Microbiology, Vol. 70, No. 2, 2001, pp. 211–216. Translated from Mikrobiologiya, Vol. 70, No. 2, 2001, pp. 253–258.

EXPERIMENTAL ARTICLES

Coexistence of Genetically Engineered *Escherichia coli* Strains and Natural Microorganisms in Experimental Aquatic Microcosms

T. V. Kargatova[1], E. E. Maksimova, and L. Yu. Popova

Institute of Biophysics, Siberian Division, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia

Received November 29, 1999; in final form, September 19, 2000

Abstract—In experimental aquatic microcosms (AMCs), the population of the *Escherichia coli* strain Z905 harboring the recombinant plasmid pPHL7 (Ap^rLux^+) was found to gradually accumulate AMC-adapted cells, which retained the plasmid but differed from the original cells in some biochemical and physiological characteristics. Both the original and the AMC-adapted *E. coli* cells could coexist with the native AMC microflora for one year or longer. When introduced into AMCs together with native pseudomonads, the AMC-adapted *E. coli* Z905-33 (pPHL7) cells were more competitive than the nonadapted cells.

Key words: *Escherichia coli*, aquatic microecosystems, recombinant plasmid.

The application of genetically engineered microorganisms (GEMs) is presently limited because of the shortage of experimental data on their interactions with native microflora in natural ecosystems [1–3]. The survival of GEMs in the environment depends on many factors, both biotic (such as population interactions) and abiotic (such as pH, salinity, temperature, illumination, humidity, and the availability of nutrients).

Of great importance are competitive interactions between introduced GEMs and native microorganisms, since the former may alter the microbial diversity in nature [4] and the latter may suppress the GEMs that were introduced, for instance, into a polluted environment for bioremediation purposes.

To be used in relevant studies, recombinant strains should contain markers that allow researchers to easily detect these strains in ecosystems. In the present study, we used *E. coli* strain Z905 harboring the recombinant plasmid pPHL7 with the reporter luminescence *lux* genes [5], whose expression allows the metabolic activity of this strain to be easily evaluated. Our interest in this strain was also stimulated by the fact that recombinant *E. coli* strains are widely used in biotechnology, and their occurrence in the environment cannot be excluded.

The aim of the present study was to evaluate the competitive ability of the *E. coli* strain Z905 (pPHL7) introduced into aquatic microcosms with reference to the typical soil bacteria *Pseudomonas fluorescens* and *P. putida.*

MATERIALS AND METHODS

Bacterial strains. Experiments were carried out with the *Escherichia coli* strain Z905 (pPHL7) harboring the recombinant plasmid pPHL7 with the ampicillin resistance genes and the bacterial luminescent system genes [5]; the adapted variant of *E. coli* Z905 (pPHL7), *E. coli* Z905-33 (pPHL7), which were isolated from a microcosm 18 weeks after introduction; and the plasmid-free *Pseudomonas fluorescens* and *P. putida* strains isolated from soil [6]. The relevant biochemical and physiological characteristics of the four strains studied are presented in the table.

Experimental aquatic microcosms (AMCs) were of three types. AMC 1 represented a 12-l glass carboy with a gravel–sand mixture at its bottom, which was filled with 2 l of aquarium water and 10 l of degassed tap water and sealed with a ground glass stopper. Therefore, the native microflora of this type of microcosms was due to the microorganisms present in the aquarium, the gravel–sand mixture, and tap water. Ten weeks after the establishment of AMC 1, it was analyzed for microflora and then inoculated with *E. coli* Z905 (pPHL7) cells as described earlier [7]. During experiments, the content of the soluble organic matter (SOM) in AMC 1 varied from 90 to 170 mg/l.

