EFFICIENCY OF NAPHTHALENE DEGRADATION BY *PSEUDOMONAS* BEARING NAH-PLASMIDS IN MODEL SOIL SYSTEM

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Abstract

A large collection of naphthalene-degrading *Pseudomonas* strains isolated from pollution sites was screened for the growth parameters in the liquid medium with naphthalene, the utilization of organic compounds (single and complex as petroleum) as the only source of carbon and energy, the activity of key enzymes of naphthalene catabolism (naphthalene dioxygenase, salicylate hydroxylase, catechol-1,2-dioxygenase and of catechol-2,3-dioxygenase). The most active strains were used as recipients of Nah-plasmids. The transfer and inheritance of Nah-plasmids of IncP-7 and IncP-9 groups in the cells of natural naphthalene utilizing bacteria and collection strains of *Pseudomonas* were studied. The efficiency of naphthalene degradation by natural and genetic constructed strains bearing Nah-plasmids in model soil system was determined. The number of microorganisms and the concentration of naphthalene in soil samples were monitored (in presence of bacteria the concentration of naphthalene in soil has been decreasing from 32,2 % to 100 % in 7 days). The best protective effect was found for the collection strain *P. putida* KT2442, bearing Nah-plasmids from group IncP-9 and naturals bacteria *P. putida* AL21, *P. putida* NL21, *P. putida* NL26, *P. putida* AL1 containing combination of two Nah-plasmids. These strains can be used as a basis for biopreparations for effective cleaning polluted soils

Key words: naphthalene, naphthalene dioxygenase, salicylate hydroxylase, catechol-1,2-dioxygenase, catechol-2,3-dioxygenase, plasmid, model soil system.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of widespread persistent pollutants which are present in effluents and gas exhausts of coke, gas and petroleum production. Naphthalene, phenanthrene, anthracene, and chrysene are components of the heavy fraction of oil, which pollutes the environment as a result of oil spills. Furthermore, PAHs occur and enter the soil, air and water due to incomplete combustion of some organic compounds and gas exhaust [1]. Though the concentration of PAHs in soil can be reduced by abiotic processes, such as evaporation and sorption, microbial populations play the major role in their degradation [2,3].Various xenobiotics, including polycyclic aromatic hydrocarbons (PAHs) can be degraded by bacteria of *Pseudomonas* [1]. In *pseudomonas* genes responsible for catabolism of PAHs including naphthalene are localisation on catabolic plasmids [4]. *Pseudomonas* plasmids that control catabolism of naphthalene and other aromatic hydrocarbons fall almost exclusively into the incompatibility groups IncP-9, IncP-7, and IncP-2 [5, 6]. The plasmid genes of naphthalene catabolism are organised into two operons that exist on a plasmid as parts of composite transposons [7-8]. Based on this fact, it could be expected that naphthalene operons to be equally distributed in *Pseudomonas* spp. Horizontal gene transfer plays an important role in evolution of bacterial populations and their adaptation to environmental pollution. Numerous studies provide circumstantial evidence that conjugative plasmids and transposons may promote the transfer of biodegradative traits within and between naturally occurring microbial communities [9, 10]. It also has been proposed that the selective pressure and active DNA recombination may form combinations of catabolic pathways that could ultimately increase the bioremediation potential of bacteria [11]. However, the question remains if certain combinations of plasmid and bacterial host increase

the efficacy of *in situ* utilization of naphthalene or provide any selective advantages to the microorganisms.The aim of this work was to assess the efficiency of naphthalene degradation by natures and genetics constructed strains bearing Nah-plasmids in model soil system. As donors or recipients of Nah-plasmids the most activity natural strains were used.

Materials and Methods

Bacterial strains: The 105 naphthalene-degrading strains previously isolated from the sites of excessive pollution with organic compounds were used. The strains *P. mendocina* BKMB1299, *P. putida* KT2442, *P. stutzeri* B975, *P. paleronii* B1328, *P. aureofaciens* B1393, *P. putida* M from the collection of microbiology department (BSU) were also used in this work.

