono and dicotyledonous plants: chickling vetch (*Lathyrussativum*), soybean (*Glycine max*), maize (*Zea mays*), alfalfa (*Medicago*

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sativa), chikpea (*Cicer arietinum*) and lettuce (*Lactuca sativa*).

B. Effect of Organochlorine Pesticides on Plant Grown Parameters and Induction of Detoxification Enzymes

The germinated plant seedlings were exposed to different concentration (0.01, 0.1 and 1mM) DDT, Lindane and PCP solutions in running water and cultivated hydroponically at ambient illumination and temperature, between 22 and 25°C. During the growing of plants on the water solutions of tested organochlorine toxicants the following parameters: length of shoots and roots of seedlings, plant biomass, germinability of seeds (the correlation between quantities of germinated and sowed seeds) and protein accumulation in plants have been determined.

Plants exposed to pesticides, after definite time were washed with distilled water, roots homogenized in 0.05M phosphate buffer, pH 7.4. Homogenates were squeezed through cheesecloth and centrifuged at 1000g, 20min. In the supernatant activities of enzymes were determined.

Peroxidase activity was determined spectrophotometrically at 470nm, according to the rate of H_2O_2-dependent oxidation of guaiacol [4].

Phenoloxidase activity was determined spectrophotometrically at 420nm, according to the rate of pyrocatechol oxidation [5].

Activity of Glutathione S-transferase was determined spectrophotometrically at 340nm according to the procedure described in Schröder et al. [6].

Monooxygenase activity was determined polarographically, by oxygen consumption rate at NADPH-dependent oxidation of N, N-dimethylaniline [7].

Protein was determined by the method of Bradford [8].

C. Screening of Microorganisms

For screening of microorganisms on their capability to degrade organochlorine pesticides, strains of bacteria, from the Durmishidze Institute of Biochemistry and Biotechnology Collection of Microorganisms Cultures were tested.

The capability of microorganisms to assimilate and degrade organic toxicants was revealed by growing strains on solid media at 28–30°C. For the screening modified Czapek's media, containing DDT, Lindane or PCP (0.01, 0.1 and 1mM) have been used.

Vegetative culture grown up to the exponential phase of growth served as inoculant. The nutrient media were inoculated with 10% of the bacterial suspension.

D. Extraction and Determination of Pesticides

The extraction of DDT, Lindane and PCP from incubation medium after the submerge cultivation of pesticides with Pseudomonas strains was carried out by using hexane. Extracts were evaporated to dry residues, obtained residues were dissolved in hexane and contents of pesticides in samples were measured by gas chromatography.

Conditions for chromatographic analysis were following: Instrument - Agilent GC; Detector – μECD; Column - Agilent 19091J-413 (HP-5 5% Phenyl Methyl Siloxane 325°C: 30mx 320μmx0.25μm), carrier gas – hydrogen, pressure 6.838psi, Flow 2.5328 mL/min; Average Velocity 50cm/sec, makeup gase – nitrogen. Temperature regime: 80°C for 1min, then 30°C/min to 175°C for 4min; then 6°C/min to 215°C for 2min; then 15°C/min to 290°C for 3min. Retention time for tested toxicants are following: DDT - 6.9min; Lindane – 7.3min; PCP – 6.8min.

III. Results And Discussion

A. Plant Growth on Organochlorine Pesticides and Induction of Enzymes

During exposure of plants on water solutions of investigated organochlorine pesticides, mostly the decrease of the growth parameters (germinability, biomass formation and length of seedlings) takes place, also the increase of protein accumulation in plant roots is observed. It should be mentioned that especially highest concentration of PCP (1mM) negatively affect on growth of all plant seedlings and induces the inhibition of growth that leads to drying and death of plants.

Among tested plants maize, soybean and alfalfa have comparatively high tolerance to organochlorine pesticides. In these plants the decrease of growth parameters by high concentrations of toxic compounds (for DDT and Lindane – 1mM; for PCP – 0.1mM) was less than 10-15%. Other plants are sensitive to all three tested pesticides. Their growth suppressed by 30-60% on mentioned concentrations.

As referred to above, during the plants growing on organochlorine toxicants containing area, enhancement of protein biosynthesis process takes place. Apparently such effect is connected with plant response reactions against to negative influence of toxicants, in particular, to the induction of detoxification enzymes that on next stage of investigation was studied (Table I).