AMC 2 represented a 10-ml test tube filled with a filter-sterilized medium from AMC 1 and sealed with a

[1] Corresponding author. E-mail: lubg@ibp.krasnoyarsk.su

0026-2617/01/7002-0211$25.00

Comparative characteristics of the bacterial strains used in this study

Characteristic	Strain			
	E. coli Z905 (pPHL7)	*E. coli* Z905-33 (pPHL7)	*P. fluorescens*	*P. putida*
Intensity of luminescence (I_{max}), μA	100			
Threshold susceptibility to ampicillin, μg/ml	500	50		
Plasmid copy number, %	100	50		
μ_{max}, h^{-1}	0.632 ± 0.117	0.841 ± 0.081	0.636 ± 0.196	0.768 ± 0.216
Utilization of				
arabinose	+	–	+	+
xylose	+	–	–	+
glucose	+	+	+	+
galactose	–	–	–	+
lactose	–	+	–	+
maltose	+	–	+	+
sucrose	–	–	+	+
raffinose	–	+	+	+
glycerol	+	+	–	–
mannitol	+	+	+	+
sorbitol	+	–	+	+

plastic screw cap. The content of the SOM in this type of the microcosms was 161.6 mg/l.

AMC 3 also represented a 10-ml tube with a plastic screw cap, but filled with a semidefined medium containing (g/l) Na_2HPO_4, 6; KH_2PO_4, 3; NaCl, 0.5; NH_4Cl, 1; and peptone, 0.05. The content of the SOM in this type of microcosms was 67.2 mg/l. Unlike the media in AMC 1 and AMC 2, the medium in AMC 3 did not contain complex organic substances.

AMC 1 was incubated at 15–20°C under constant illumination for a year. After the inoculation of bacteria, AMC 2 and AMC 3 were incubated at 20°C in the dark for 2 months.

The microbial population in the microcosms was evaluated by plating samples taken from them on solid M9P medium [8]. The results were expressed in colony-forming units (CFU) per ml.

Utilization of carbohydrates and alcohols was assayed by inoculating the Hiss medium containing phenol red with samples taken from the microcosms. The ability of bacteria to utilize carbohydrates and alcohols was judged from the Hiss medium changing colors [9]. Arabinose, xylose, maltose, raffinose, glycerol, and sorbitol were added to the Hiss medium at a concentration of 5 g/l; glucose, lactose, sucrose, and mannitol were added at a concentration of 10 g/l.

Evaluation of the selection rates. The selection rate of microorganisms in the experimental microcosms was expressed in the selection rate constants S_{ij}, which were calculated by the slightly modified formula of Velicer and Lenski [10]:

$$S_{ij} = \ln[N_i(t)N_i(0)] - \ln[N_j(t)/N_j(0)],$$

where $N(0)$ and $N(t)$ are the initial and final population densities, respectively; and i and j denote competitive bacterial strains (in the given case, i denotes the *E. coli* strains and j denotes the *Pseudomonas* strains). If $S_{if} > 0$, the ith strain is more competitive than the jth strain; if $S_{if} < 0$, the ith strain is less competitive than the jth strain.

Statistics. The experiments were performed in triplicate. The data obtained were statistically processed using the Microsoft Excel 97 program.

RESULTS AND DISCUSSION

After the introduction of the *E. coli* strain Z905 (pPHL7) into AMC 1, its population decreased to a density of 10^2 CFU/ml within a week and remained at this level for a year (Fig. 1). Within the incubation period of the microcosm, *E. coli* Z905 (pPHL7) gradually changed its phenotypic characteristics: the variants

of this strain isolated from the microcosm at different times of its incubation exhibited a poorer expression of the luminescence and the ampicillin resistance genes, as well as altered growth characteristics and carbohydrate utilization patterns. This can easily be seen from the table, which presents the comparative characteristics of the introduced strain, *E. coli* Z905 (pPHL7) and one of its variants, *E. coli* Z905-33 (pPHL7), isolated from AMC 1. Some authors also found that the long-term incubation of bacterial cells under extreme conditions may affect their metabolic activity [11–13].