Media: Bacteria were grown in LB broth and liquid M9 medium [12]. The ability of microorganisms to degrade aromatic hydrocarbons was tested using M9 agar or liquid M9 mediumcontaining different aromatic hydrocarbons as the only source of carbon and energy (sodiumsalicylate, sodium succinate, naphthalene, naphthalene bromide, naphthylamine, phenanthrene, anthracene, pyrene, biphenyl, chlorinated bithenyl, toluene, benzole, xylene (*meta*-, *para*-, *ortho*-), camphor, benzyl alcohol, hexadecane, octane, kerosene, diesel fuel and petroleum in the concentration of 0,1 - 0,2 %). Commercial preparations of antibiotics (kanamycin, streptomycin, chloramphenicol and nalidixic acid) were used in the final concentrations of 25, 50, 25, and 100 μg/ml, correspondingly.

Physiological growth parameters: were studied in batch culture with naphthalene(2 g l^{-1}) as the only source of carbon and energy according to [13].

Enzyme activity assay: The specific activities of naphthalene dioxygenase, salicylate hydroxylase, catechol-1,2-dioxygenase and of catechol-2,3-dioxygenase were measured using procedures described in the works [14-15]. Protein concentration was determined by the spectrophotometric method [16].

Transposon mutagenesis: was carried out by the method proposed in [17].

Conjugal filter matings: The cultures of donor and recipient bacteria from the exponential growth phase were condensed 100-fold, mixed in a 1 : 1 ratio, and applied to nitrocellulose filters (Synpor 6, pore size 0.45 m, Czech Republic) placed onto the surface of complete or minimal agarized media. Bacteria were incubated for 3–18 h, and then the cells were washed off with physiological saline and inoculations on selective media were made from appropriate dilutions. The results were scored after 48–72 h of incubation at28 ˚C. The frequency of transfer of plasmid markers was determined by the ratio of the number of transconjugates to the total number of donor cells.

Determination of plasmid inheritance stability: Plasmid inheritance stability was tested by cultivation of plasmid-containing bacteria (initial quantity 10^3 cells cm^{-3}) under nonselective conditions (LB broth) to the stationary growth phase at temperatures 28 ˚C followed by planting on rich agarized medium and testing the formed colonies for the integrity of plasmid markers.

Soil preparation: Soil model systems were prepared from gray forest soil taken near Minsk (Belarus).The soil was sieved (2.0mm pore diameter), then was sterilized three times for 30min at 121 ˚C under pressure (101.3 kPa) with a one-day interval. Naphthalene was added into a flask with 100 g of sterile dry soil, and then the flask was shaken (200 rpm) for 2 h at room temperature (20 ˚C) to distribute the naphthalene. The final concentration of naphthalene in the soil was about mg (g dry soil)$^{-1}$.

Inoculation: Bacteria were grown in liquid M9 medium with naphthalene as the only source carbon and energy until the end of the exponential growth phase (2 x 10^9 cells cm^{-3}). Then, using the turbidity standard, the bacterial suspension was diluted with phosphate buffer (NaH_2PO_4 x $2H_2O$ – 0.04 mol dm^{-3}, Na_2HPO_4 x $12H_2O$ – 0.06 mol dm^{-3}) at pH 6.9 to the concentration of 5 x 10^8 cells cm^{-3}. The bacterial

suspension was added to sterile water and used to inoculate the soil placed into the flask. The soil in the flask was thoroughly mixed using by a spatula and incubated at 18–20 °C for one week.

Microbial quantification: Three to four samples were taken from different soil sites of each flask. These samples were mixed to achieve the average sample (0.5 g) which was used to determine the total number of microorganisms. The average sample was suspended in the same phosphate buffer (4.5 cm^3) using a Paramix 2 mixer (Juchheim Labortechnik KG, Germany) for 1 min at room temperature. Then the sample was diluted and plated on LB-agar. The plates were incubated at 28 °C overnight. The number of colony forming units (CFU) per 1 g dry soil was counted.

Naphthalene assay in soil: Soil samples (1 g) were extracted in a Soxhlet device (40 cm^3 methanol) for 30min at 65 °C. The extract was adjusted by methanol to 50 cm^3 in a measuring flask. High pressure liquid chromatography (HPLC) of assaying naphthalene in soil was used. The methanol extract of soil samples free of mechanical admixtures was loaded into a column and analyzed by LCMS-QP8000α (Shimadzu, Japan) under the following conditions: column, Restec Allure C18 (5 μm; 4,6 mm x 150 mm); flow rate, 0.5 cm^3 min^{-1}; eluent, 85 % (v/v) methanol and 0,1 % (v/v) acetic acid.

