ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 4, pp. 433–439. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © A.A. Ovchinnikova, A.A. Vetrova, A.E. Filonov, A.M. Boronin, 2009, published in Mikrobiologiya, 2009, Vol. 78, No. 4, pp. 484–490.

EXPERIMENTAL ARTICLES

Phenanthrene Biodegradation and the Interaction of *Pseudomonas putida* BS3701 and *Burkholderia* sp. BS3702 in Plant Rhizosphere

A. A. Ovchinnikova[a,1], A. A. Vetrova[a,b], A. E. Filonov[a,b], and A. M. Boronin[a,b]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

[b] *Pushchino State University, pr. Nauki 3, Pushchino, Moscow oblast, 142290 Russia*

Received July 15, 2008

Abstract—The interaction between the strains degrading polycyclic aromatic hydrocarbons, *Pseudomonas putida* BS3701 and *Burkholderia* sp. BS3702, was studied in the course of phenanthrene degradation in plant rhizosphere. Strain BS3702 was shown to accumulate 1-hydroxy-2-naphthoic acid (which is toxic for plants); it was then utilized by strain BS3701, which thereby increased the resistance of plants to the pollutant and to the toxic intermediate. With this type of interaction (cooperation), the efficiency of phenanthrene degradation was noted to decrease.

Key words: polycyclic aromatic hydrocarbons, phytoremediation, rhizosphere.

DOI: 10.1134/S0026261709040067

As a result of industrial development, the biosphere has become unable to cope with anthropogenic contamination. One of the modern approaches to environmental contamination is phytoremediation, i.e., application of plants and associated microorganisms for purification of soil and aquatic ecosystems from various pollutants. This technology is based on the so-called rhizospheric effect, which intensifies microbial degradation of a pollutant in soil. This process is facilitated by exudates of plant roots, which provide the populations of rhizospheric microorganisms–destructors with additional sources of carbon, energy, sometimes oxygen, trace elements, and enzymes [1], while the penetration of roots into soil facilitates the interaction between the plants, microorganisms, and pollutant. The major virtues of phytoremediation are possible recultivation of vast territories, relatively low cost as compared with other technologies, high efficiency, and an absence of a negative impact on the environment [2].

Polycyclic aromatic hydrocarbons (PAH) are widespread and stable environmental pollutants, which result from the burning of fossil fuel and are by-products of industrial activity. The main contribution to their degradation is made by those heterotrophic microorganisms which can utilize oil hydrocarbons as the major energy and carbon source. It becomes presently more and more evident that the rate of PAH degradation in the environment may depend on the metabolism of particular microbial species and the capabilities of entire microbial communities [3]. Based on the above, the process of bioremediation more and more often employs bacterial associations rather than monocultures. Co-metabolism and cooperation are very important. The application of more complex microbial systems for biotechnological purposes requires consideration of the rearrangements of internal succession and inter-population dynamics, which result in substantial modifications of the properties of the community in time [3].

The studies on phytoremediation are carried out in many countries worldwide, but the interaction of the components of this biotechnology (microorganisms, plants, and pollutants) has been poorly studied.

The goal of the present work was to study the interaction of destructor microorganisms and its influence on the efficiency of phenanthrene biodegradation in plant rhizosphere.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in the work were *Pseudomonas putida* BS3701 (pBS1141, pBS1142) and *Burkholderia* sp. BS3702 (pBS1143) (Table) isolated from the soil samples contaminated with oil products and from the wastes of a by-product of a coke plant collected in the Moscow oblast and described as naphthalene and phenanthrene destructors [4, 5].

[1] Corresponding author; e-mail: Anastasia_777@rambler.ru

Bacterial strains and plasmid used in the work

Strain	Characteristics	Source
Pseudomonas putida BS3701 (pBS1141, pBS1142)	Nah^+ Sal^+ $2MeNah^+$ Gnt^+ Phn^+ Hna^+	LPB, IBPM RAS
Burkholderia sp. BS3702 (pBS1143)	Nah^+ Sal^+ Phn^+ Ant^+ Hna^-	LPB, IBPM RAS

Note: Nah^+, the ability to grow on naphthalene; $2MeNah^+$, on 2-methyl naphthalene; Gnt^+, on gentisate; Phn^+, on phenanthrene; Ant^+, on anthracene; Hna^+, on 1-hydroxy-2-naphthoic acid; Sal^+, on salicylate; LPB, Laboratory of Plasmid Biology; IBPM RAS, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences.