The population density of indigenous microflora in AMC 1, which was dominated by bacteria of the genera *Arthrobacter*, *Caulobacter*, *Flavobacterium*, *Micrococcus*, *Enterobacter*, and *Pseudomonas* [14], remained at a level of 10^4–10^5 CFU/ml throughout the observation period (Fig. 1). In this case, the population of bacteria belonging to the last three genera somewhat decreased.

In further studies, we used (along with the *E. coli* strains) two pseudomonads (*P. fluorescens* and *P. putida*), which are most abundant in natural ecosystems. Experiments were performed with the microcosms AMC 2 and AMC 3. Bacteria were introduced into the microcosms to a concentration of 10^4–10^5 cell/ml, i.e., to the density of the indigenous microorganisms observed in AMC 1. When the four bacterial strains studied were separately introduced into AMC 2 and AMC 3, their populations remained at approximately constant levels for 2 months (Fig. 2), except the population of the AMC-adapted strain *E. coli* Z905-33 (pPHL7) in AMC 2, which increased by about tenfold.

When the pseudomonads were introduced in the microcosms either with the nonadapted *E. coli* strain Z905 (pPHL7) or with the AMC-adapted *E. coli* strain Z905-33 (pPHL7), the population of *E. coli* Z905-33 (pPHL7) in AMC 2 remained at a level of 10^4 CFU/ml, while the population of the nonadapted *E. coli* strain decreased about tenfold (Fig. 3). The population densities of the introduced *P. fluorescens* and *P. putida* species did not considerably decrease.

It should be noted that the number of cells introduced into the microcosms greatly varied, making it difficult to compare the dynamics of bacterial populations there. For this reason, in the further analysis of the population dynamics data, we used the so-called selection rate constants (SRCs), which allow for a more easy interpretation of these data. As can be seen from Fig. 4, the nonadapted *E. coli* strain Z905 (pPHL7) dominated the pseudomonads only in AMC 3 with the semidefined medium, whereas the pseudomonads *P. fluorescens* and *P. putida* were dominant in AMC 2. At the same time, the AMC-adapted *E. coli* strain Z905-33 (pPHL7) dominated *P. fluorescens* and *P. putida* in AMC 2 (Fig. 4). In AMC 3, the competitive ability of *E. coli* Z905-33 (pPHL7) was lower than that of the pseudomonads within 35 days of the experiment but then tended to increase with the exhaustion of the nutrients in the medium.

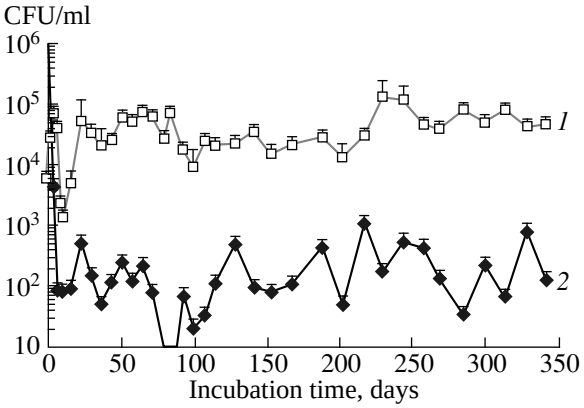

Fig. 1. Dynamics of (*1*) indigenous microflora and (*2*) the introduced strain *E. coli* Z905 (pPHL7) in the microcosm AMC 1.