Results and Discussion

The biological treatment of soils contaminated with PAH should be an efficient, economic, and alternative to physicochemical treatment, because it offers potential advantages such as the complete destruction of the pollutants, lower treatment cost, greater safety, and less environmental disturbance. Therefore, many researchers have been focusing on the biodegradation of PAHs. One of the ways to create effective technologies of cleaning environment from polycyclic aromatic hydrocarbons is utilization of active degrading strains, containing catabolic plasmids [18]. The transposon organization of catabolic operons, localized in conjugation plasmids, causes horizontal biodegrading genes transfer between natural bacterial populations [9]. The plasmid-containing bacteria, introduced in polluted soil, are the source of distribution of catabolic genes, which provide selective advantage to microorganisms containing them in stressful environmental conditions. In the soil, where microbial community is established, bacteria-destructors will remain or gradually eliminate. In both cases, thanks to appearance of the new combinations of plasmid and chromosomal origin, the intensification of processes of soil bioremediation can be observed. At the same time, the horizontal gene transfer can go in any direction, therefore new plasmids-containing strains appear, arising as on the basis of introduced and resident bacteria [19-20].In the work in question the efficiency of naphthalene degradation in model soil systems, when bacteria, representing various combinations "plasmid-host", are introduced in, was studied to modeling processes, occurring in natural environment. We assumed that it will allow us to reveal the most active strains-destructors, which can serve as a basis for creation biological products for bioremediation of polluted soils.Earlier 105 strains of gram-negative naphthalene-utilizing bacteria, which after primary identification were referred to the genus *Pseudomonas*, were extracted from 363 samples of soil from territory of Belarus, Ukraine and Iraq. Ability of the isolated bacteria to grow on intermediate products of naphthalene degradation and presence of catechol-2,3-dioxygenase have allowed to establish that salicylate, which then oxidizes via *meta*-pathway, is an intermediate product of naphthalene degradation for all investigated bacteria [21]. Selection of the most active strains-destructors (for utilizing them as recipients in D-plasmid transfer) and search of the plasmids possessing effective systems of biodegradation (for their transfer to cells of natural and collection bacteria *Pseudomonas*) were carried out among natural bacteria.At the first stage

the growth rate of bacteria in the medium with naphthalene was defined. It was assumed, that the given index can be directly or indirectly connected with physiological peculiarities of the isolated microorganisms, in particular, can depend on time of cell cycle in which reduplication and distribution of genetic material occur, and on ability of biodegrading genes to express in one or another genetic environment.Time, which is necessary for bacterial population to reach stationary phase, served as an indicator of growth rate in the medium with naphthalene (initial concentration of bacteria was10^3 cells cm^{-3}). On grounds of obtained data natural naphthalene-utilizing bacteria have been approximately divided into three groups: bacteria from the first group reached stationary growth phase in 48 h of cultivation (60 strains), from the second group – in 72 h (21 strains) and from the third group – in 96 h (24 strains). It should be mentioned that established growth rate is rather low. It is caused by small concentration of brought bacteria, which did not provide fast accumulation of intermediate product of metabolism of naphthalene (salicylate), necessary for induction of *nah*-genes' expression. Nevertheless, the obtained data, certainly, is useful for the primary description of the bacteria, capable to use naphthalene as a source of carbon and energy.It is well-known that bacteria of genus *Pseudomonas* utilize a wide range of organic substrata [1]. As investigated microorganisms are extracted from polluted soils, it is possible to assume that for successful existence in such conditions they should contain biodegrading genes. Besides, the naphthalene-1,2-dioxigenaze, which carries out the first stage of naphthalene oxidation, also participate in decomposing of other polycyclic aromatic hydrocarbons, phenanthrene, for example. For the majority of gram-negative bacteria the degradation way of phenathrene is a modification of the naphthalene degradation pathway only [21, 22].For studying metabolic potential of the isolated bacteria their ability to use monocyclic aromatic hydrocarbons (camphor, benzol, toluene, xylene and their derivatives), polycyclic aromatic hydrocarbons (phenanthrene and anthracene, pyrene, biphenyl, chlorinated biphenyl, naphthalene bromide), acyclic hydrocarbons (hexadecane, octane), oil and products of its processing (diesel fuel, kerosene) as the only source of carbon and energy was defined. As a result of studying 9 strains, capable to use from six to thirteen various organic substrata as the only source of carbon and energy, were revealed. Other strains utilized from one to five compounds, and 7 strains did not possess additional biodegradation possibilities. Strains *P. putida* NL21, *P. putida* NL26, *P. putida* AL21 were characterized by the widest spectrum of utilized substrata. In particular, strain *P. putida* NL26 has grown in the medium with naphthalene bromide, naphthalene, phenanthrene, anthracene, biphenyl, chlorinated biphenyl, *meta*- and *para*-xylene, octane, hexadecane, kerosene, diesel fluel, and strain *P. putida* AL21, besides these compounds also utilized camphor. It should be mentioned that these strains were characterized by relatively high growth rate in the medium with naphthalene (are referred to I group). No one of investigated strains possessed the ability to utilize oil.Genes, determining synthesis of enzymes involved in the conversion of naphthalene, which provide naphthalene utilisation, form a part of two operons [21]. It was established that the "upper" operon is conservative enough and, as a rule, has plasmid localisation. The first gene of the "upper" operon determines synthesis of naphthalene-1,2-dioxygenase (NO) – an enzyme with a wide substratum specificity. Salicylic acid is a product of naphthalene oxidation, which is determined by genes of the " upper " operon. Further splitting of salicylic acid is determined via the "lower" operon. The first gene of the "lower" operon also determines synthesis of enzymes with wide substratum specificity – salicylate hydroxygenase (SH), providing transformation of salicylate into catechol. Catechol-2,3-dioxygenase (C2,3O) and catechol-1,2-dioxygenase (C1,2O) provide splitting of catechol via *meta*- and *ortho*-cleavage pathway, correspondingly. It has

