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EXPERIMENTAL ARTICLES

Rhizosphere Strain of *Pseudomonas chlororaphis* Capable of Degrading Naphthalene in the Presence of Cobalt/Nickel

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Abstract—Combination of genetic systems of degradation of polyaromatic hydrocarbons, resistance to heavy metals, and promotion of plant growth/protection is one of the approaches to the creation of polyfunctional strains for phytoremediation of soils after co-contamination with organic pollutants and heavy metals. A plant-growth-promoting rhizosphere strain *Pseudomonas chlororaphis* PCL1391(pBS216*, pBS501) has been obtained, in which the *nah* operon of plasmid pBS216 provides naphthalene biodegradation and the *cnr*-like operon of plasmid pBS501 provides resistance to cobalt and nickel due to the extrusion of heavy metal cations from the cells. In the presence of 100 μM of nickel, the viability, growth rate, and naphthalene biodegradation efficiency of the resistant strain PCL1391(pBS216*, pBS501) were much higher as compared with the sensitive PCL1391(pBS216). During the growth of the resistant strain, in contrast to the sensitive strain, nickel (100 μM) had no inhibiting effect on the activity of the key enzymes of naphthalene biodegradation.

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At present, plant-growth-promoting rhizobacteria *Pseudomonas* (PGPR *Pseudomonas*) are quite an attractive research object in view of their potential application for phytoremediation. Combination of the genetic systems encoding the degradation of polyaromatic hydrocarbons (PAH), resistance to heavy metals, and promotion of plant growth/protection is one of the approaches to the creation of multifunctional strains for phytoremediation of soils with co-contamination by organic pollutants and heavy metals. Heavy metals are known to inhibit the degradation of xenobiotics [1, 2]. In addition, the changes in the composition of microbial populations under complex soil contamination may result in a predominance of phytopathogenic fungi and therefore a considerable decrease in the phytoremediation efficiency. It has previously been shown that strains of *Alcaligenes eutrophus* bearing plasmids of metal resistance and plasmids of biodegradation of polychlorinated biphenyls and 2,4-dichlorophenoxyacetic acid degrade these xenobiotics more effectively in the presence of nickel or zinc as compared with sensitive strains [3, 4]. However, to date, there have been no data on the interaction of the genetic systems of PAH degradation and metal resistance and their effect on physiology, biodegradation efficiency, and the activity of the key enzymes in multifunctional strains.

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The goal of this work was to study the physiological and biochemical features of naphthalene biodegradation in the presence of cobalt and nickel in variants of the rhizospheric strain *P. chlororaphis* PCL1391 sensitive and resistant to heavy metals.

MATERIALS AND METHODS

Bacterial strains. Strain *Pseudomonas chlororaphis* PCL1391, kindly provided by Professor B.J.J. Lugtenberg (Leiden, The Netherlands), was used in the work as the plasmid recipient. The strain belongs to the group of pseudomonades synthesizing the antibiotic phenazine carboxamide which inhibits the growth of many phytopathogenic fungi. Strain PCL1391 was shown to possess no plasmid DNA and exhibits a chromosome-controlled catechol-1,2-dioxygenase activity [5]. Previously obtained plasmid-bearing variants of strain PCL1391 were also used in the work. In the variant PCL1391(pBS216), plasmid pBS216 carrying the *nah* operon provides cell growth on naphthalene and salicylate [5]. In the variant PCL1391(pBS501), plasmid pBS501 containing a *cnr*-like operon determines resistance to nickel and cobalt. Plasmids pBS216 [6] and pBS501 [7] were isolated from environmental strains (collection of the Laboratory of Plasmid Biology, IBPM, Pushchino).

Cultivation media and conditions. Bacteria were grown in LB medium [8] and in Tris–mineral medium (TMM) [9] at 24°C. Glucose (2 g l^{-1}) or naphthalene (1 g l^{-1}) was added as a carbon source. Metal salts $CoCl_2 \cdot 7H_2O$ and $NiCl_2 \cdot 7H_2O$ were added in concentrations of 20 μM to 2 mM.