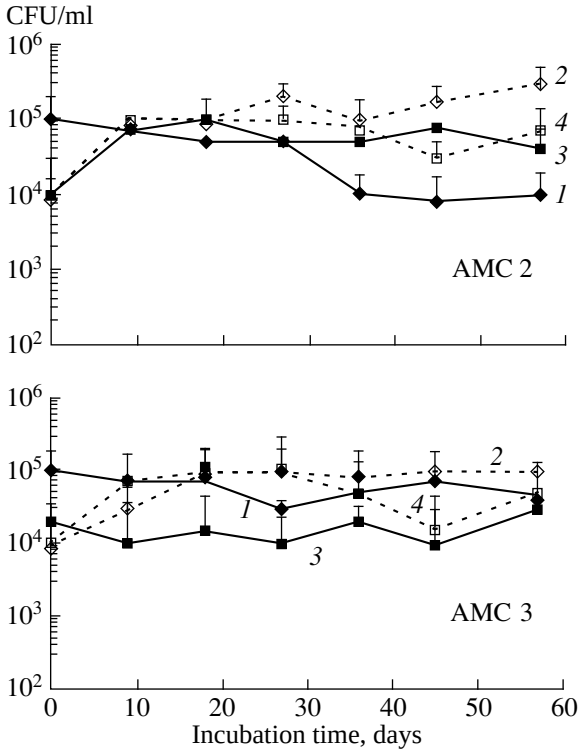

Fig. 2. Dynamics of (*1*) *E. coli* Z905 (pPHL7), (*2*) *E. coli* Z905-33 (pPHL7), (*3*) *P. fluorescens*, and (*4*) *P. putida* introduced separately into the microcosms AMC 2 and AMC 3.

In spite of the numerous relevant investigations, the mechanism of bacterial adaptation to altered environments remains unclear. Some authors believe that nonspore-forming bacteria occurring under unfavorable conditions die or transit to a nonculturable state. The probability of the existence of nonculturable bacterial

