

ASSESSMENT OF PHENOL DETOXIFICATION BY *RHODOCOCCUS AETHERIVORANS* UCM AC-602 USING THE PHYTOTESTING METHOD

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Monoaromatic compounds are related to widespread pollutants of soil and groundwater. Among them phenol is one of the most toxic and carcinogenic compounds. Therefore biodestruction of phenol is of much importance for environment protection. The use of metabolic potential of microorganisms for depolluting environment is a safe and economical alternative to widely used physicochemical methods. **Aim of study.** To assess efficacy of phenol detoxification with strain *Rhodococcus aetherivorans* UCM Ac-602 using the phytotesting method. **Methods.** Bacteria were cultivated in liquid mineral medium with initial concentration of phenol 500, 750 and 1000 mg/L as a single source of carbon and energy. Cultivation time was 24 h, 48 h and 72 h respectively. Phytotoxicity was determined in express-test with use of seeds of spring wheat variety “Pecheryanka” (*Triticum aestivum* L.). Plant seeds were incubated by temperature 20 ± 2 °C during 7 days in Petri dishes with filter paper treated with respective phenol aqueous solutions or post-fermentative cultural fluids (PFCFs). PFCFs were obtained after cultivation of strain in growth medium with same concentration of phenol. Morphometric parameters of wheat were assessed against control plants cultivated on distilled water. Comparative analysis of samples toxicity and toxicity class determination was performed according to Kabirov method by calculation of index of test factor toxicity (ITF). **Results.** Phenol aqueous solutions and PFCFs were much different in effect on wheat. Phenol solutions 500 and 700 mg/L have shown significant inhibitory effect on all initial growth parameters of test plants. The weakest growth inhibition was induced by phenol concentration of 500 mg/L which caused decrease in number of germinated seeds by 59.6 %, shoot length – by 59.7 %, root length – by 84.5 %, sprout dry weight – by 35.0 %. In the presence of phenol concentration of 750 mg/L these indicators increased by 7–30 %; roots of test plants were the most sensitive to effect of phenol. Phenol concentration of 1000 mg/L caused total seed mortality. Unlike phenol aqueous solutions PFCFs have shown insignificant effect on all morphometric indicators of plants compared to control. Similar effects on plants were observed in the presence of PFCFs obtained from cultivation of strain *R. aetherivorans* UCM Ac-602 in the growth medium with initial concentrations of phenol of 500 and 750 mg/L. Under the influence of these PFCFs, the number of germinated seeds decreased on average by 15.8 %, root length decreased by 19.8 %, at the same time shoot length and their dry weight increased by 17.8 % and 7.2 % respectively. More negative effect on wheat was shown by PFCF obtained after strain cultivation on medium with phenol concentration 1000 mg/L. It caused reduction in number of germinated seeds by 18.0 %, shoot length – by 25.3%, root length – by 29.0 %, sprout dry weight – by 7.2%. For phenol aqueous solutions ITFs had much lower values 0–0.40 than for PFCFs (0.71–1.0). **Conclusions.** Based on data obtained in this research it was concluded that strain *R. aetherivorans* UCM Ac-602 performs active detoxification of high-concentrated phenol-containing media. Analysis of calculation results for ITF medium values (ITF_m) had shown that under the influence of studied strain there was a decrease in toxicity of phenol solutions (500, 750 and 1000 mg/L). According to Kabirov toxicity scale it was assessed that toxicity of phenol solutions with initial values of classes II (high) and I (very high) was decreased to IV (low) and V (normal level). Our results demonstrate ecological safety of the end products of phenol destruction with strain *R. aetherivorans* UCM AC-602 and prospects of its use in biotechnologies for environment detoxification from phenol pollutions.

Keywords: *Rhodococcus aetherivorans*, phenol, detoxification, phytotoxicity.

Among numerous groups of toxic compounds getting into environment with wastewaters and industrial wastes the important place is held by monocyclic aromatic compounds – phenol and its derivatives. One of the most ecologically dangerous monoaromatic compounds is phenol. It is widely used in various branches of industry and medicine, particularly in manufacturing of antiseptics, dyes, pesticides and pharmaceutical products. Phenol is also present in large amounts in wastewaters from oil- and wood-processing and pharmaceutical manufacturing [1]. According to US Environmental Protection Agency (US EPA) phenol constitutes the 11th of the 126 chemicals, which has been designated as priority pollutants [2]. Getting into water bodies, phenolic compounds have negative impact on natural biocoenoses causing many ecological problems. US EPA and World Health Organization (WHO) have set a guideline of 1 µg/L while the European Community defined a limit of 0.5 µg/L for phenolic compounds in drinking water [1]. In Ukraine maximum permissible concentration (MPC) value for phenols for drinking water is 1 µg/L [3], and for industrial wastewaters – 0.25 mg/L [4]. However, the content of phenolic compounds in a polluted environment significantly exceeds the permissible standards. For instance, phenol concentration from petroleum refinery effluents vary between 50 and 2000 mg/L, from olive mill wastewater between 1200 and 4300 mg/L and waste solutions generated from coal conversion processes phenol concentrations of 200–600 mg/L are usually detected [5, 6]. Therefore, the phenol-containing waste water must undergo a mandatory local purification in factory wastewater treatment plant and then transferred to the city sewer system for post-purification.