Plasmid DNA was isolated by alkaline lysis [8].

Transformation of the strain by plasmid DNA was performed as described previously [5].

Physiological parameters of bacterial growth. The specific growth rate (μ) was determined after the lag phase in 3 h interval according to the equation $\mu = \ln(x_t/x_0)/(t - t_0)$, where x_t and x_0 are the optical densities (OD) of the culture at the moments of time t and t_0, respectively. The duration of the lag period was determined graphically [10]. The optical density of the culture of the sensitive strain PCL1391(pBS216) in the presence of nickel was measured relative to the control, for which a stained medium after cell precipitation was used.

Induction of the *nah* operon of plasmid pBS216 was achieved by growing strain PCL1391(pBS216*, pBS501) in TMM with naphthalene (1 g l^{-1}). To induce the system of resistance to cobalt and nickel of plasmid pBS501, this strain was grown in TMM with glucose, with the addition of 100 μM nickel. Noninduced cells were grown in TMM with glucose. Bacteria were grown to the optical density of 0.4 (10^9 cells ml^{-1}), precipitated by centrifugation, and transferred into a fresh TMM with naphthalene and 200 μM of nickel or cobalt (to OD 0.03).

Stability of plasmids was determined after 3, 6, and 10 passages of the culture in a liquid LB medium. Using the replica technique, one hundred separate colonies were transferred to selective TMM media with naphthalene and LB with 2 mM of cobalt (nickel). The plasmid stability was defined as the percentage ratio of the number of clones retaining the characteristic to the total number of tested clones. The presence of plasmids was determined by electrophoresis.

Enzyme activities were determined in cell-free extracts on a UV-160A spectrophotometer (Shimadzu, Japan). The activities of naphthalene dioxygenase and salicylate hydroxylase were determined from the decrease in NADH extinction (λ = 340 nm, ε = 6.22 μM cm^{-1}) [11, 12]. The activity of catechol-2,3 dioxygenase was determined from the rate of α-oxymuconic semialdehyde formation (λ = 375 nm, ε = 33.4 μM cm^{-1}) [13]. The activity of catechol-1,2 dioxygenase was determined from the rate of *cis-cis*-muconate formation (λ = 260, ε = 16.9 μM cm^{-1}) [14]. The specific enzyme activity was expressed in nanomoles of the cofactor consumed or the product formed in 1 min per 1 mg of total bacterial protein. The protein concentration was determined spectrophotometrically [15].

Concentration of naphthalene and its oxidation products. The bacteria were grown in 5 ml of TMM with naphthalene (1 g l^{-1}) in the presence of 100 μM Ni^{2+} or without the metal. An equal volume of chloroform was used for extraction. The chloroform fraction was evaporated under vacuum and dissolved in methanol. Naphthalene and the intermediates were identified by HPLC. The fractionation conditions were as follows: a Spherisorb ODS-2 C18 (Supelco, United States) column with inverted phase, in the gradient of 1% acetic acid/0–100% methanol for 10 min. The naphthalene concentration was calculated with allowance for its natural evaporation in the control without the bacteria.

RESULTS

Obtaining of strain *P. chlororaphis* PCL1391 (pBS216*, pBS501). To obtain a polyfunctional strain capable of naphthalene oxidation in the presence of heavy metals, plasmid pBS501 was transformed into strain PCL1391/(pBS216). After the transformation, clones were selected on LB medium with 2 mM $CoCl_2$. The transformation frequency was 10^2 CFU/μg of plasmid DNA. Two plasmids (65 and 85 kb) with molecular weights corresponding to pBS216 (85 kb) and pBS501 (65 kb) were visualized in the strain.