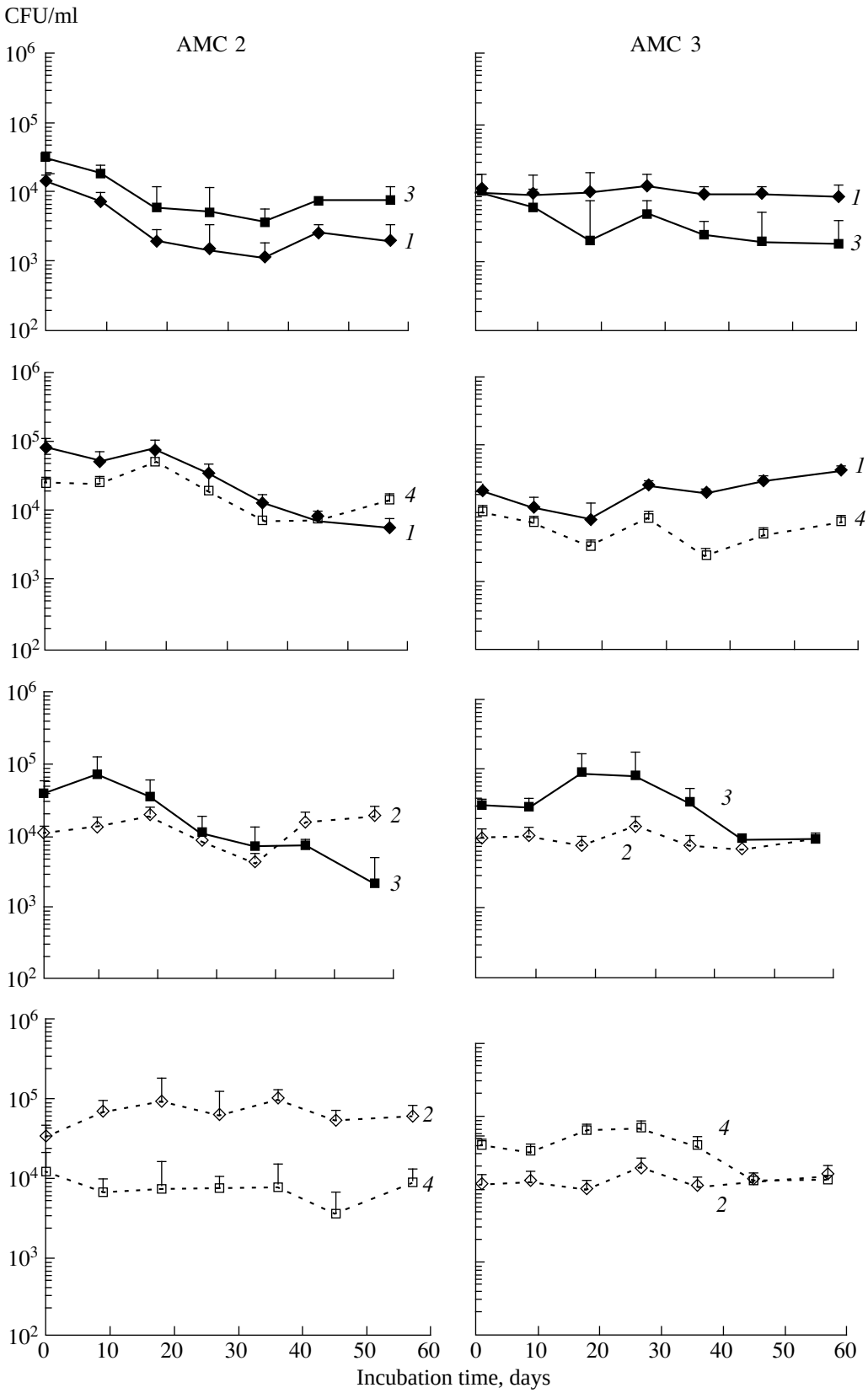

Fig. 3. Dynamics of (*1*) *E. coli* Z905 (pPHL7), (*2*) *E. coli* Z905-33 (pPHL7), (*3*) *P. fluorescens*, and (*4*) *P. putida* introduced by pairs into the microcosms AMC 2 and AMC 3.