Nutrient media. Bacteria were grown on complete nutrient media, LB agar and LB broth [6], containing the following (g/l): bactotryptone (Difco), 10.0; yeast extract (Difco), 5.0; NaCl, 10.0.

The minimal medium for bacteria was synthetic Evans medium [7] of the following composition (g/l): K_2HPO_4, 8.71; 5 M NH_4Cl solution, 1; 0.1 M Na_2SO_4 solution, 1; 62 mM $MgCl_2$ solution, 1; 1 mM $CaCl_2$ solution, 1; 0.005 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ solution · $4H_2O$, 1; and trace elements, 1. The solution of trace elements in 10% HCl contained (g/l): ZnO, 0.41; $FeCl_2 \cdot 6H_2O$, 5.4; $MnCl_2 \cdot 4H_2O$, 2.00; $CuCl_2 \cdot 2H_2O$, 0.17; $CoCl_2 \cdot 6H_2O$, 0.48; H_3BO_3, 0.06; pH 7.0.

For solid media, 20 g/l agar (Pronadisa, Spain) was added.

For cultivation with naphthalene or phenanthrene as a sole carbon and energy source, the microorganisms were grown on an agarized mineral medium in the vapors of these compounds. King B medium (KB) [8] was used for the monitoring of the strains. For cultivation in a liquid medium, naphthalene and phenanthrene were added as powder in the concentrations of 2 and 0.5 g/l, respectively.

Gnotobiotic system for plant cultivation [9]. Plants were cultivated in closed plastic vessels, $77 \times 77 \times 97$ mm (Magenta vessel, Sigma), in 150 g of sand. Pheanthrene was added as powder in the concentration of 500 μg/g of sand.

The "Murashige and Skoog basal salt" medium (Sigma) was used for mineral nutrition of the plants. The plants grown without phenanthrene and without inoculation were used as a positive control and the plants grown with phenanthrene but without inoculation were used as a negative control.

Sterilization of the seeds. The seeds of white mustard (*Sinapis alba* L.) were sterilized with 5% sodium hypochlorite solution for 3 h and then washed four times with sterile tap water for 2 h. The seeds were placed on the plates with LB agar and incubated for 18–20 h at 24°C for sterility control.

Introduction of degrading microorganisms into model systems. In the experiment, a microbial suspension was introduced directly into sand together with the mineral medium; the concentration of microorganisms was 1.5×10^8 CFU/g of sand.

Twenty mustard seedlings were planted into one pot. The plants were grown in the following regime: 12-h light period and 12-h dark period at 20°C. After 7 days, washouts were made from the roots and rhizosphere on LB agar and KB medium for the qualitative and quantitative analysis of bacteria.

Scheme of the experiment. The following model systems were used for the study of interaction of degrader strains and assessment of phenanthrene decrease:

1. with phenanthrene, without microorganisms and plants: for estimation of abiotic decrease of phenanthrene;
2. with plants, without microorganisms and phenanthrene: as a control of biometric characteristics of white mustard;
3. with plants, without microorganisms, with phenanthrene: for estimation of the phytotoxic effect of phenanthrene on plants;
4. with plants, phenanthrene, and strain BS3701: for the study of the effect of introduction of a PAH-degrading strain on the biometric properties of white mustard in the presence of the pollutant, the study of the content of bacteria in plant rhizosphere and rhizoplane, and for the study of the effect of strain BS3701 on the efficiency of phenanthrene degradation;
5. with plants, phenanthrene, and strain BS3702: for the study of the effect of introduction of a PAH-degrading strain on the biometric properties of white mustard in the presence of the pollutant, the content of bacteria in plant rhizosphere and rhizoplane, and the effect of strain BS3702 on the efficiency of phenanthrene degradation;
6. with plants, phenanthrene, strains BS3701 and BS3702: for the study of the effect of joint introduction of PAH-degrading strains on the biometric properties of white mustard in the presence of the pollutant and on the efficiency of phenanthrene degradation, for the study of the content of microorganisms in plant rhizosphere and rhizoplane.