Compatibility and stability of plasmids. In the previously obtained variants PCL1391(pBS216) and PCL1391(pBS501), each plasmid remained stable after ten passages in a nonselective medium. In the variant PCL1391(pBS216*, pBS501), both plasmids were also maintained stably and retained the determined phenotype of Nah^+, Co^r/Ni^r after ten passages in a nonselective medium.

The effect of induction of the *nah* and *cnr* operons on the growth of strain PCL1391(pBS216*, pBS501) on naphthalene in the presence of cobalt/nickel. The obtained strain *P. chlororaphis* PCL1391(pBS216*, pBS501) grows on the minimal medium with naphthalene as the sole carbon source and has the level of resistance to cobalt/nickel typical of strain PCL1391(pBS501). For resistant variants PCL1391(pBS501) and PCL1391(pBS216*, pBS501) grown in TMM with glucose, the minimal inhibitory concentrations (MIC) of cobalt (0.5 mM) and nickel (2 mM) were much higher than for the sensitive strain PCL1391(pBS216) (0.05 and 0.15 mM for cobalt and nickel, respectively). When the strains were grown in TMM with naphthalene, the MIC of the metals and the character of growth changed depending on the method of cell induction. It is known that the *nah* operon on plasmid pBS216 is inducible [5]. Resistance to nickel/cobalt determined by the *cnr*-like operon of plasmid pBS501 is induced by nickel (unpublished data). In the medium with naphthalene in the presence of 200 μM of either cobalt or nickel, growth of the resistant strain PCL1391(pBS216*, pBS501) was inhibited in the variant when both plasmid operons were not induced. However, after the induction of the *cnr*-like operon, the character of the growth curves depended on

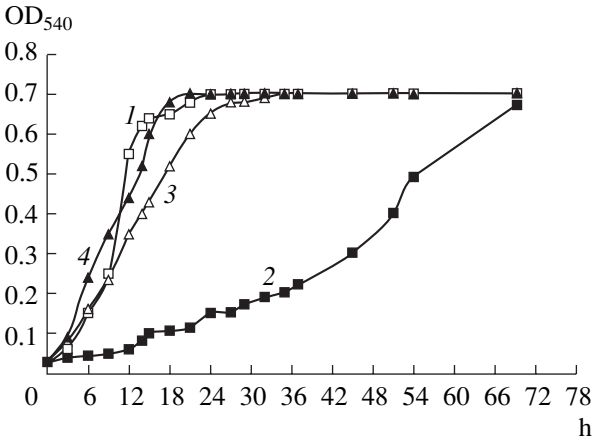
Table 1. Growth characteristics of strain *P. chlororaphis* PCL1391(pBS216*, pBS501) at different modes of induction of *nah* and *cnr* operons

Induction	Naphthalene + Co^{2+}*			Naphthalene + Ni^{2+}		
	Lag phase, h	μ, h^{-1}	OD_{max}	Lag phase, h	μ, h^{-1}	OD_{max}
No induction	No growth			No growth		
Glucose + Ni	48	0.14	0.10	3	0.30	0.80
Naphthalene	3	0.23	0.18	None	0.30	0.80
Naphthalene + Ni	None	0.27	0.60	None	0.27	0.75

* Concentrations of naphthalene, 1 g l^{-1}; metals, 200 μM.

which cation (Co^{2+} or Ni^{2+}) was present in the growth medium. At a 200 μM concentration of Co^{2+}, strain growth was inhibited for 48 h; however, at the same concentration of Ni^{2+}, the culture started to grow after a 3 h lag phase (μ = 0.3 h^{-1}) and reached the maximal OD of 0.75 after 27 h. The induction of the *nah* operon resulted in growth after a 3 h lag phase in the presence of 200 μM Co^{2+}; however, after 3 h of growth at μ = 0.23 h^{-1}, the culture passed into the stationary phase with low OD (0.18). In the presence of 200 μM Ni^{2+}, exponential growth started immediately, without the lag phase, and the culture reached the maximal OD of 0.8. After the induction of both genetic systems, *nah* and *cnr*, the strain grew without the lag phase (μ = 0.27 h^{-1}) and the exponential growth phase lasted for 27 h (in the medium with Co^{2+}) or for 18 h (in the medium with Ni^{2+}); the final OD was 0.6 and 0.75, respectively (Table 1). Since Co^{2+} was shown to be more toxic for both strains than Ni^{2+}, only nickel was used in further experiments.