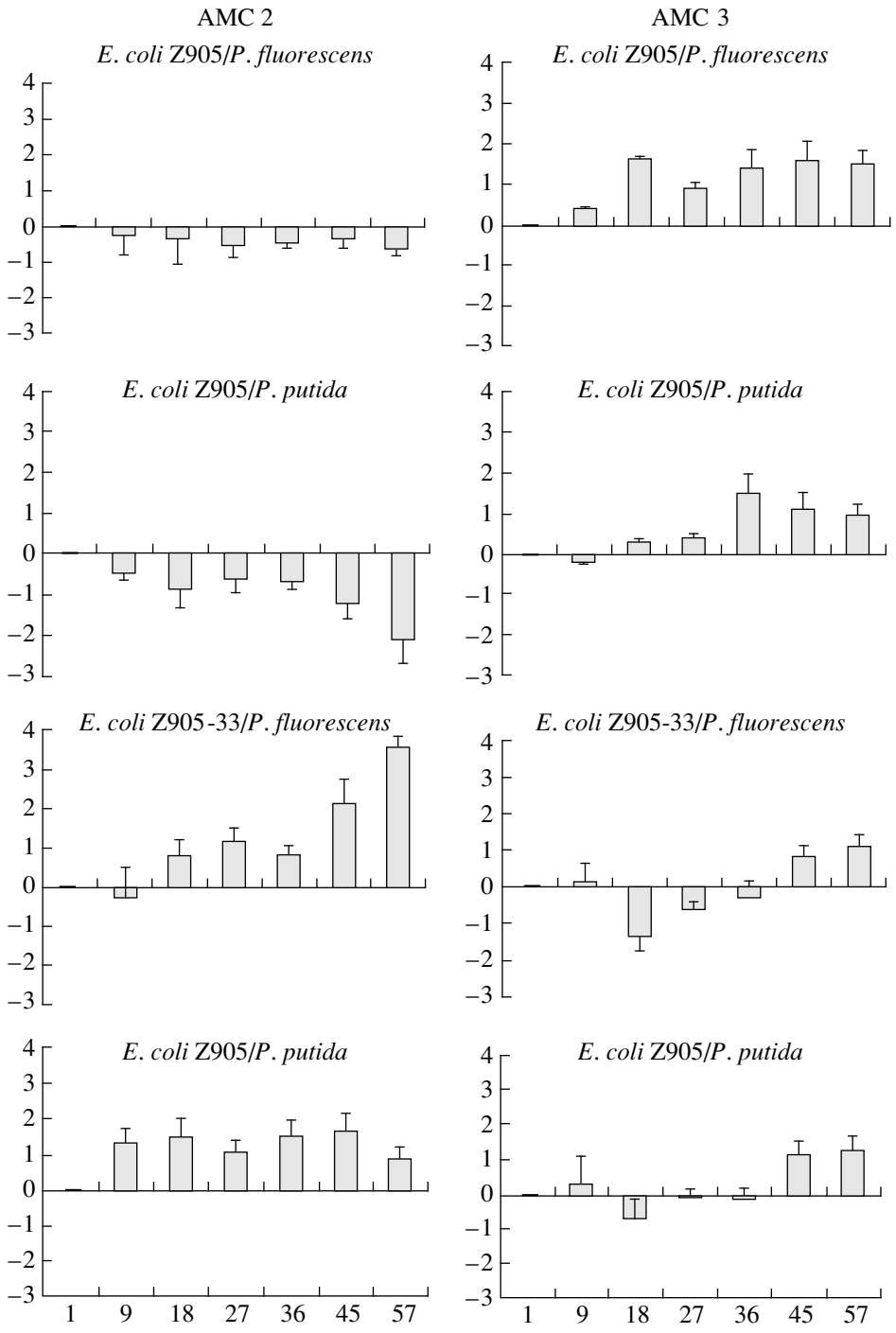

Fig. 4. Selection rate constants of *E. coli* Z905 (pPHL7), *E. coli* Z905-33 (pPHL7), *P. fluorescens*, and *P. putida* introduced by pairs into the microcosms AMC 2 and AMC 3.

forms is the subject of extensive discussion in literature [12, 13, 15–17]. In this paper, we will not consider this problem. It should be noted, however, that the observed decrease in the population density of the introduced bacteria may be due, at least partially, to the formation of nonculturable bacterial forms.

Thus, the recombinant bacterial strains introduced into microcosms undergo adaptation, which facilitates their coexistence with indigenous microflora. The concurrent decrease in the expression of the cloned genes promotes the adaptation of the introduced recombinant strains. Therefore, it is necessary to evaluate in relevant

studies the competitive ability not only of the original recombinant strains but also of their variants adapted to the environment into which these strains are to be introduced.

ACKNOWLEDGMENTS

This work was supported by grant no. 8F0006 from the Krasnoyarsk Scientific Foundation.