The microorganisms play an important role in the processes of purification of phenol-contaminated water and soil. Among them actinobacteria of the genus *Rhodococcus* occupy a significant place. They are widely distributed in nature, exhibit significant catabolic versatility, resistance to a wide range of toxic substances and are often used to purification of persistent organic pollutants environment [7]. Microbial methods detoxification of phenol-polluted environments has significant advantages over physical and chemical approaches by being relatively cheaper and ensuring complete phenol degradation. So for example, physico-chemical treatment of contaminated industrial sewage at petrochemical enterprises by adsorption, evaporation and extraction reduces the phenol content from 2000 to 6000 mg/L to

only 400–80 mg/L which still requires additional post-treatment using biochemical methods to reduce the phenol content to 5–2 mg/L [6]. The mandatory characteristic of microorganisms, which determines the possibility of their use for cleaning toxic substances contaminated objects, is the ability to completely degrade the contaminants to environmentally friendly substances. In previous studies, we have shown that the strain of *R. aetherivorans* UCM Ac-602 isolated from oil-contaminated soil was able to efficiently assimilate phenol at its content in the medium up to 2000 mg/L [8]. However, the environmental safety of phenol degradation products contained in the culture medium after its assimilation by the strain *R. aetherivorans* UCM Ac-602 has not been previously studied. One of the most common methods of toxicity determination is phytotesting, which makes it possible to obtain an integral toxicological characteristic not only of natural environments, such as soil and water, but also to assess the toxicity of various chemical compounds, products of microbial synthesis and industrial waste [9].

Considering the above **the purpose of this study** was to investigate the effectiveness of phenol detoxification by the *R. aetherivorans* UCM Ac-602 strain using a phytotesting method.

Materials and Methods. The object of the study was the strain *Rhodococcus aetherivorans* UCM Ac-602 (IMV Ac-5035) deposited in the Depository of Microorganisms of Danylo Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine. The strain was isolated from lubricant contaminated soil and it was selected in our previous studies as an active destructor of phenol and hydrocarbons [8].

The cultures were grown on a mineral medium with an initial phenol concentration 500, 750 and 1000 mg/L as described previously [8]. A post-fermentation culture fluid (PFCF) obtained after grown of the strain at the mentioned phenol concentrations designated respectively as PFCF-500, PFCF-750 and PFCF-1000. Phytotoxicity of phenol aqueous solutions and PFCF was studied according to methods described by Pimenova et al. [10] with some modification. Seeds of spring wheat variety “Pecheryanka” (*Triticum aestivum* L.) were used as phytotest-object. Seeds were placed on filter paper in 10 cm Petri dishes, 20 seeds per each. Test samples were applied in volume of 10 mL in each Petri dish. Seeds cultivated in equal volume of distilled water were served as a control. Each

experiment was repeated 5 times. Experiments were carried out at the temperature 20 ± 2 °C during 7 days. After the end of exposition the following indicators (test-functions) were assessed: number of germinated seeds, shoot length, root length, dry weight of sprout. Comparative analysis of toxicity levels of samples was carried out using the index of test factor toxicity (ITF) calculated with following formula: $ITF = (TF_0 / TF_c)$, where TF_0 – value of test-function in experiment, and TF_c – value of control [11]. To consolidate all parameters obtained after biotesting, mean value of ITF for each experiment (ITF_m) was calculated according to the formula $ITF_m = (ITF_1 + ITF_2 + \dots + ITF_n) / n$, where ITF_1, ITF_2, ITF_n – indexes of toxicity calculated for each test-function; n – number of test-responses involved in experiment for each function. Obtained results were assessed according to R.R. Kabirov et al. toxicity scale [11] consisting of 6 classes.

Statistical processing of results. The experiments were repeated three times. The analysis of research results was performed using the MS Excel 2010 software. The significance of difference between the mean values was determined with t -test and was considered reliable at $p \leq 0.05$.

Results. There were significant differences between obtained data for level of phytotoxic effect of phenol aqueous solutions and PFCF on test-plants of wheat. For phenol concentrations 500 and 750 mg/L we have observed considerable decrease

in all morphometric parameters of test-plant against control (Fig. 1). Namely, in presence of phenol 500 mg/L number of germinated seeds reduced by 59.6 %, shoot length by 59.7 %, root length by 84.5 %, sprout dry weight – by 65 %. Under the influence of phenol 750 mg/L these indicators were lower than control by 91.0, 66.5, 95.5 and 74.7 % respectively. It should be noted that roots of test-plants were much more susceptible to the effect of phenol than sprouts. Increase of phenol concentration up to 1000 mg/L led to absolute (100 %) inhibition of seeds germination and caused blackening and death.

Observed results of changes in morphometric parameters of wheat under influence of all studied PFCFs indicate insignificant effect on seed germination. It was reduced by 14.6–18.0 % in experiment groups in comparison with the control (Fig. 1). The lowest effect on test-plants of wheat caused PFCF-500: seed germination in this group was reduced by 14.6 %, root length by 17.7 %, shoot length increased by 18.8 %, sprout dry weight – by 8.5 % compared to the control values (Fig. 1). Results of studied effects of phenol aqueous solution 500 mg/L and PFCF-500 on wheat test-plants are presented on Fig. 2.