The effect of nickel on bacterial viability. To compare the growth parameters of the *nah*-induced resistant PCL1391(pBS216*, pBS501) and sensitive PCL1391 (pBS216) strains, the medium with naphthalene was supplemented with nickel in a concentration which was subinhibitory for the sensitive strain (100 μM) (Figure). In the presence of nickel, the specific growth rates (μ) of the sensitive strain (0.18 and 0.24 h^{-1}) were lower than those of the resistant strain (0.29 and 0.33 h^{-1}) in the beginning (up to OD 0.2) and in the middle (up to OD 0.4) of exponential growth.



Growth of variants of the strain *P. chlororaphis* under different conditions: PCL1391(pBS216) on naphthalene without metal (*1*) and in the presence of 100 μM nickel (*2*); PCL1391(pBS216*, pBS501) on naphthalene without metal (*3*) and in the presence of 100 μM nickel (*4*).

The counting of viable bacteria (colony-forming units, CFU) in the culture medium at different points of exponential growth showed that nickel (100 μM) significantly inhibited the growth of the sensitive strain PCL1391(pBS216). The CFU number increased insignificantly after 36 h (OD 0.2) and then, under an increase in optical density, the death of bacteria was observed and the CFU number decreased by 2 and 4 orders of magnitude (1.4×10^5 at OD 0.4 and 2.0×10^3 at OD 0.6) (Table 2). Nickel had no negative effect on the viability of the resistant strain PCL1391 (pPBS216*, pBS501): the CFU number did not decrease but rather increased both in the control and in the presence of the metal at the same culture densities (Table 2).

The effect of 100 μM nickel on the activity of the enzymes of naphthalene oxidation was studied in three points during the period of exponential growth of the strains. At the early point (OD 0.2), the activities of the key enzymes of naphthalene oxidation (naphthalene dioxygenase (NO), salicylate hydroxylase (SH), catechol-1,2 dioxygenase (C-1,2-O), and catechol-2,3 dioxygenase (C-2,3-O) were comparable in the sensitive and resistant strains independently of the presence of nickel cations in the medium (Table 3). In the middle of exponential growth (OD 0.4) in the presence of nickel, the sensitive strain PCL1391(pBS216) had much lower NO and SH activities (0.2 and 4.1 nmol min^{-1} mg^{-1} protein, respectively) as compared with the resistant strain PCL1391(pBS216*, pBS501) (NO and SH activities of 1.6 and 36.8 nmol min^{-1} mg^{-1} protein, respectively). At OD 0.6, nickel completely inhibited the activity of the analyzed enzymes in the sensitive strain. The resistant strain had no C-1,2-O and NO activities but showed a low C-2,3-O activity (1.9 nmol $^{-1}$ mg^{-1} protein) with SH activity

Table 2. Viability (CFU ml^{-1}) of variants of the strain *P. chlororaphis* PCL1391

OD	PCL1391(pBS216)		PCL1391 (pBS216*, pBS501)	
	naphthalene	naphthalene + Ni	naphthalene	naphthalene + Ni
0.03	1.6×10^7 (0)	3.7×10^7 (0)	3.5×10^7 (0)	1.5×10^7 (0)
0.2	1.3×10^8 (9)	7.0×10^7 (36)	4.0×10^8 (9)	2.4×10^8 (7)
0.4	2.1×10^9 (12)	1.4×10^5 (54)	30×10^9 (15)	1.0×10^9 (11)
0.6	1.3×10^{10} (18)	2.0×10^3 (69)	8.2×10^{10} (21)	1.2×10^{10} (21)

Note: Growth time in hours in given in parentheses; nickel concentration in the medium is 100 μM.

remaining at the previous level (30.8 nmol min^{-1} mg^{-1} protein) (Table 3).