REFERENCES

1. Devanas, M.A., Rafaeli-Eshkol, D., and Stotzky, G., Survival of Plasmid-containing Strains of *E. coli* in Soil: Effect of Plasmid Size and Nutrients on the Survival of Hosts and Maintenance of Plasmids, *Curr. Microbiol.*, 1986, vol. 13, pp. 269–277.
2. Awong, J., Bitton, G., and Chaudhry, G.R., Microcosm for Assessing the Survival of Genetically Engineered Microorganisms in Aquatic Environments, *Appl. Environ. Microbiol.*, 1990, vol. 56, no. 4, pp. 977–983.
3. Barcina, I., Arana, I., Fernandez-Astorga, A., Iriberri, J., and Egea, L., Survival Strategies of Plasmid-Carrier and Plasmidless *Escherichia coli* Strains under Illuminated and Nonilluminated Conditions in a Freshwater Ecosystem, *J. Appl. Bacteriol.*, 1992, vol. 73, pp. 229–236.
4. Thompson, J.P., Ellis, R.J., and Bailey, M.J., Autecology of Genetically Modified Fluorescent Pseudomonad on a Sugar Beet, *FEMS Microbiol. Ecol.*, 1995, vol. 17, no. 1, pp. 1–14.
5. Illarionov, B.A. and Protopopova, M.V., Cloning and Expression of the Luminescent System Genes of *Photobacterium leiognathi* in the Plasmid Vector PUC18, *Genetika*, 1985, no. 6, pp. 10–13.
6. Pechurkin, N.S., Somova, L.A., Polonskii, V.I., Pis'man, T.I., and Sarangova, A.B., Effect of Rhizosphere Bacteria of the Genus *Pseudomonas* on the Growth of Wheat Seedlings under the Conditions of Complete Mineral Supply and Nitrogen Deficiency, *Mikrobiologiya*, 1997, vol. 66, no. 4, pp. 553–557.
7. Bril'kov, A.V., Popova, L.Yu., Baranova, E.A., Maksimova, E.A., Repeta, T.V., and Pechurkin, N.S., Prognostic Investigation of the Survival and Distribution of Genetically Engineered Microorganisms in Laboratory Aquatic Ecosystems, *Dokl. Akad. Nauk*, 1995, vol. 341, no. 3, pp. 429–430.
8. Popova, L.Yu., Repeta, T.V., Maksimova, E.E., Bril'kov, A.V., and Pechurkin, N.S., Growth Dynamics of *Escherichia coli* in Experimental Aquatic Microcosms before and after Green Algal Bloom, *Biotekhnologiya*, 1993, no. 10, pp. 35–38.
9. Sidorov, M.A., Skorodumov, D.I., and Fedotov, V.B., *Opredelitel' zoopatogennykh mikroorganizmov* (A Practical Guide to Ithe dentification of Zoopathogenic Microorganisms), Moscow: Kolos, 1995.
10. Velicer, G.J. and Lenski, R.E., Evolutionary Trade-Offs under Conditions of Resource Abundance and Scarcity: Experiments with Bacteria, *Ecology*, 1999, vol. 80, no. 4, pp. 1168–1179.
11. Zinser, E.R. and Kolter, R., Mutations Enhancing Amino Acid Catabolism Confer a Growth Advantage in Stationary Phase, *J. Bacteriol.*, 1999, vol. 181, pp. 5800–5807.
12. Debabov, V.G., Bacterial Life beyond Laboratories, *Mol. Biol.* (Moscow), 1999, vol. 33, no. 6, pp. 1074–1084.
13. Golovlev, E.L., Introduction into the Biology of Stationary-Phase Bacteria: The Mechanism of General Response to Stresses, *Mikrobiologiya*, 1999, vol. 68, no. 5, pp. 623–631.
14. Kargatova, T.V., Maksimova, E.E., Krylova, T.Yu., and Popova, L.Yu., Comparative Investigation of the Growth of Recombinant *Escherichia coli* Variants and Indigenous Microorganisms in Aquatic Microcosms under Selective and Nonselective Conditions, *Izv. Akad. Nauk. Ser. Biol.*, 1999, no. 2, pp. 152–157.
15. Mizunoe, Y., Wai, S.N., Takade, A., and Yoshida, S., Restoration of Culturability of Starvation-Stressed and Low-Temperature–Stressed *Escherichia coli* 0157 Cells by Using H_2O_2-degrading Compounds, *Arch. Microbiol.*, 1999, vol. 172, pp. 63–67.
16. Golovlev, E.L. and Golovleva, L.A., Microbial Physiology and Metabolic Engineering, *Mikrobiologiya*, 2000, vol. 69, no. 2, pp. 149–163.
17. Bogosian, G., Morris, P.J.L., and O'Neil, J.P., A Mixed Culture Recovery Method Indicates that Enteric Bacteria Do Not Enter the Viable but Nonculturable State, *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 1736–1742.