TABLE I
Units for Magnetic Properties the Induction of Oxidative Enzymes in Roots of 10-Days Old Seedlings after Growing on Solutions of Pesticides

Pesticide	Enzyme	Maize	Soybean	Alfalfa
DDT	Cytochrome P450	100 ± 5	131 ± 7	112 ± 6
	Peroxidase	198 ± 10	115 ± 6	150 ± 8
	Phenoloxidase	260 ± 13	140 ± 7	108 ± 5
	Glutathione S-transferase	135 ± 7	177 ± 9	150 ± 8
Lindane	Cytochrome P450	100 ± 5	105 ± 5	105 ± 5
	Peroxidase	200 ± 10	118 ± 6	146 ± 7
	Phenoloxidase	214 ± 11	100 ± 5	138 ± 7
	Glutathione S-transferase	143 ± 7	107 ± 5	182 ± 9
PCP	Cytochrome P450	105 ± 5	108 ± 5	106 ± 5
	Peroxidase	82 ± 4	113 ± 6	115 ± 6
	Phenoloxidase	116 ± 6	100 ± 5	112 ± 6
	Glutathione S-transferase	178 ± 9	108 ± 6	125 ± 7

Concentration of pesticides: DDT and Lindane – 1mM; PCP – 0.1mM.Duration of incubation on solution of pesticide – 5 days. Activities of enzymes are presented in percent. The activities of enzymes in control variants (without toxicant) are considered as 100 %.

As it seem from results presented on Table I, in maize all investigated enzymes are induced for transformation of DDT

and Lindane, but for PCP only the activation of glutathione S-transferase takes place. It can be supposed that for detoxification of non-polar molecules of DDT and Lindane the initial oxidation and subsequent conjugation is necessary. In difference from DDT and Lindane, PCP contain polar hydroxyl group and so this pesticide maybe undergoes direct conjugation with glutathione. Almost analogous results have been obtained for other plants, but such induction effects are less expressed.

B. Microorganisms Ability to Degrade DDT

For selection of rhizospheric microorganisms degrading organochlorine pesticides, more than 70 strains from genera *Pseudomonas* were screening on solid nutrient area with DDT, Lindane or PCP.

The obtained results show that after cultivation of strains on DDT containing area, 35 strains from genera *Pseudomonas* reveals best growth with glucose; 15 strains were grown better with DDT, than with glucose. As a result, 11 strains of *Pseudomonas* that reveals best grown as with glucose, as without it, are selected.

In case of Lindane, as a result of the screening, 14 strains from genera *Pseudomonas* reveals best growth with glucose; 13 strains were grown better with Lindane, than with glucose. Finally, 11 strains of *Pseudomonas* that reveals best grown as with glucose, as without it, are selected.

After cultivation with PCP, 14 strains from genera *Pseudomonas* reveals best growth with glucose; 4 strains were grown better with PCP, than with glucose. Finally, 10 strains that reveals best grown as with glucose, as without it, are selected.

The influence of DDT, Lindane and PCP preparations on biomass accumulation by selected strains were estimated. It has been selected the strains for quantitative analysis of tested pesticides utilization due to results of screening. According to obtained results, some strains (*Pseudomonas* sp.6R21 in case of DDT, *Pseudomonas* sp. 4G14 in case of Lindane and *Pseudomonas* sp. GN28 in case of PCP) accumulate biomass better on tested pesticides containing area, than on Czapek's medium without glucose.

The detoxification potentials of strains that selected according to biomass accumulation ability at submerge cultivation on pesticides containing areas, have been estimated. According to results of gas chromatographic analyses of residual pesticides in incubation medium after cultivation, the best strains with high pesticide assimilating capacities have been revealed (Table II). These strains can degrade and/or uptake of organochlorine pesticides from cultivation area (more than 80% from initial concentration) and are usable for application in developed phytoremediation technology.

In future, investigation of compatibility of selected microorganisms and plants, and the model testing of new phytoremediation technology by using of joint action of selected microorganisms and plants for cleaning of soils artificially polluted with organochlorine pesticides are planning.

TABLE II
CONTENT OF PESTICIDES IN INCUBATION MEDIUM AFTER SUBMERGE CULTIVATION OF SELECTED PSEUDOMONAS STRAINS WITH PESTICIDES

Pesticide	Conventional name of *Pseudomonas* strains	Residual content of pesticide, % from initial concentration
DDT	751-6D	15.3 ± 0.8
	8JL63	15.7 ± 0.8
	TBM5	14.8 ± 0.8
	TBM6	9.9 ± 0.6
	4JL50	8.9 ± 0.5
Lindane	4G14	6.1 ± 0.4
	PS63	19.8 ± 1.0
PCP	8JL63	3.8 ± 0.2
	PS8D	12.7 ± 0.7
	PS66	9.9 ± 0.6
	TP335	2.1 ± 0.1
	GN28	5.4 ± 0.3

Incubationconditions: Czapek's medium; temperature – 30ºC; initial concentration of pesticides – 1mM; speed of shaker – 180rpm, duration of cultivation – 72h.

IV. CONCLUSION

Thus, as a result of carried out work, alfalfa, maize and soybean as plants phytoremediators and 11 strains from genera *Pseudomonas* as pesticide degrading agents for phytoremediation method targeted to cleaning environment polluted with organochlorine pesticides have been selected.

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