been established that *meta*- cleavage pathway is always coded by plasmid genes, and *ortho*- cleavage pathway of catechol metabolism is defined by chromosomal determinants and rare by plasmid genes [21].Regulation of *nah*-operons expression is carried out with participation of regulatory proteins and demands presence of an inductor in the medium. Primary or intermediate metabolism products act as such inductors. Addition of salicylate (or naphthalene, which can be slowly metabolized into salicylate) to the medium leads to activation of biodegrading genes' transcription, mediated by *nah*R gene product. Presumably, NahR protein binds with salicylate or salicylic aldehyde and according to the model of positive regulation passes to active form, providing activation of a transcription of *nah*-operons [21]. Activity of key enzymes of naphthalene catabolism, namely, naphthalene dioxygenase, salicilate hydroxigenase, catechol-2,3-dioxygenase and catechol-1,2-dioxygenase characterizes efficiency of naphthalene degradation. Besides, studying of the named enzymes allows to immediately characterize genes of plasmid (NO, SH, C2,3O) and chromosomal (C1,2O) origins that can further provide purposeful designing of effective strains-destructors, containing definite combinations of chromosomal and extrachromosomal genetic determinants.It has been established that all investigated strains possessed induced system of *nah*-genes expression, namely, activity of key enzymes (NO and SH) depended on presence of inductors, which are salicylate or naphthalene. There was no difference between actions of salicylate and naphthalene as inductors, that is salicylate was used in this capacity.From the analysis of key enzymes' activities of naphthalene metabolism (tab. 1) it is possible to draw some conclusions. Activity of key enzymes of naphthalene metabolism doesn't correlate with growth rate of bacteria in the medium with naphthalene in the capacity of the only source of carbon and energy. For example, activity NO in strains of I group varies from 13,8 to 170,0 nm/min/mg of protein, in strains of II and III groups – from 22,7 to 172,9 nm/min/mg of protein. The same picture is observed for SH which activity in strains of I group is in the range from 2,5 to 79,3 nm/min/mg of protein and in strains of II and III groups – 3,2 to 103,3 nm/min/mg of protein. The obtained data allows to assume that physiological parameters of bacteria growth in the medium with naphthalene depend on features of the cell cycle (speed of cell fission) for the most part and aren't connected with efficiency of naphthalene metabolism genes'expression. Nevertheless, it could be preferable to use bacteria of I group in capacity of strains-destructors. Namely these bacteria are capable to utilize a wide range of organic substrata and, in comparison with other strains, can possess selective advantage in the medium, containing polycyclic aromatic hydrocarbons. Rapid increase in number of the bacterial population, caused by peculiarities of cell cycle and wide metabolic possibilities, can provide given bacteria with high competitiveness in struggle for existence in the developed microbial communities. In our opinion, it is more optimal to use fast-growing bacteria (I group), which are capable to utilize a wide range of organic compounds and possess relatively high activities of key enzymes of naphthalene metabolism, as strains-destructors (strains *P. putida* AL21, *P. putida* NL26,*P. putida* NL21). Rather high activity of key enzymes of naphthalene metabolism of plasmid origin (NO, SH, C2,3O) is characteristic for bacteria *P. putida* NL4,*P. fluorescens* NL61 and *P. putida* AL1, containing D-plasmids of IncP-9 and IncP-7 groups. Plasmids from these strains were used for their transfer to the cells of various bacterial hosts in order to find strains-destructors, containing an optimum combination of determinants of plasmid and a chromosomal origin. Besides strains *P. putida* AL21,*P. putida* NL21, *P. putida* NL26, utilizing a wide range of organic compounds, bacteria, on basis of which biological stimulators of plants' growth are made (*P. mendocina* BKMB1299, *P. putida* M), were used as recipients for D-plasmids' transfer. Also