Determination of phenanthrene content in the samples. Sand (150 g) was transferred form the model system into a flask and extracted with 100 ml of methanol for 16 h at 25°C. Then, 200 μl of methanol extract was analyzed by HPLC on a LKB-2150 chromatograph (Sweden): C18 column (Nova Pak Waters, United States), the system of 60% methanol–40% water, UV detector, working wavelength 280 nm, and flow rate 1 ml/min. The phenanthrene concentration was calcu-

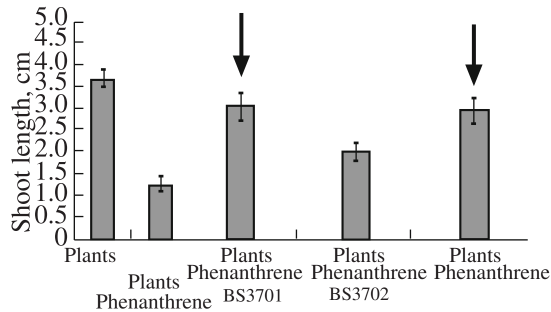

Fig. 1. Length of a white mustard shoots after 7 days of cultivation. Arrows indicate the variants with the best protective effect.

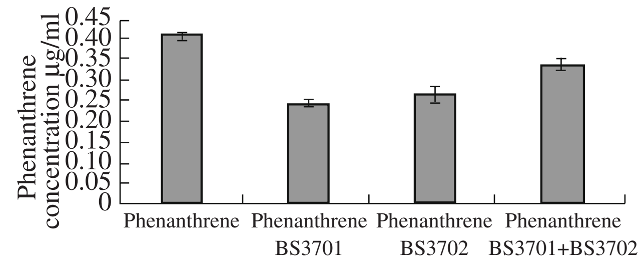

Fig. 2. Residual phenanthrene concentration after 7 days of plant cultivation.

lated by the peak area as compared with the peak area of the control sample.

Kinetics of microbial growth on phenanthrene. The specific maximum growth rate in batch culture on phenanthrene was determined at cultivation of microorganisms in flasks with Evans medium (250 ml). Phenanthrene was introduced as powder (500 mg/l). The cultures grown on Evans medium with phenanthrene were used as inoculum. The initial concentration of bacteria in flasks was $1–4 \times 10^5$ cells/ml. The optical density of the culture liquid was determined spectrophotometrically in a FEK-56M-U42 colorimeter at 540 nm in a 0.5-cm cuvette. Concentrations of microorganisms in the growth medium were determined by the method of standard serial dilutions with plating on King B medium (KB [8]). On this medium, the colonies of two strains were morphologically different; moreover, strain BS3701 exhibited fluorescence under UV illumination. The samples were taken every 3 h. The specific maximum growth rate (μ_{max}) was defined as $\mu_{max} = \frac{\ln\frac{X_0}{X}}{t - t_0}$, where X_0 is the initial cell number, X is the final cell number, and t and t_0 are time points [10].

Determination of the content of gentisic acid and 1-hydroxy-2-naphthoic acid. The culture grown in a liquid Evans medium with phenanthrene was sampled (1.5 ml), the cells were precipitated, and the supernatant was transferred into a clean Eppendorf test tube and extracted with methanol.

Gentisate and 1-hydroxy-2-naphthoate concentrations were analyzed in a HPLC chromatograph LKB-2150 (Sweden), column Symmetry300^{TM} C_4 (150 × 3.9 mm) (Waters, United States), mobile phase: methanol–water–acetic acid (34:66:1), elution rate 1.2 ml/min, and working wavelength 230 nm.

For identification, the retention times of the analyzed compounds and standards were compared. Quantitative calculation of the chromatograms was carried out by the method of absolute calibration. Variation coefficients for gentisic acid and 1-hydroxy-2-naphthoic acid were 5.2% and 7.6%, respectively. The detection limit was 5 ng for gentisic acid and 11.2 ng for 1-hydroxy-2-naphthoic acid.