PFCF-750 caused similar effect on the sprouts of test-plants: shoot length was increased by 16.9 %, and sprout dry weight – by 5.8 %. Higher susceptibility to PFCF-750 effect was observed in wheat roots growth of which was inhibited by

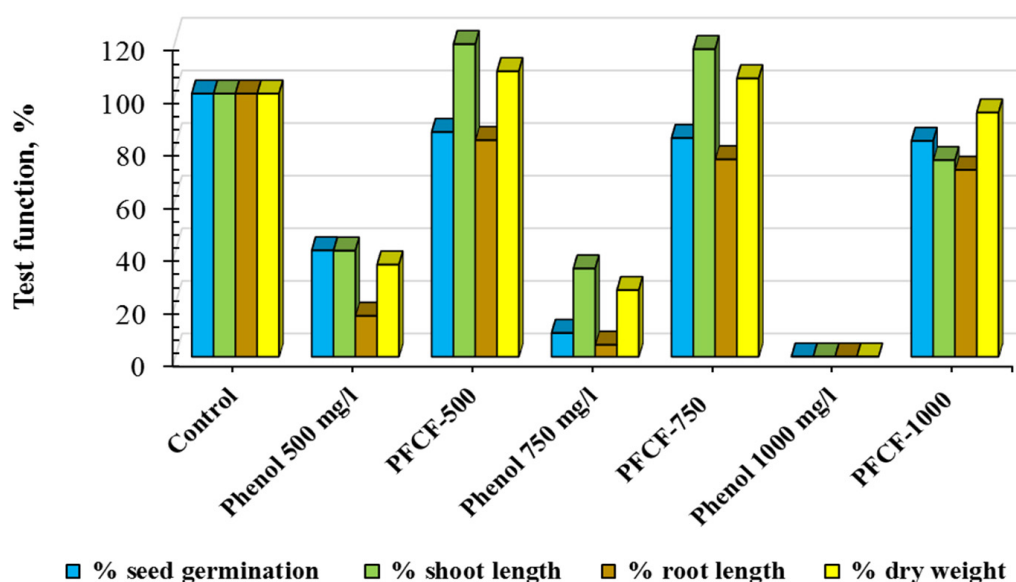


Fig. 1. The effect of phenol and PFCF on test functions (seed germination, shoot length, root length and dry weight) of spring wheat cultivar “Pecheryanka” (*Triticum aestivum* L.).
Notes: PFCF-500, PFCF-750, PFCF-1000 – post-fermentation culture fluid after growth *R. aetherivorans* UCM Ac-602 on a mineral medium at an initial phenol concentration 500, 750 and 1000 mg/L, respectively.

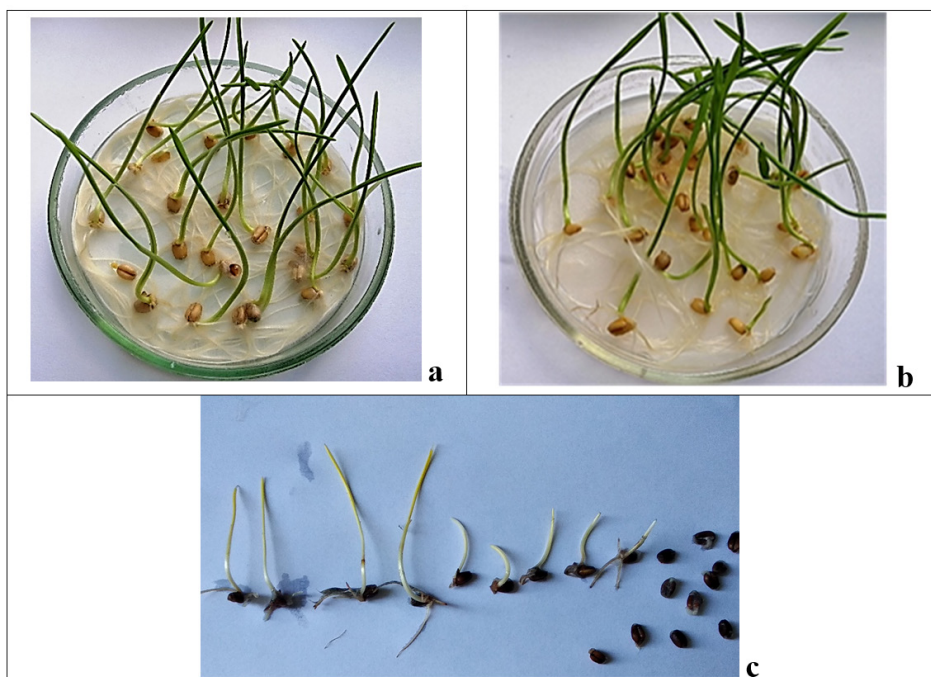


Fig. 2. Testing of phenol aqueous solution 500 mg/L and PFCF-500 effect on spring wheat cultivar «Pecheryanka» (*Triticum aestivum* L.). Notes: a – control (distilled water); b – PFCF-500 (post-fermentation culture fluid after growth *R. aetherivorans* UCM Ac-602 on a mineral medium at an initial phenol concentration 500 mg/L); c – phenol aqueous solution 500 mg/L.

25.0 % compared to the control. Increase of initial phenol concentration in growth medium up to 1000 mg/L led to more significant growth inhibition of shoot and roots. In presence of PFCF-1000 length of sprouts and roots was reduced by 25.3 % and 29.0 %, respectively, and sprout dry weight – by 7.2 %.