some collection bacteria *Pseudomonas*, which represent soil microflora (*P. putida* KT2442,*P. aureofaciens* B1393, *P. stutzeri* B975, *P. paleronii* B1328), and, hence, represent potential hosts of biodegrading plasmids, were used for these purposes.It is known that the property of naphthalene utilization does not always express effectively in the bacteria, which differ from natural hosts, and absence of well diagnosed selective markers doesn't allow to study their inheritance in foreign bacteria cells. Because of this transposons mini-Tn5, determining resistance to kanamycin or streptomycin, have been introduced into plasmid replicons. As a result of conjugational crossings of transposon-containing plasmids have been transferred to the cells of all investigated bacteria. The transfer frequency of plasmids varied in big enough intervals. For example, the plasmid pAL1 was transferred with frequency 2,9 x 10^{-2} in bacteria *P. putida* KT2442 and with frequency 1,5 x 10^{-8} in bacteria *P. putida* NL26. The plasmid pNL4 was transferred with frequency 1,7 x 10^{-3} in bacteria *P. mendocina* BKMB1299 and with frequency 2,7 x 10^{-6} in bacteria *P. putida* AL1. The plasmid pNL61 was transferred with frequency 7,4 x 10^{-2} in bacteria *P. putida* M and with frequency 1,3 x 10^{-4} in bacteria *P. putida* AL1 (tab. 2).The analysis of plasmids' inheritance has shown that all of them are maintained stably in foreign hosts' cells and, hence, received plasmid-containing strains can be used for analysis of their biodegrading abilities in soil (tab. 2).The dynamics of growth and efficiency of utilizing naphthalene by plasmid-containing bacteria, when they are introduced in model soil system, has been studied. Samples of soil with naphthalene and without bacteria served as a positive control, allowing to define natural evaporation of naphthalene, and samples of soil without naphthalene served as negative control.As a result of conducted experiments has been established that introduced in soil naphthalene utilizing bacteria in concentration 10^6 - 10^7 CFU (g dry soil)$^{-1}$ has provided increase of their number at the average to 10^8 CFU (g dry soil)$^{-1}$ in 7 days. And genetically constructed strains possessed the highest efficiency of naphthalene degradation in soil (tab. 3). In particular, high efficiency of naphthalene degradation is peculiarity for strain*P. putida* KT2442, containing plasmid pNL61 (IncP-9 group). At the same time, combinations of two plasmids of IncP-9, IncP-7 groups and IncP-2, IncP-7 groups in the cells of natural strains *P. putida* AL21, *P. putida* NL21, *P. putida* NL26 and *P. putida* AL1 have authentically increased their biodegrading potential (concentration of naphthalene has been decreased to negative control level). The lowest degradation of naphthalene occurred, when bacteria of strain *P. paleronii* B1328, containing a plasmid pNL4, were brought in soil (IncP-9 groups).Thus, as a result of the conducted research it has been established that plasmids of InP-7 and InP-9 groups are transferred by conjugation in bacteria of genus *Pseudomonas* effectively enough, demonstrating stability of inheritance in foreign hosts' cells. This fact gives grounds to assume that, when effective bacteria-destructors, containing Nah-plasmids of given classification groups, are introduced in soil, horizontal biodegrading genes transfer between naturally occurring microbial communities, which will provide increase in their adaptive abilities, will be observed. In the capacity of effective strains-destructors the bacteria, capable to utilize a wide range of organic compounds as a source of carbon and energy and containing plasmid of biodegradation in cells (in particular, *P.putida* AL21, *P.putida* NL21, *P.putida* NL26), can be used.