The study of 1-hydroxy-2-naphthoic acid toxicity for plants. The toxic effect of 1-hydroxy-2-naphthoic acid on plants was studied in gnotobiotic systems with sterile sand, plants (white mustard), Murashige mineral medium, and 1-hydroxy-2-naphthoic acid introduced in two concentrations, 15 and 150 mg/l. The model systems with plants but without 1-hydroxy-2-naphthoic acid served as a positive control.

RESULTS

The study of the protective effect on plants at introduction of degrader strains *Pseudomonas putida* BS3701 (pBS1141, pBS1142) and *Burkholderia* sp. BS3702 (pBS1143). The toxic effect of phenanthrene was demonstrated by suppression of the growth of the shoots of white mustard.

After 7 days of incubation of the plants in the model systems, the shoot length was determined, which correlated with the positive effect of microorganisms on plants. The best protective effect on plants (Fig. 1) was observed in the systems inoculated by strain BS3701 and by the mixture of degrading microorganisms, BS3701 and BS3702.

The model system containing strain BS3702 showed a lower protective effect.

Phenanthrene biodegradation. Under joint cultivation of BS3701 and BS3702 in a phenanthrene-contaminated system, degradation was lower (17%) than in the presence of one of the degrader strains (37%) (Fig. 2).

The dynamics of the numbers of degrading microorganisms and root colonization. The capacity for root colonization and the dynamics of the quantities of monocultures and the mixed culture of degrading microorganisms were determined in the study of bacterial numbers in the rhizoplane and rhizosphere of plants under contamination of a model system with phenanthrene.

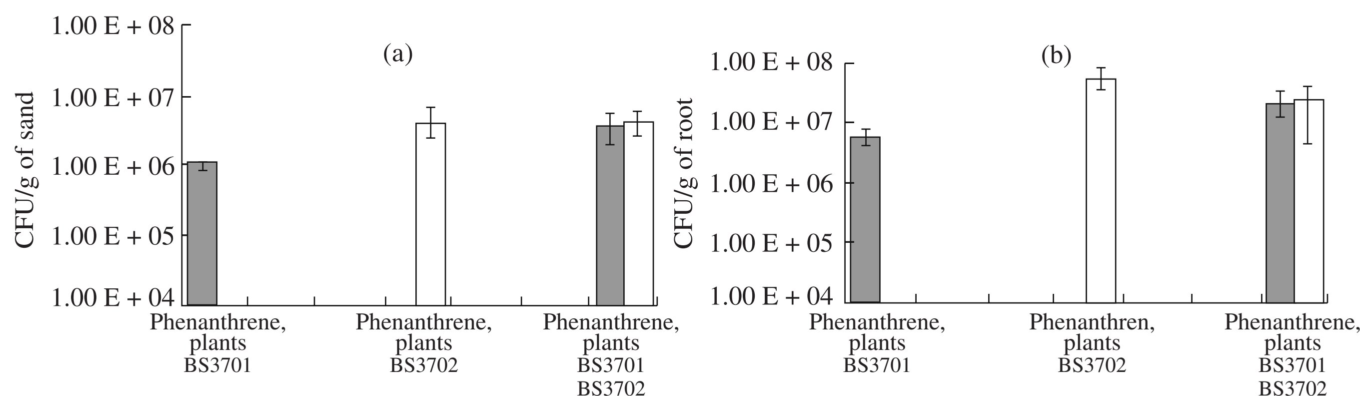

Fig. 3. Microbial numbers after 7 days of plant cultivation: in the rhizosphere (a) and in the rhizoplane (b). Light columns, BS3702; dark columns, BS3701.