Results of index of test factor toxicity (ITF) for each test-function of wheat presented on Fig. 3 confirm significant differences of these indicators for phenol aqueous solutions and PFCF.

ITF values for phenol solutions were much lower (0–0.40) then for PFCF (0.71–1.0). The lowest susceptibility to effect of phenol in concentrations 500 mg/L and 750 mg/L had the sprouts of test plants. ITFs for shoot length in presence of these concentrations constituted 0.40 and 0.33, and for sprout dry weight – 0.35 and 0.25 respectively. The highest from all ITF values were indicated for wheat sprouts after seed treatment with PFCF-500 and PFCF-750. ITF for shoot length were 1.19 and 1.16; for sprout dry weight – 1.10 and 1.10 respectively. Based on the obtained data mean values of index of test factor toxicity (ITF_m) were calculated. Phenol detoxification rate after strain cultivation on this substrate was evaluated using 6-class toxicity scale of Kabirov et al. [11] (Table 1).

Data shown in this table indicate that aqueous solutions of phenol of concentrations 500 and 750 mg/L (ITF_m < 0.5) belong to toxicity class II (high toxicity), and solutions of 1000 mg/L – to toxicity class I (very high toxicity) causing absolute seed mortality. Unlike phenol aqueous solutions PFCF obtained after strain cultivation with tested concentrations of toxicant had no substantial impact or caused insignificant inhibition on investigated test-functions of wheat. Calculated mean values of (ITF_m) for PFCF-500 and PFCF-750 permit to refer them to toxicity class V (normal) (ITF = 0.91–1.10), meaning absence of toxic effect. By the level of toxic effect PFCF-1000 belongs to toxicity class IV (ITF = 0.71–0.90) characterized by insignificant decrease in test-function value of study object compared to control.

Discussion. In current research we have used phytotesting method for assessment of level of phenol detoxification with strain *R. aetherivorans* UCM Ac-602 after cultivation on liquid mineral medium with initial phenol concentrations of 500, 750 and 1000 mg/L. Previously we have found [8] that phenol concentrations of 500 and 750 mg/L had not caused inhibitory effect on growth and development of this test strain, while concentration of 1000 mg/L caused insignificant delay in culture

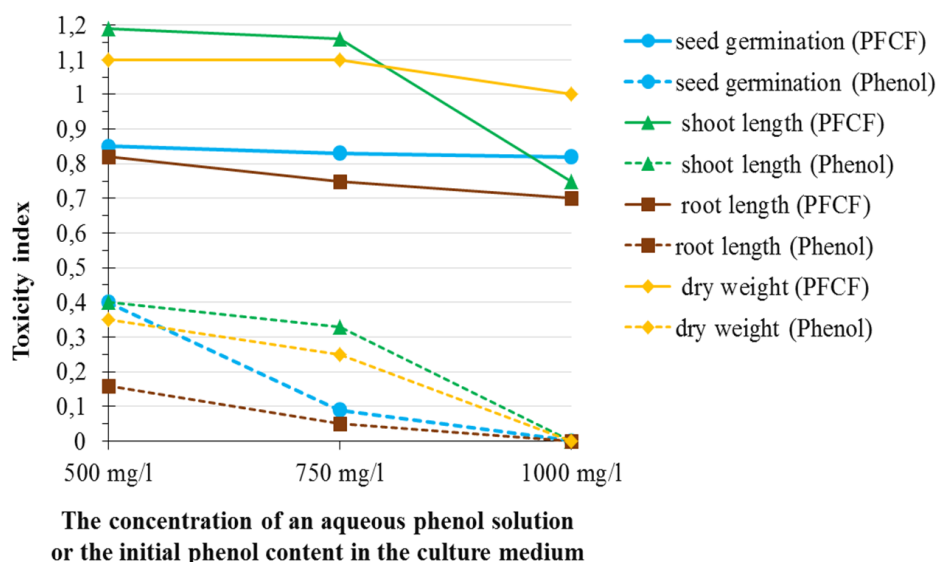


Fig. 3. Indexes of test factor toxicity (ITF) for test-function wheat cultivar «Pecheryanka» (*Triticum aestivum* L.). Notes: PFCF – post-fermentation culture fluid after the growth of *R. aetherivorans* UCM Ac-602 on a mineral medium with phenol.

Table 1
Toxicity assessment of aqueous phenol solutions and PFCFs of strain *R. aetherivorans* UCM Ac-602 after cultivation on mineral medium with phenol

Experimental group	ITF _m	Toxicity assessment according to Kabirov scale (R.R. Kabirov and al.) [11]	
		Class of toxicity	Level of toxicity
Phenol, 500 mg/L	0.33	II	High toxicity. Significant decrease of test-function values in experiment compared to control (ITF <0.5)
Phenol, 750 mg/L	0.18	II	
Phenol, 1000 mg/L	0	I	Very high toxicity causing death of test object (ITF=0)
PFCF- 500*	0.99	V	Normal. Factor does not cause substantial impact on development of test organism, values of test function remains on the control level (ITF = 0.91 – 1.10)
PFCF-750*	0.96	V	
PFCF-1000*	0.80	IV	Low toxicity, negligible decrease in test-function values in experiment compared to control (ITF = 0.71– 0.90)