The measurement of enzyme activities at different points during the exponential growth phase revealed their dynamics. Thus, SH activity was observed to decrease in one-plasmid strain PCL1391(pBS216) while increasing in two-plasmid strain PCL1391 (pBS216*, pBS501). In the beginning of exponential growth, the C-1,2-O activity in both strains was much higher than the C-2,3-O activity, independently of the presence of nickel in the medium. During the growth of the one-plasmid strain against a significant decrease in the C-1,2-O activity (from 97.7 to 4.2 nmol min^{-1} mg^{-1} protein), the activity of plasmid C-2,3-O increased slightly. However, the C-1,2-O activity in the two-plasmid strain remained at a high level both at the beginning and at the end of exponential growth (56.5 and 23.1 nmol min^{-1} mg^{-1} protein, respectively) while the C-2,3-O activity did not increase.

The difference in the C-2,3-O activities in the initial PCL1391(pBS216) and the two-plasmid PCL1391 (pBS216*, pBS501) variants could be due either to structural rearrangements of plasmid pBS216 or to the effect of plasmid pBS501 on the expression of the *nah* operon. To determine the reasons for the changes in the C-2,3-O activity, plasmid pBS216 was isolated from strain PCL1391(pBS216*, pBS501) and transformed into plasmid-free strain PCL1391. The measurement of enzyme activities at the end of exponential growth showed that in the obtained variant PCL1391 (pBS216*), like in the two-plasmid strain, the activity of chromosomal C-1,2-O was maintained at a high level (35 nmol min^{-1} mg^{-1} protein), while the activity of plasmid C-2,3-O was not registered. The restriction analysis of plasmids pBS216* and pBS216 with restriction endonucleases *Sal*1, *Hind*III, and *Bgl*II revealed differences in the *Bgl*II restriction profiles (data not shown). Therefore it should be supposed that the structural rearrangements in plasmid pBS216* were the reason for the decreased C-2,3-O activity in strain PCL1391(pBS216*, pBS501).

Naphthalene biodegradation. The inhibitory effect of nickel influenced both the enzyme activity and the naphthalene biodegradation efficiency of the sensitive strain. In the presence of 100 μM nickel, strain PCL1391(pBS216) oxidized only 12% of naphthalene in 36 h at a density of 7.0×10^7 cells ml^{-1} (Table 4). Further increase of OD resulted in a collapse of the strain's viability, so that the decrease in naphthalene concentration in the medium was caused only by its evaporation. The advantage of the resistant strain over the sensitive one was obvious. In the presence of nickel, resistant strain PCL1391(pBS216*, pBS501), which had reached the density of 1.2×10^{10} cells ml^{-1} in 21 h, oxidized 98% naphthalene, i.e., as much as the sensitive PCL1391(pBS216) grown without the metal (Table 4). When the sensitive PCL1391 (pBS216) was grown in the presence of nickel, the culture medium changed color from yellow (in the first hours) to dark brown. On reaching OD 0.2, an oily brown precipitate was formed in the medium. The analysis of numerous intermediates of the culture liquid of the sensitive strain showed that their concentrations were much higher than in the resistant strain. Salicylate was found in the extract of the brown precipitate, along with unidentified compounds.