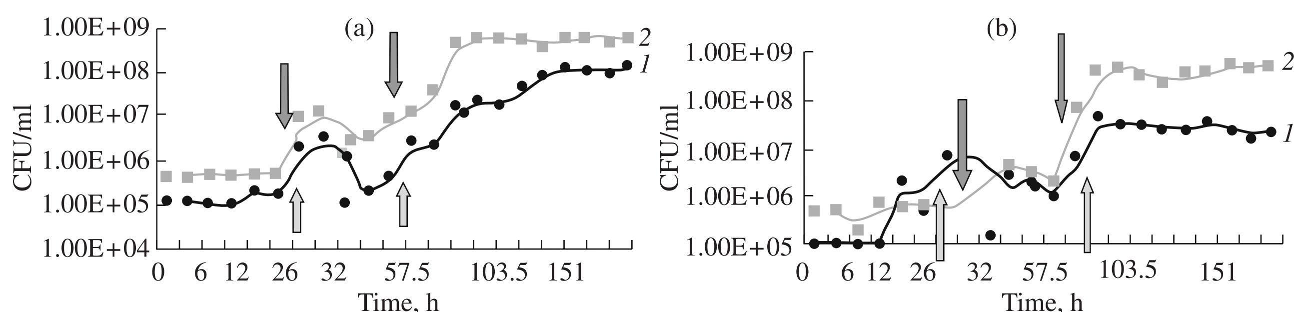

Fig. 4. Cultivation of BS3701 and BS3702 in liquid mineral medium with phenanthrene: separate (a) and joint (b). Arrows show the parts of the growth curves for which the maximum specific growth rates were calculated. BS3701 (*1*); BS3702 (*2*).

A significant difference was observed in the concentrations of microorganisms on the roots (Fig. 3a) and in the rhizosphere of mustard (Fig. 3b). The numbers of BS3701 in both rhizosphere and rhizoplane were higher at joint cultivation with BS3702 than in case of the system with BS3701 alone. At separate cultivation of the monocultures, the content of strain BS3702 was higher as compared with the quantity of BS3701 both on the roots and in the near-root zone of the plants.

Growth kinetics of strains *Pseudomonas putida* BS3701(pBS1141, pBS1142) and *Burkholderia* sp. BS3702(pBS1143). The experiment was performed under batch cultivation in a liquid Evans mineral medium with phenanthrene as a carbon and energy source. Apart from the pure cultures of strains BS3701 and BS3702, the mixed culture of these microorganisms (in equal ratio) was studied in the experiment. The maximum specific growth rates were calculated for the two parts of the growth curves with the maximum increase of microbial numbers.

At separate cultivation of degrading microorganisms BS3701 and BS3702 in liquid Evans mineral medium with phenanthrene (Fig. 4a), the value of the maximum specific growth rate for the first part of the growth curve was higher than for the second one (0.5 and 0.1 h^{-1}, respectively) for both strains. At joint cultivation (Fig. 4b), the values of the maximum specific growth rate for the first part of the growth curve for both BS3702 and BS3701 were lower than in monocultures (0.24 and 0.22 h^{-1}, respectively), while the values determined for the second part of the growth curve did not decrease that much (0.25 and 0.17 h^{-1}, respectively).

Accumulation of metabolites at phenanthrene degradation in liquid mineral medium by strains *Pseudomonas putida* BS3701(pBS1141, pBS1142) and *Burkholderia* sp. BS3702(pBS1143). Accumulation of the intermediates of the pathway of phenanthrene degradation was assessed by HPLC the analysis of liquid samples of the culture.

At separate cultivation of degrader strains BS3701 and BS3702, the latter accumulated both gentisate and 1-hydroxy-2-naphthoic acid; 1-hydroxy-2-naphthoic acid was produced in greater amounts than gentisate (14.57 and 0.58 mg/l, respectively). Strain BS3701 was able to consume both intermediates practically without their accumulation, which resulted in relatively low concentrations of these substrates in the systems at separate cultivation of the microorganisms (0.5 mg/l for gentisate and 0.11 mg/l for 1-hydroxy-2-naphthoate).

As can be seen from Figure 5, the system containing both degrader strains at first showed the accumulation of 1-hydroxy-2-naphthoic acid; its uptake began after 35 h of cultivation with concurrent accumulation of gentisic acid.

Toxicity of 1-hydroxy-2-naphthoic acid to plants. In view of accumulation of 1-hydroxy-2-naphthoic acid, which is known to be nontoxic for microorganisms [5], the following stage of the work pursued the study of toxicity of 1-hydroxy-2-naphthoate to plants.