* PFCF-500, PFCF-750, PFCF-1000 – post-fermentation culture fluid after growth *R. aetherivorans* UCM Ac-602 on a mineral medium at an initial phenol concentration 500, 750 and 1000 mg/L, respectively; ITF_m – mean value of index of test factor toxicity for all test-functions in one experimental group.

growth. It should be noted that the process of phenols biodegradation is characterized by the formation of several intermediate compounds, some of them are quite stable and toxic, in particular, quinones and some polymers products. The analysis of phenol biodegradation under action of microorganisms showed that some intermediate products of phenol transformation are: pyrocatechol (40 %) and pyrogallol (20 %), and also hydroquinone (10 %). Among the products of phenol biotransformation (about 15 %) the polymer fraction corresponding to oligophenylene with molecular mass ~3500 has been fixed [12]. It was established, for example, that among monoatomic and diatomic phenols,

pyrocatechol and hydroquinone are the most toxic to higher aquatic plants [13]. These data confirm that determination of toxicity level of phenol-polluted media after its biodestruction with microorganisms is of high ecological importance.

It should be pointed out that phytotesting with use of higher plants seeds as test-object is widely used for assessment of ecological safety of various substances, in particular for products of microbial synthesis and degradation-resistant environment pollutants. Literature contains evidences of the use of this method for evaluation of biosurfactants toxicity [14, 15], biodestruction products from unusable pharmaceuticals [16–18], hydrocarbons

[19–24], herbicides [10, 25], heavy metals [26, 27] and other compounds.

In our study we used express-method of phytotesting on grain crops seeds (spring wheat variety “Pecheryanka”) with direct contact of tested substances with seeds. In such conditions phytotoxicity of investigated substances is assessed by biological effect of their aqueous solutions or extracts. Such approach to exposure is the most technically simple and convenient in use [28]. There exist literature data on successful use of this method for determination of phytotoxicity of aliphatic (n-tridecane, n-decane, n-hexane) as well as aromatic (isopropylbenzene, 1,2,4-trimethylbenzene) hydrocarbons [22], polycyclic aromatic hydrocarbons [19, 24, 29], herbicide “Tornado” [10], glycolipid biosurfactants of rhodococci [14], products of drotaverine hydrochloride biodestruction by these bacteria [17] and sodium diclofenac [18]. We have also used this method before for determination of phytotoxicity of paracetamol (N-(4-Hydroxyphenyl)acetamide) and products of its biotransformation by strain *Rhodococcus erythropolis* UCM Ac-23 [30].

In the present research toxicity of studied samples was assessed by R.R. Kabirov et al. toxicity scale [11]. This method unlike the standard scale used for routine evaluation [23, 31], takes into account the possibility of stimulation effect on plants which can be caused by small dosages and their destruction products. R.R. Kabirov et al. toxicity scale [11] is widely used in researches of other authors for eco-toxicological evaluation of different environmental objects with technogenic pollutions [27, 32, 33].

According to literature data the level of phytotoxicity of aromatic and other hydrocarbons depends on their chemical structure, exposure time on test-plants seeds and experimental conditions [22]. In particular, in the literature there are the data on the absence of a significant effect of low concentrations (200 mg/L) of polyaromatic hydrocarbons (PAHs) on seed germination of certain grain crops and wild plants [19]. There are data about stimulatory effect of fluoranthene (50 mg/L) on development of sprouts of sorghum – *Sorghum bicolor* (L.) and increase of values of majority of morphometric parameters of this plant under the influence of cultural fluid of micromycete *Fusarium oxysporum* after cultivation on this substrate [24]. Stimulatory effect on development of sprouts of sorghum and alfalfa (*Medicago sativa* L.) was shown by chrysene, fluoranthene and pyrene in concentration 250 mg/L [29]. It is found that some

metabolites produced in microbial degradation of phenanthrene (9,10-phenanthrenequinone, 1-hydroxy-2-naphthoic and benzoic acids) were more toxic for these plants in comparison with source PAHs [29].

According to data from other authors, aromatic and polyaromatic hydrocarbons show toxicity on cultural and wild plants. In presence of these compounds in concentration range 50–1200 mg/L there is observed inhibition of seed germination by 100 % [22]. This corresponds with the results obtained in the present research where 100 % wheat seed mortality was caused by phenol concentration 1000 mg/L. There are literature data that direct contact of spring wheat seeds variation “Kostanaiskaia-12” with aromatic compounds (isopropylbenzene and 1,2,4-trimethylbenzene) caused significant reduction of their germination. In case of long-term contact of seeds (240–480 min) with isopropylbenzene inhibition of seed germination constituted 60.0 %, and for 1,2,4-trimethylbenzene – more than 80.0 % [22]. These data are corresponding with our results for phenol concentrations 500 and 700 mg/L.

Our research has shown that all concentrations of phenol aqueous solution caused significantly weaker growth inhibition on wheat sprouts than on roots. In presence of phenol 500 mg/L root length of test-plants was 6.4 times less than control, and shoot length – 2.5 times. With the increase of phenol concentration up to 750 mg/L mean root length decreased 22.2 times, and concentration 1000 mg/L caused death of test-plant. Our data for effect of different concentration of phenol on root length of wheat test-plants are corresponding with results obtained for cress (*Lepidium sativum*). Phenol in concentration of 1 mM (94.11 mg/L) had no significant inhibitory effect on cress root length, and with increase of concentration to 4 mM (376.4 mg/L) root length decreased 5 times compared to control plants [34].