Table 1.The activity of key enzymes of naphthalene catabolism in the cells of naphthalene-degrading *Pseudomonas*

Strain	Substratum utilised, quantity	Growth rate with naphthalene, group	Enzyme activity, nM/min/mg protein			
			NO	SH	C2,3O	C1,2O
AL21	13	I	31,36	79,3	19,11	103,89
NL26	12		73,44	30,18	193,73	59,98
NL21	10		67,90	50,51	2231,14	39,01
NL3	6		49,7	33,1	158,4	6,1
AL30	5		19,7	2,5	231,4	12,7
AL1	5		109,76	60,87	1177,29	124,91
NL1	5		36,1	14,2	6,5	4,7
AL32	4		28,5	4,2	793,7	31,6
NL42	4		15,9	15,9	2,7	5,3
AL38	1		13,8	13,8	55,0	0
NL61	1		117,0	60,9	1333,6	31,7
AL37	1		20,7	27,6	88,1	0
NL4	6	II	172,9	83,6	1171,9	113,5
AL11	4		49,0	63,7	0,9	27,0
NL49	4	III	50,6	4,5	229,0	11,8
NL5	4		90,6	103,3	617,49	35,77
AL35	3		22,7	3,2	501,1	19,4
NL9	3		170,0	34,0	3,6	23,5

Notes: Activity of NO – naphthalene dioxygenase, SH – salicylate hydroxylase, C2,3O - catechol -2,3-dioxygenase, C1,2O - catechol -1,2-dioxygenase was determined by adding salicilate as inductor.

Table 2.Inheritance of IncP-7 and IncP-9 groups Nah-plasmids in the cells of *Pseudomonas* bacteria

Strain-recipient	Resident plasmid (IncP-group) *	Transfered plasmid (IncP-group)	Frequency of plasmid transfer	Stability of inheritance, %
P. putida AL21	IncP-9	pAL1 (IncP-7)	$1,8x10^{-4}$	100
P. putida NL21	IncP-9		$1,9x10^{-6}$	100
P. putida NL26	IncP-2		$1,5x10^{-8}$	100
P. putida NL4	IncP-9		$1,3x10^{-4}$	100
P.mendocina BKMB1299	not		$4,2\times10^{-6}$	100
P.putida KT2442	not		$2,9\times10^{-2}$	100
P.stutzeri B975	not		$1,1\times10^{-4}$	100
P. putida AL1	IncP-7	pNL4 (IncP-9)	$2,7x10^{-6}$	100
P. putida NL26	IncP-2		$7,2x10^{-6}$	100
P.putida KT2442	not		$7,0\times10^{-4}$	100
P.mendocina BKMB1299	not		$1,7\times10^{-3}$	100
P.paleronii B1328	not		$7,2x10^{-6}$	100
P. putida AL1	IncP-7	pNL61 (IncP-9)	$1,3x10^{-4}$	100
P.mendocina BKMB1299	not		$1,1\times10^{-3}$	100
P.putida M	not		$7,4\times10^{-2}$	100
P.aureofaciens B1393	not		$1,5\times10^{-4}$	100
P.putida KT2442	not		$7,4\times10^{-3}$	100

Notes:*– plasmids were detected and characterised by the restriction analysis of *rep*-gene amplification products.