The toxic effect of 1-hydroxy-2-naphthoic acid on shoots of white mustard was studied in the experiment with gnotobiotic systems consisting of sterile sand, plants, and 1-hydroxy-2-naphthoic acid introduced as a solution together with the mineral medium for plant growth.

Our results revealed (Fig. 5) that 1-hydroxy-2-naphthoic acid had a toxic effect on the shoots even at 15 mg/l, inhibiting the growth of white mustard by 18%; at 150 mg/l, the length of plant shoots decreased by 64% as compared with the control.

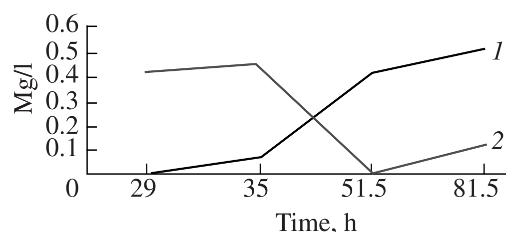

Fig. 5. The dynamics of accumulation of gentisate (*1*) and 1-hydroxy-2-naphthoate (*2*) under joint cultivation of *P. putida* BS3701 (pBS1141, pBS1142) and *Burkholderia* sp. BS3702(pBS1143).

DISCUSSION

The interaction of degrader strains was studied in model systems in accordance with the scheme described in Materials and Methods. Such artificial ecosystems, being simplified models of natural systems, make it possible to establish the basic interrelations between their components.

As is known, PAH are consumed by bacteria in the water phase [11] and, hence, their uptake depends on their solubility in water. Besides, sorption by soil particles protects these molecules from degradation by microbes and is one of the main reasons of their long-term stability in soil. Phenanthrene is a poorly soluble PAH (1.3 mg/l) and, therefore, is not easily accessible to microorganisms.

One of the methods of introducing microorganisms into model systems such as bacterization of plant seedlings has been described in the literature previously [12, 13]. This method of introduction showed the protective effect on plants at phenanthrene contamination of model systems, but the degradation of weakly sublimated phenanthrene was practically absent. Probably, microorganisms were localized on the roots and in the rhizosphere, thus protecting plants from the negative effect of PAH, while the major volume of contaminated sand was not engaged in the biodegradation process. In this connection, at the next stage of work the degrader strains were introduced by direct inoculation of a bacterial suspension into sterile sand. Such a method proved to be more effective, and the data obtained indicated phenanthrene degradation in the whole model system.

Originally it was expected that phenanthrene degradation under joint cultivation of strains BS3701 and BS3702 will be more effective (Fig. 2) because the microbes were not antagonists, judging from compatibility tests and their pathways of PAH utilization were different [5]. Strain BS3701 degrades phenanthrene with the formation of salicylate, which is further oxidized to catechol and then to the intermediates of the Krebs cycles, while strain BS3702 oxidizes salicylate to gentisate, which is further cleaved to the intermediates of the Krebs cycle. However, phenanthrene decomposition was worse under joint cultivation of two strains than at separate introduction of each destructor into a model system.

Introduction of strains BS3701 and BS3702 into model systems resulted in several effects on the shoots of white mustard (Fig. 1). Under joint introduction of the microorganisms, as well as at inoculation of the system with BS3701 alone, the effect of plant protection from the action of phenanthrene was visualized by an increase in shoot length relative to the negative control (with the pollutant but without the microorganisms). With BS3702 alone, the shoot length was lower by 33% than in the combinations described above.

Since phenanthrene degradation at introduction of BS3702 was 37% (Fig. 2), while the protective effect on plants was insignificant (Fig. 1), it was suggested that this microorganism accumulated an intermediate in the course of phenanthrene degradation, which was probably toxic to plants. At joint cultivation of BS3701 and BS3702, the former is probably able to consume the accumulated intermediate, thus eliminating the toxic effect and concurrently lessening the degree of phenanthrene degradation (17%) due to utilization of the most advantageous substrate.