Studies of phenol detoxification processes with strains isolated from seawater and sediments of Far East seas have shown that speed of this process depends of growth medium, temperature and cultivation time [35]. In study for toxicity determination of cultural fluid from strain *Bacillus pumilus* MMM 21 cultivated on medium with initial phenol concentration 1000 mg/L as single carbon source it was shown that detoxification process reached only up to the category “toxic”. Notable that after strain cultivation on media with additional carbon sources there was observed much lower toxic effect of microbial phenol metabolites

on seed germination of *Tradescantia albiflora* and radish (*Raphanus sativus*). In our experimental conditions cultural fluid of strain *R. aetherivorans* UCM Ac-602 after cultivation on medium with initial phenol concentration 1000 mg/L as single carbon source and energy was able to reduce toxicity of medium from class “very high toxicity” to “low toxicity”.

Analysis of literature data and our results shows that phenol biodegradation do not necessarily match in time with clearance of growth medium as it can still contain toxic destruction products. Therefore in sense of biological effect it is much more significant to assess not just destruction, but detoxification of polluted medium. Previously we have shown that full digestion of phenol by strain *R. aetherivorans* UCM Ac-602 occurs after stationary growth phase namely after 24h, 48h and 72h with initial phenol concentrations 500, 750 and 1000 mg/L respectively. [8]. Our data obtained in this research indicate that under described growth conditions efficacy of phenol detoxification is directly related to initial concentration in medium. According to our data PFCF obtained after strain cultivation with tested concentrations of toxicant had no impact or caused insignificant inhibition on morphometric parameters of wheat. Thus in accordance with toxicity scale of R.R. Kabirov et al. [11], investigated PFCFs belongs to non-toxic or low-toxicity classes of substances.

Conclusions. It was found that strain *R. aetherivorans* UCM Ac-602 performs active detoxification of high-concentrated phenol-containing media. Under the influence of studied strain there was a decrease in toxicity of phenol solutions (500, 750 and 1000 mg/L) that was accessed as class II (high) and I (very high) was decreased to IV (low) and V (normal level). Obtained results demonstrate prospects of the use of *R. aetherivorans* UCM Ac-602 in biotechnologies for environment detoxification from phenolic pollutions.

ОЦІНКА ДЕТОКСИКАЦІЇ ФЕНОЛУ ШТАМОМ *RHODOCOCCUS AETHERIVORANS* УКМ АС-602 МЕТОДОМ ФІТОТЕСТУВАННЯ

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Резюме

Моноароматичні речовини відносяться до широко розповсюджених забруднювачів ґрунту та підземних вод. Серед них – фенол, який є однією з найбільш токсичних та канцерогенних сполук. Його біодеструкція має важливе значення для захисту навколишнього середовища. Використання метаболічного потенціалу мікроорганізмів для очищення довкілля від забруднюючих речовин є безпечною та економічно вигідною альтернативою фізико-хімічним методам, які широко застосовуються. **Мета.** Дослідити ефективність детоксикації фенолу штамом *Rhodococcus aetherivorans* УКМ Ас-602 із використанням методу фітотестування. **Методи.** Бактерії вирощували в рідкому мінеральному середовищі при початковій концентрації фенолу 500, 750 та 1000 мг/л як єдиного джерела вуглецю та енергії протягом 24, 48 і 72 г росту відповідно. Фітотоксичність визначали за допомогою експрес-тесту з використанням насіння ярої пшениці сорту «Печерянка» (*Triticum aestivum* L.). Насіння рослини інкубували за температури 20±2 °С протягом 7 діб у чашках Петрі із фільтрувальним папером, обробленим відповідним водним розчином фенолу, або постферментаційною культуральною рідиною (ПФКР) після росту штаму в середовищі з такими ж концентраціями фенолу. Морфометричні параметри пшениці оцінювали відносно дистильованої води. Порівняльний аналіз ступеня токсичності зразків і визначення класу їх токсичності проводили з використанням методики розрахунку індексу токсичності фактору (ІТФ), який оцінюється по Кабірову. **Результати.** По дії на пшеницю водні розчини фенолу і ПФКР значно різнилися між собою. Розчини фенолу 500 і 750 мг/л проявляли значну інгібувальну дію на всі початкові ростові параметри тест-рослини. Найменше пригнічення росту викликала концентрація фенолу 500 мг/л, під дією якої кількість пророслого насіння зменшилася на 59.6 %, довжина пагонів – на 59.7 %, довжина ко-

ренів – на 84.5 %, а суха маса пагонів – на 35.0 %. За концентрації фенолу 750 мг/л ці показники збільшувалися на 7–30 %. При цьому найбільшу чутливість до дії фенолу проявляли коріння рослини. Концентрація фенолу 1000 мг/л викликала повну загибель насіння рослини. На відміну від водних розчинів фенолу ПФКР проявляла незначний, у порівнянні з контролем, вплив на всі морфометричні показники рослини. Близькими за дією на рослину були ПФКР після росту штаму *R. aetherivorans* УКМ Ас-602 на середовищі з початковими концентраціями фенолу 500 і 750 мг/л: кількість пророслого насіння зменшилася в середньому на 15.8 %, довжина коренів – на 19.8 %, а довжина пагонів і їх суха маса збільшилися на 17.8 % і 7.2 % відповідно. ПФКР після росту штаму на середовищі з концентрацією фенолу 1000 мг/л проявляла більш негативну дію щодо пшениці: кількість пророслого насіння зменшувалася на 18.0 %, довжина пагонів – на 25.3, довжина коре-