Table 3.Efficiency of naphthalene degradation by *Pseudomonas* bearing Nah-plasmids in model soil system

Strain	Plasmid (IncP group)		Number of bacteria in soil, CFU (g dry soil)$^{-1}$		Naphthalene concentration in soil in 7 days, %
	resident	transferred	Initial	In 7 days	
P. putida AL1	Inc-7	not	$6,3x10^7$	$3,0x10^8$	1,5
P. putida AL21	IncP-9	pAL1 (IncP-7)	$2,9x10^7$	$8,0x10^8$	0
P. putida NL21	IncP-9		$5,1x10^7$	$3,9x10^8$	0
P. putida NL26	IncP-2		$1,1x10^7$	$8,0x10^8$	0
P. putida NL10	IncP-9		$1,3x10^7$	$5,0x10^7$	32
P.mendocina BKMB1299	not		$3,7x10^6$	$7,8 x10^8$	6,9
P.putida KT2442	not		$2,2 x10^6$	$2,3 x10^8$	0,1
P.stutzeri B975	not		$2,0 x10^6$	$3,0 x10^7$	0,1
P. putida NL4	IncP-9	not	$2,6 x10^6$	$1,3 x10^8$	0,2
P. putida AL1	IncP-7	pNL4 (IncP-9)	$3,6x10^7$	$4,0x10^8$	0,1
P. putida NL26	IncP-2		$1,0x10^8$	$2,6x10^9$	0,1
P.mendocina BKMB1299	not		$3,5 x10^6$	$1,6 x10^8$	1,1
P.putida KT2442	not		$2,5 x10^6$	$1,7 x10^8$	0,1
P.paleronii B1328	not		$3,6 x10^6$	$2,3 x10^8$	67,8
P. fluorescens NL61	IncP-9	not	$3,7 x10^6$	$7,1 x10^8$	0,1
P. putida AL1	IncP-7	pNL61 (IncP-9)	$1,5x10^7$	$1,0x10^8$	0
P.mendocina BKMB1299	not		$3.4 x10^6$	$1,4 x10^8$	21,3
P.putida M	not		$3,2 x10^6$	$2,0 x10^8$	0,1
P.aureofaciens B1393	not		$2,9 x10^6$	$2,0 x10^8$	0,1
P.putida KT2442	not		$1,2x10^6$	$1,1x10^8$	0
P. putida NL26	IncP-2	not	$2,7 x10^6$	1,9 x108	0,3
P. putida AL21	IncP-9	not	$3,2 x10^6$	$2,8 x10^8$	0,2
P. putida NL21	IncP-9	not	$3,1x10^6$	$1,4 x10^8$	0,3

Notes: naphthalene concentration in soil was determined taking into account its natural evaporation

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كفاءة بكتريا الزائفة *Pseudomonas* الحاملة للبلازميدات النفثالينية Nah-PLASMIDS في تحليل النفثالين في أنظمة التربة النموذجية

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الخلاصة

تم عزل مجموعة كبيرة من سلالات بكتريا الزائفة *Pseudomonas* القادرة على تحليل مركبات النفثالين من عدة مناطق ملوثة ,و التي أظهرت نمو ثابت ومعتدل في الأوساط السائلة ألحاويه على النفثالين. حيث تتخذ هذه السلالات من تحلل المركبات العضوية البسيطة منها والمعقدة(النفط) كمصدر وحيد للكربون والطاقة في نموها, ولأنزيماتها فاعليه تحليلية للمركبات النفثالينة: (naphthalene dioxygenase, salicylate hydroxylase, catechol-1,2-dioxygenase and catechol-2,3-dioxygenase).

استخدمت السلالات الأكثر فعالية كمستقبلات recipients للبلازميدات نوع Nah-plasmids مجموعة IncP-7 و IncP-9 . حيث تم النقل الوراثي للبلازميدات الى داخل خلايا البكتريا الطبيعية المحللة للنفثالين (المعزولة من المناطق الملوثة) والى سلالات أخرى من بكتريا الزائفة *Pseudomonas* المعلمة والمدروسة مختبريا . أظهرت المجموعتين كفاءة محددة في تحليل النفثالين في نماذج التربة المختبرة , حيث تناقص تركيز النفثالين من 32.2 % إلى 100 % خلال 7 أيام في نموذج التربة .أفضل كفاءة للسلالات ألمختبريه في تحليل النفثالين *P.putida* KT2442 , وللبكتريا الطبيعية المعزولة *P. putida* AL1, *P. putida* NL21, *P. putida* NL26, والحاوية على نوعين من البلازميدات Nah-plasmids.هذه السلالات يمكن استخدامها في عملية التطهير الحيوي بصوره فعالة في المناطق البيئية الحاوية على تربة ملوثة.