At the following stage of the work, the changes in the number of microorganisms on the roots and in the rhizosphere of the plants were analyzed (Fig. 3). The results showed that the content of introduced bacteria on the roots (Fig. 3b) was higher nearly by an order of magnitude than in the rhizosphere (Fig. 3a). This phenomenon is probably associated with exudation of the plant roots, resulting in creation of an ambient medium enriched in nutrients, where microbial activity is stimulated. Plant exudates contain such basic components as sugars, organic acids, and amino acids. The ability for effective colonization of plant roots by different strains of the same genus varies significantly [11]. Pre-

viously it has been shown that the chemotaxis of microorganisms with respect to some organic acids and amino acids present in root exudates plays a key role in the colonization process. At joint cultivation of the two microbes, the quantity of strain BS3701 increased both on the roots and in the rhizosphere of plants as compared with its concentration in the system with introduced monoculture. This fact probably also indicates the presence in the medium of a substrate stimulating the increase of BS3701 content.

For interpretation of the reported results, an experiment was performed under batch cultivation in the Evans liquid mineral medium with phenanthrene as a sole carbon and energy source. In addition to the pure cultures of strains BS3701 and BS3702, the mixed culture of these microorganisms (equal ratio) was analyzed in the experiment. The maximum specific growth rates were calculated for two parts of the growth curves with the maximum increase of microbial biomass (Fig. 4). At separate cultivation of the microorganisms (Fig. 4a), the maximum specific growth rate for the first part was five times higher than for the second one in both strains. At joint cultivation (Fig. 4b), the values of the maximum specific growth rate for BS3702 were lower for both parts and were comparable with the values for BS3701. It could be associated with the character of interaction of both strains, namely with secretion of intermediates by one strain and their uptake by the other strain as more accessible carbon sources.

The samples of the culture liquid were analyzed by HPLC to confirm the hypothesis of a toxic-to-plants intermediate secreted at phenanthrene degradation. As has been shown previously [14], one of major initial metabolites of phenanthrene degradation by strains BS3701 and BS3702 is 1-hydroxy-2-naphthoic acid, and its accumulation was observed in our work in case of strain BS3702 with simultaneous accumulation of gentisate. Gentisate accumulated in the cultures of both strains under separate cultivation in small and nearly equal amounts (0.50 mg/l for BS3702 and 0.58 mg/l for BS3701), while no decrease of its amount was observed at the point of maximum biomass growth under joint cultivation (0.50 mg/l).

In case of 1-hydroxy-2-naphthoic acid, under separate cultivation of BS3701 and BS3702, the latter produces and accumulates this acid in greater amounts as compared with BS3701. Under joint cultivation of two strains, the concentration of accumulated 1-hydroxy-2-naphthoate was 133 times less. This fact is evidence of more efficient uptake of this intermediate in the mixed culture.

Thus, the interactions between the microorganisms observed under joint introduction of BS3701 and BS3702 at phenanthrene contamination were expressed in cooperation, i.e., strain BS3702 degraded phenanthrene, accumulating high amounts of an intermediate (1-hydroxy-2-naphthoic acid), while BS3701 utilized 1-hydroxy-2-naphthoate as a more advantageous substrate than phenanthrene.

At the following stage of the work, the toxicity of 1-hydroxy-2-naphthoate to white mustard was estimated. This metabolite indeed proved to be toxic to plants, which was demonstrated by suppression of shoot growth (Fig. 5).

Based on the above, it may be concluded that selection of microbial–plant associations for the most effective biodegradation of polycyclic aromatic hydrocarbons requires the study of and allowance for the interaction of degrading bacteria with each other and with the plants in order to avoid undesirable events, which may have a negative effect both on microbial–plant associations and on the process of remediation of contaminated territories as a whole.

ACKNOWLEDGMENTS

The work was supported by the International Science and Technology Center (project ISTC no. 2366), state contract Tema RNP 2.1.1.7789, CRDF RUB2-010001-PU-05, RNP 2.1.1.9290, and the Russian Foundation for Basic Research (projects RFFI 08-04-99019-r_ofi and RFFI 08-04-90028-Bel_a).