нів – на 29.0, а суха маса пагонів – на 7.2 %. Для водних розчинів фенолу ІТФ мали значно нижчі значення (0–0.40), ніж для ПФКР (0.71–1.0). **Висновки.** На основі отриманих у цій роботі даних встановлено, що штам *R. aetherivorans* УКМ Ас-602 активно проводить детоксикацію висококонцентрованих фенолвмісних середовищ. Аналіз результатів розрахунку середніх значень ІТФ (ІТФ_{ср}) показав, що під впливом цього штаму токсичність розчинів фенолу з концентрацією 500, 750 та 1000 мг/л, які, відповідно до шкали Кабірова, відносяться до II (високий) та I (надвисокий) класів, знизилася до IV (низька токсичність) та V (норма) класів. Це свідчить про екологічну безпечність кінцевих продуктів деструкції фенолу штамом *R. aetherivorans* УКМ Ас-602 та перспективність його використання у біотехнологіях очищення довкілля від фенольних забруднень.

Ключові слова: *Rhodococcus aetherivorans*, фенол, детоксикація, фітотоксичність.

- Gami AA, Shukor MY, Khalil KA, Dahalan FA, Khalid A, Ahmad SA. Phenol and its toxicity. J Environ Microbiol Toxicol. 2014; 2(1):11–24.
- Sarker N, Fakhruddin ANM. Removal of phenol from aqueous solution using rice straw as adsorbent. Appl Water Sci. 2017; (7):1459–65.
- State sanitary norms and rules 2.2.4-171-10. [Hygienic requirements for drinking water intended for human consumption]. Available from: <https://zakon.rada.gov.ua/laws/show/z0452-10#Text>. Ukrainian.
- Rules for accepting wastewater into centralized drainage systems. Ukrainian. Available from: <https://zakon.rada.gov.ua/laws/show/z0056-18#n192>.
- Paisio CE, Oller ALW, Ibañez SG, Talano MA, González PS, Medina MI, et al. Bioremediation as a useful biotechnological strategy for the treatment of phenolics: advances, challenges and future prospects. Chapter 4. In: Bioremediation: Processes, Challenges and Future Prospects. J.B. Velázquez-Fernández, S. Muñoz-Hernández, editors. Nova Science Publishers, New York. 2014. p. 57–79.
- Smirnova VS, Khudorozhkova SA, Ruchkinovala OI. [Dephenolization of highly concentrated wastewater from industrial enterprises]. Bull Perm National res Polytechnic Univ Construction and architecture. 2017; 8:52–63. Russian.
- Solyanikova IP, Golovleva LA. Physiological and biochemical properties of actinobacteria as the basis of their high biodegradative activity (review). Appl Biochem Microbiol 2015; 51(2):143–149.
- Nogina T, Fomina M, Dumanskaya T, Zelena L, Khomenko L, Mikhalovsky S, et al. A new *Rhodococcus aetherivorans* strain isolated from lubricant-contaminated soil as a prospective phenol-biodegrading agent. Appl Microbiol Biotechnol. 2020; 104(8):3611–3625.
- Lisovitskaya OV, Terekhova VA. [Phytotesting: basic approaches, problems of the laboratory method and modern solutions]. Doklady po ekologicheskomu pochvovedeniyu. 2010; 1(13):1–18. Russian.
- Pimenova EV, Kozlova GA, Burkova MV. [Assessment of microbial detoxification of tornado herbicide based on glyphosate by phytotesting method]. Bulletin of the Perm National Research Polytechnic University. Chemical technology and biotechnology. 2011; (12):160–4. Russian.
- Kabirov RR, Sagitova AR, Sukhanova NV. [Development and use of a multicomponent test system for assessing the toxicity of the soil cover of