Table 3. Activity of the key enzymes of naphthalene biodegradation, nmol min^{-1} mg^{-1} protein

Enzyme	OD	PCL1391(pBS216)		PCL1391 (pBS216*, pBS501)	
		naphthalene	naphthalene + + Ni^{2+}	naphthalene	naphthalene + + Ni^{2+}
NO	0.2	4.6	4.6	4.0	3.4
	0.4	2.2	0.2	3.6	1.6
	0.6	4.5	0	3.6	0
SH	0.2	32.0	32.8	26.8	44.6
	0.4	26.1	4.1	14.3	36.8
	0.6	3.2	0	125.6	30.8
C-1,2-O	0.2	97.7	84.4	56.5	87.1
	0.4	6.4	7.5	18.4	55.3
	0.6	4.2	0	23.1	0
C-2,3-O	0.2	8.5	4.1	1.8	0
	0.4	15.0	27.4	2.0	1.2
	0.6	27.0	0	0	1.9

Table 4. Naphthalene biodegradation by variants of the strain *P. chlororaphis* PCL1391 under various growth conditions

Strains	PCL1391(pBS216*, pBS501)		PCL1391(pBS216)	
Growth conditions	naphthalene	naphthalene + Ni	naphthalene	naphthalene + Ni
Time, h	21	21	18	36
Naphthalene, mg l^{-1}	59.0 ± 0.1	9.0 ± 0.1	9.0 ± 0.1	168.0 ± 17.0
Control*	870.0 ± 2.0	870.0 ± 22.0	900.0 ± 15.0	191.0 ± 40.0
% of degradation	93	98	99	12

* Naphthalene concentration in the medium without bacteria, mg l^{-1}.

Table 5. The content of salicylate in cultivation media of the variants of strain *P. chlororaphis* PCL1391 at different growth phases, mg l^{-1}

OD	PCL1391(pBS216)		PCL1391(pBS216*, pBS501)	
	naphthalene	naphthalene + Ni^{2+}	naphthalene	naphthalene + Ni^{2+}
0.2	6.0 ± 0.1 (9 h)	52.0 ± 4.9 (36 h)	114.0 ± 12.0 (9 h)	3.0 ± 0.4 (7 h)
0.4	13.0 ± 0.1 (12 h)	44.0 ± 0.1 (54 h)	79.0 ± 7.8 (15 h)	5.0 ± 1.8 (11 h)
0.6	32.0 ± 0.1 (18 h)	82.0 ± 8.5 (69 h)	45.0 ± 0.1 (21 h)	23.0 ± 4.9 (21 h)

Salicylate was also found in the culture medium of both strains. In PCL1391(pBS216), salicylate accumulated by the end of growth, coinciding with the decrease in SH activity. The maximal accumulation of salicylate (82 mg l^{-1}) was noted at the end of exponential growth in the presence of nickel. In strain PCL1391(pBS216*, pBS501) without the metal, on the contrary, the salicylate concentration was maximal (114 mg l^{-1}) at the beginning of exponential growth, and then gradually decreased to 45 mg l^{-1} (Table 5).

DISCUSSION

The study of resistance to cobalt and nickel in sensitive and resistant strains has shown that the MIC of metals in the medium with naphthalene was much lower than in the medium with glucose. The growth curves of the resistant strain PCL1391(pBS216*, pBS501) on naphthalene in the presence of 200 μM Co^{2+} or Ni^{2+} depended on the method of cell induction. Metal cations inhibited the growth of the resistant strain with an induced *nah* operon to a lesser extent as compared with the strain with an induced *cnr*-like operon (Table 3, Figure).

The activities of naphthalene oxidation enzymes in the sensitive strain were significantly inhibited at levels as low as 100 μM Ni^{2+}. Thus, the NO activity in PCL1391(pBS216) was not revealed beginning from the mid-exponential growth phase, whereas in the resistant strain PCL1391(pBS216*, pBS501), it was registered at the end of this phase as well. The SH activity in the sensitive strain also decreased from 32.8 to 0 nmole min^{-1} mg^{-1} protein, which resulted in an accumulation of salicylate in the culture medium from 52 to 82 mg l^{-1}. The SH activity in the resistant strain was unchanged (44.6 and 30.8 nmol min^{-1} mg^{-1} protein at the beginning and at the end of exponential growth, respectively), and salicylate accumulated in the medium in lower amounts (3 to 23 mg l^{-1}).