REFERENCES

1. *Molekulyarnye osnovy vzaimootnoshenii assotsiativnykh mikroorganizmov s rasteniyami* (Molecular Basis of the Interaction of Associated Microorganisms with Plants), Ignatov, V.V, Ed., Inst. Biochem. Physiol. Microorg., Moscow: Nauka, 2005.
2. Nazarov, A.V. and Ilarionov, S.A., Potential Application of Plant–Microbial Interaction for Bioremediation, *Biotekhnol.*, 2005, no. 5, pp. 54–62.
3. Pechurkin, N.S., Bril'kov, A.V., and Marchenkova, T.V., *Populyatsionnye aspekty biotekhnologii*, (Populational Aspects of Biotechnology), Novosibirsk: Nauka. Sib. otd, 1990.
4. Balashova, N.V., Kosheleva, I.A., Filonov, A.E., Gayazov, R.R., and Boronin, A.M., Phenanthrene- and Naphthalene-degrading Strains of *Pseudomonas putida, Mikrobiologiya*, 1997, vol. 66, no. 4, pp. 488–493 [*Microbiology* (Engl. Transl.), vol. 66, no. 4, pp. 408–412].
5. Balashova, N.V., Degradation of Phenanthrene and Naphtalene by Bacteria of the Genera *Pseudomonas* and *Burkholderia, Cand. Sci. (Biol.) Dissertation*, Pushchino, 2000.
6. Miller, J.H., *Experiments in Molecular Genetics*, Cold Spring Harbor: Cold Spring Harbor Laboratories, 1972 [Russ. Transl. Moscow: Mir, 1976].
7. Evans, C.G.T., Herbert, D., and Tempest, D.B., The Continuous Cultivation of Microorganisms. Construction of a Chemostat, *Methods Microbiol.*, 1970, vol. 2, pp. 277–327.
8. King, O.E., Ward, W., and Raney, D.E., Two Simple Media for Demonstration of Pyocyanin and Fluorescin, *J. Lab. Clin. Methods*, 1954, vol. 44, no. 2, pp. 301–307.

9. Simons, M., Van der Bij, A.J., Brand, J., de Weger, L.A., Wijffelman, C.A., and Lugtenberg, B.J., Gnotobiotic System for Studying Rhizosphere Colonization by Plant Growth-Promoting *Pseudomonas* Bacteria, *Mol. Plant–Microbe Interact.*, 1996, vol. 9, no. 7, pp. 600–607.
10. Pirt, S.J., *Principles of Microbe and Cell Cultivation*, Oxford: Blackwell, 1975 [Russ. Transl. Moscow: Mir, 1978].
11. Kuiper, I., Lagendijk, E.L., Bloemberg, G.V., and Lugtenberg, B.J.J., Rhizoremediation: a Beneficial Plant-Microbe Interaction, *Mol. Plant-microbe Interactions*, 2004, vol. 17, no. 1, pp. 6–15.
12. Kravchenko, L.V., Azarova, T.S., Makarova, N.M., and Tikhonovich, I.A., The Effect of Tryptophan Present in Plant Root Exudates on the Phytostimulating Activity of Rhizobacteria, *Mikrobiologiya*, 2004, vol. 73, no. 2, pp. 195–198 [*Microbiology* (Engl. Transl.), vol. 73, no. 2, pp. 156–158].
13. Ignatova, A.A., Vetrova, A.A., Lisov, A.V., Filonov, A.E., Puntus, I.F., and Boronin, A.M., Dynamics of Numbers and Interaction of Plant Growth-Stimulating Pseudomonads and of Naphthalene-Degrading Strains in White Mustard Rhizosphere, *Biotekhnol.*, 2006, no. 6, pp. 35–43.
14. Puntus, I.F., Filonov, A.E., Kosheleva, I.A., Gayazov, R.R., Karpov, A.V., and Boronin, A.M., Isolation and Characterization of Microorganisms Degrading Polycyclic Aromatic Hydrocarbons, *Mikrobiologiya*, 1997, vol. 66, no. 2, pp. 269–272 [*Microbiology* (Engl. Transl.), vol. 66, no. 2, pp. 222–225].