- urban areas]. Ecology. 1997; 6:408–11. Russian.
12. Veliyev MG, Salmanov MA, Bektashi NR, Aliyeva SR, Gasanova AR. Investigation of the process of biodegradation of aromatic hydrocarbons and phenols by a method of reversed-phase high-efficient liquid chromatography. Science Journal of Analytical Chemistry. 2013; 1(2):7–11.
 13. Kislitsina MN, Borisova GG. [Effect of hydroquinone and resorcinol on the morphometric characteristics of *Elodea canadensis* Michx. and *Potamogeton perfoliatus* L]. In: Proceedings of the IX International Symposium Phenolic Compounds: Fundamental and Applied Aspects; 2015 Apr 20–25; Moscow, Russia. Moscow: IPP RAS, 2015. p. 292–5. Russian.
 14. Koretska N, Karpenko O, Baranov V, Lubenets V, Nogina T. [Biological properties of surface-active metabolites of *Rhodococcus erythropolis* Au-1 and their prospects for crop technology]. Innovative Biosystems and Bioengineering. 2019; 3(2):77–85. Ukrainian.
 15. Rychkova MI. [Influence of *Rhodococcus*-biosurfactants on the desorption and degradation of petroleum hydrocarbons in the soil]. Abstract of dissertation. ... candidate biol. science. Perm. 2007. 27p. Russian. Available from: https://static.freereferats.ru/_avtoreferats/01003319309.pdf.
 16. Mishenina II. [Development of a biological method for the disposal of drugs unsuitable for medical use, phenol derivatives]. Abstract of dissertation. ... candidate biol. science. Perm, 2008. 21p. Russian. Available from: <http://medical-diss.com/docreader/241424/a/#?page=1>
 17. Mukhutdinova AN. [Biodegradation of drotaverine hydrochloride by actinobacteria of the genus *Rhodococcus*]. Abstract of dissertation. ... candidate biol. science. Perm. 2014. 27p. Russian. Available from: <http://earthpapers.net/preview/589441/a/#?page=1>
 18. Tyumina EA. [Biodegradation of sodium diclofenac by actinobacteria of the genus *Rhodococcus*] [dissertation]. Perm (Russia): Perm State University; 2019. Russian. Available from: http://perm-sc.ru/images/docs/disser/D.999.219.02/Tyumina/Tyumina_disser.
 19. Henner P, Schiavon M, Druelle V, Lichtfouse E. Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. Organic Geochemistry. 1999; 30:963–9.
 20. Sindireva AV, Lovinetskaya SB. [Evaluation of phytotoxicity of soil polluted by oil products]. Vestnik NSAU (Novosibirsk State Agrarian University). 2017; 1(42):116–21. Russian.
 21. Njoku KL, Akinola MO, Taiwo BG. Effect of gasoline diesel fuel mixture on the germination and the growth of *Vigna unguiculata* (Cowpea). African Journal of Environmental Science and Technology. 2009; 3(12):466–71.
 22. Kulagin NV, Archipova NS, Breus IP. Assessment of phytotoxicity of hydrocarbons of different chemical nature in their direct contact with seeds, and indirectly, through soil. Bulletin of TGSPU. Biological sciences. 2011; 4(26):70–5. Russian.
 23. Dzhura NM. [Potentials of using plant test systems for biomonitoring oil polluted soils]. Biol Stud. 2011; 5(3):183–96. Ukrainian.
 24. Turkovskaya OV, Dubrovskaya EV, Golubev SN, Bondarenkova AD, Balandina SA, Pozdnyakova NN. [Influence of soil-dwelling fungi on seedlings of broom sorghum in the presence of polycyclic aromatic hydrocarbons]. Plant physiology. 2019; 66(5):384–93. Russian.
 25. Fedenko VS, Shemet SA, Struzhko VS. [Functional dependences of phytotoxicity of cross herbicide and accumulation of phenolic compounds in corn seedlings]. Bulletin of the Kharkov National Agrarian University. Biology series. 2012; 2(26):85–91. Ukrainian.
 26. Valerko RA. [The use of the biotesting method for anthropically polluted soils to their estimates ecotoxicological]. Bulletin of KhNAU. Ecology of soils. 2013; (2):262–6. Ukrainian.
 27. Yakovyshyna TF. [Biotesting system of the toxicity of the soil contaminated by the heavy metals]. Bulletin of the Sumy National Agrarian University. Series Agronomy and Biology. 2014; 3(27):70–3. Ukrainian.
 28. MR 2.1.7.2297-07 [Substantiation of hazard class of production and consumption waste by phytotoxicity. Methodical recommendations].

- Russian. Available from: http://www.infosait.ru/norma_doc/52/52957/index.htm.
29. Dubrovskaya EV, Pozdnyakova NN, Muratova AY, Turkovskaya OV. Changes in phytotoxicity of polycyclic aromatic hydrocarbons in the course of microbial degradation. *Russian Journal of Plant Physiology*. 2016; 63(1):172–9.
 30. Khomenko LA, Nogina TM. [Biodegradation of paracetamol by nocardioform actinobacteria]. *Mikrobiol Z*. 2019; 81(4):76–89. Ukrainian.
 31. Beshley ZM, Beshley SV, Baranov VI, Terek OI. [Use of plants test systems for assess the toxicity of polluted substrates]. *The Bulletin of Kharkiv national agrarian university. Series Biology*. 2014; (31):97–102. Ukrainian.
 32. Mironova NG. [Estimation of radiation background and phytotoxicity of ecotope of technogenic lakes of Small Polesye]. *Scientific Bulletin of UNFU*. 2013; 23(13):145–51. Ukrainian.
 33. Yakovishina TF. [Ecotoxicological assessment of urban soils by the biotesting method]. *Universum: chemistry and biology: electron scientific zhurn*. 2015; 8(16). Russian. URL: <https://7universum.com/ru/nature/archive/item/2491>.
 34. Kurnik K, Treder K, Skorupa-Kłaput M, Tretyn A, Tyburski J. Removal of phenol from synthetic and industrial wastewater by potato pulp peroxidases. *Water Air Soil Pollution*. 2015; 226(8): 226–54.
 35. Drozdovskaya OA. [Search of microorganisms – indicators and destructors of phenols in the coastal waters of the Far Eastern seas]. Abstract of dissertation. ... candidate biol. science. Vladivostok. 24 p. Russian. Available from: https://static.freereferats.ru/_avtoreferats/01000303000.pdf

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