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EXPERIMENTAL ARTICLES

The Construction and Monitoring of Genetically Tagged, Plasmid-Containing, Naphthalene-Degrading Strains in Soil

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Abstract—A genetically tagged, plasmid-containing, naphthalene-degrading strain, *Pseudomonas putida* KT2442(pNF142::Tn*Mod*-OTc), has been constructed. The presence of the *gfp* gene (which codes for green fluorescent protein) and the kanamycin and rifampicin resistance genes in the chromosome of this strain allows the strain's fate in model soil systems to be monitored, whereas a minitransposon, inserted into naphthalene biodegradation plasmid pNF142 and containing the tetracycline resistance gene, makes it possible to follow the horizontal transfer of this plasmid between various bacteria. Plasmid pNF142::Tn*Mod*-OTc is stable in strain *P. putida* KT2442 under nonselective conditions. The maximal specific growth rate of this strain on naphthalene is found to be higher than that of the natural host of plasmid pNF142. When introduced into a model soil system, the genetically tagged strain is stable and competitive for 40 days. The transfer of labeled plasmid pNF142::Tn*Mod*-OTc to natural soil bacteria, predominantly fluorescent pseudomonads, has been detected.

Key words: naphthalene biodegradation, labeled plasmids, conjugal transfer, *Pseudomonas.*

The contamination of soil and water with polycyclic aromatic hydrocarbons (PAHs) in the industrially developed regions of the world is of particular environmental concern, as many PAHs possess carcinogenic and mutagenic properties [1].

PAHs are mainly degraded by microbial populations [2, 3], although abiotic processes can also play a role in PAH degradation. The efficiency of biological PAH degradation can be significantly improved by introducing active bacterial PAH degraders into a contaminated environment [3, 4]. Bacterial PAH degradation is often related to plasmids. Most of the plasmids that control PAH degradation have been found in bacteria of the genus *Pseudomonas* [5, 6]. Many such plasmids are conjugative; i.e., they are able to transfer genetic information [7]. This allows the propagation of biodegradation characteristics inside and between microbial populations [5, 8]. The horizontal transfer of biodegradation genes can enhance the bioremediation of contaminated soils [3] and promote the evolution of the genetic systems responsible for biodegradation. Study of the horizontal transfer of biodegradation genes and plasmids in microbial populations can be considerably facilitated by labeling these plasmids and host strains.

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The aim of this work was to construct a genetically tagged, plasmid-containing, naphthalene-degrading strain; to study its stability, survival, and competitiveness in mixed microbial populations; and to monitor the horizontal transfer of biodegradation plasmids in soil.

MATERIALS AND METHODS

The bacterial strains and plasmids used in this work are listed in the table.

Media and cultivation conditions. Bacteria of the genus *Pseudomonas* were grown at 24°C in nutrient-rich media (LB broth [12] and King B medium [13]) for 1 day or in a mineral Evans medium [14] for 3–5 days. Agar plates were prepared by adding 2% Difco agar to the media.

Glucose, succinate, and salicylate were added to the media at final concentrations of 0.1–0.2% to provide carbon sources. When the bacteria were grown on agar plates, naphthalene was placed on the inner side of the lids of inverted petri dishes. When the bacteria were grown in liquid media, naphthalene was added to these media at concentrations of 0.2–0.5 g/l.

The selective nutrient media contained antibiotics in the following concentrations (μg/ml): tetracycline (Tc),

Bacterial strains and plasmids used in this work

Strain	Plasmid	Relevant phenotype	Source
–	pTn*Mod*-OTc	–	G. Zylstra, USA [9]
Escherichia coli S17–1	pTn*Mod*-OTc	Tc^r	This work
Pseudomonas sp. 142NF	pNF142	Nah^+ Rif^r Sm^r	Laboratory of Plasmid Biology
Pseudomonas putida KT2442	pNF142::Tn*Mod*-OTc	Nah^+ Gfp^+ Km^r Tc^r Rif^r	This work
Pseudomonas putida KT2442	pBS216	Nah^+ Gfp^+ Km^r Rif^r	This work
Pseudomonas putida KT2442	pNF142	Nah^+ Gfp^+ Km^r Rif^r	This work
Pseudomonas putida KT2442	–	Gfp^+ Km^r Rif^r	K. Smalla, Germany [10]
Pseudomonas putida BS394	pNF142::Tn*Mod*-OTc	Cys^- Nah^+ Tc^r Sm^r	This work
Pseudomonas putida G7	NAH7	Nah^+	I. Gunsalus, USA [11]
Pseudomonas putida BS394	–	Cys^- Rif^r Sm^r	This work

Note: Nah^+ indicates the ability to grow on naphthalene. Km^r, Rif^r, Sm^r, and Tc^r stand for kanamycin, rifampicin, streptomycin, and tetracycline resistance, respectively. Gfp^+ indicates the presence of green fluorescent protein. Cys^- is cysteine auxotrophy.

10, 50, and 100; streptomycin (Sm), 50 and 100; kanamycin (Km), 100; rifampicin (Rif), 50 and 100.