Further cleavage of the catechol aromatic ring can proceed by the *ortho*- or *meta*-pathway with the participation of chromosomal C-1,2-O or plasmid C-2,3-O, respectively. Plasmid pBS216 encodes the expressed gene of C-2,3-O [6]. The chromosomal *ortho*-pathway was shown to dominate in the beginning of PCL1391 (pBS216) growth, but its contribution to catechol cleavage significantly diminished from the middle of the exponential growth phase, so that the plasmid *meta*-pathway was predominant at the late point. However, strain PCL1391(pBS216*, pBS501) showed no such "switching" of enzyme activities. During the exponential growth, catechol oxidation proceeded mainly by the *ortho*-pathway. The decrease in C-2,3-O gene expression was unchanged upon the transfer of plasmid pBS216 from the two-plasmid strain back to the recipient PCL1391. The change in the restriction profile of plasmid pBS216* isolated from the two-plasmid strain could be evidence of rearrangements that occurred in the *nah* operon upon the cultivation of strain PCL1391(pBS216,pBS501) in the presence of heavy metals, but not of the effect of the second plasmid pBS501.

The *meta*-pathway is considered to dominate in cells in the presence of both genetic systems of catechol oxidation [16]. However, the activities of naphthalene degradation enzymes can change in strains in the course of their cultivation [17, 18]. Usually, enzyme activity is measured in cells with high initial density (OD_0 = 0.1–0.2) that have reached the stationary

growth phase. At equal optical densities of PCL1391 (pBS216*), variants with high (OD_0 = 0.2) and low (OD_0 = 0.03) initial densities, in the former case the culture passed through a smaller number of generations and the C-1,2-O activity was much higher. Thus, the activity of chromosomal C-1,2-O in the strain PCL1391(pBS216) depended on the number of generations but not on the culture density and dominated at the beginning of the exponential growth. However, the C-1,2-O activity in the strain PCL1391(pBS216*, pBS501) did not depend on the number of generations. The absence of plasmid C-2,3-O in this case was apparently compensated by high C-1,2-O activity.

Nickel in a concentration of 200 μM is known to inhibit aerobic degradation of naphthalene, biphenyl, xylene, and a number of other xenobiotics in *Alcaligenes* and *Pseudomonas* strains [2]. The toxic effect of 100 μM nickel on the sensitive strain PCL1391 (pBS216) was accompanied by a decrease in its enzymatic activity and in endogenous NADH consumption and, as a consequence, by possible accumulation of more toxic intermediates in the medium, decline of bacterial viability, and decrease in naphthalene biodegradation efficiency. In the resistant strain PCL1391 (pBS216*, pBS501), the *cnr*-like operon seemed to provide active efflux of nickel cations from cells; therefore, the metal had no marked inhibiting effect. Previously a similar finding has been reported for the multifunctional strains of *Alcaligenes eutrophus* that bear plasmids of resistance to bivalent cations, pMOL28 (*cnr* – Co^r Ni^r) and pMOL30 (*czc* – Co^r Zn^r Cd^r), and catabolic plasmids of biodegradation of polychlorinated biphenyls, pSS50 (Bph^+/Cbp^+), and 2,4-dichlorophenoxyacetic acid, pJP4 (Tfd^+). Resistant strains effectively degraded the above pollutants in the presence of zinc and nickel [3]. Our findings demonstrate the possibility of combining the plasmids of PAH catabolism, plasmids of resistance to heavy metals, and systems of plant growth protection/promotion. This approach can be used for the creation of polyfunctional PGPR *Pseudomonas* strains effective for the phytoremediation of soils polluted by oil in combination with PAH and heavy metals.

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