Cell transformation. Competent cells of *Escherichia coli* S17-1 were transformed by plasmid pNF142::Tn*Mod*-OTc as described in the handbook [15] and plated onto LB agar containing tetracycline at a concentration of 10 μg/ml.

The conjugal transfer of bacterial plasmids. Donor and recipient cells, taken in a proportion of 1 : 2, were plated onto LB agar and incubated for 12 h. Then, the cells were washed off the plates with 0.85% NaCl. The washings were appropriately diluted and plated onto selective agar media.

Plasmid DNA was isolated using a slightly modified alkaline lysis method [16]. Plasmid-containing bacterial strains were grown at 28°C for 16 h on a shaker in 200 ml of Evans medium containing naphthalene.

Restriction analysis was carried out according to the method described by Sambrook *et al.* [15] using buffer solutions and restriction endonucleases purchased from Amersham (United Kingdom). The total volume of the reaction mixture was 20–40 μl. The DNA samples were digested by the restriction enzymes at 37°C for 15–120 min, after which the reaction was stopped by incubating the mixture at 65°C for 15 min.

Electrophoresis was carried out in 0.8% agarose gel with a 0.5× Tris–borate buffer (89 mM H_3BO_3, 89 mM Tris–HCl, and 2 mM EDTA). The agarose gel contained ethidium bromide at a final concentration of 0.5 μg/ml. The sample buffer contained 0.025% xylenecyanol, 0.025% bromphenol blue, and 2.5% Ficol-400. The marker used was 1-kb DNA Ladder (Fermentas, Lithuania).

The specific growth rate of the naphthalene-degrading strains was determined by growing them in a batch mode in Evans medium supplemented with 2 g/l naphthalene powder. The medium was inoculated with $(1–2) \times 10^6$ cells/ml. The samples were taken at 3-h intervals. The concentration of bacterial cells was determined by plating serial sample dilutions onto LB agar. The maximum specific growth rate was calculated by minimizing the square deviations of the exponential function approximating the concentration of bacterial cells in the exponential growth phase. The minimization was carried out with the aid of the MINUIT program [17].

The stability of phenotypic traits (utilization of naphthalene and salicylate and resistance to tetracycline) was determined as the percentage of clones that retained the ability to grow on naphthalene and salicylate or in the presence of tetracycline compared to the total number of clones grown on LB agar. In this case, the strains were subcultured in LB broth, with a regular (at 1-day intervals) transfer of cells to a fresh medium and the analysis of 200 clones for the ability to grow on selective media with succinate, salicylate, 2-methylnaphthalene, naphthalene, 100 μg/ml kanamycin, and 50 μg/ml tetracycline.

The monitoring of genetically tagged, plasmid-containing, naphthalene-degrading strains in soil was carried out on an experimental plot on the territory of the municipal sewage-treatment plant in Pushchino. The plot consisted of two separate blocks of soil that were each 1 m^2 in area and 10 cm in depth. Both blocks were supplemented with naphthalene powder at a concentration of 2 mg/g of soil. The first block was used as the control, whereas the soil of the second block was inoculated with strain *Pseudomonas putida* KT2442, harboring naphthalene degradation plasmid pNF142::Tn*Mod*-OTc. At 7-day intervals, the control and experimental soils were sampled for analysis.

Analysis of naphthalene in the soil samples. An aliquot (5 g) of soil was suspended in 50 ml of methanol and extracted by incubating the suspension at 25°C for 24 h. An aliquot (0.8 μl) of the extract was then analyzed using an LKB-2150 high-performance liquid

chromatograph (LKB–Pharmacia Biotechnology) equipped with a column (4 × 250 mm) packed with 5-μm Spherisorb ODS-2. The column was eluted with 75% methanol at a flow rate of 0.7 ml/min. The eluate was monitored at 275 nm.

RESULTS

1. Construction of genetically tagged, naphthalene-degrading strains *P. putida* KT2442 (pNF142::Tn*Mod*-OTc) and *P. putida* BS394 (pNF142::Tn*Mod*-OTc). In order to derive *P. putida* KT2442(pNF142::Tn*Mod*-OTc), *E. coli* S17-1 cells were transformed with plasmid pTn*Mod*-OTc, which harbors minitransposon Tn*Mod*-OTc. Plasmid pTn*Mod*-OTc was then transferred to strain *Pseudomonas* sp. 142NF, which carries naphthalene degradation plasmid pNF142. The sole clone produced, *Pseudomonas* sp. 142NF Nah^+ Tc^r, was crossed with *P. putida* KT2442 *gfp*, and eight *P. putida* KT2442 transconjugants with the phenotype Nah^+ Tc^r Km^r Gfp^+ were selected. The phenotype of the transconjugants indicated that minitransposon Tn*Mod*-OTc was indeed incorporated into plasmid pNF142 (Figs. 1a, 1b). Two of the transconjugants (clones 5 and 8) were used in further experiments. The strain tagged by the *gfp* gene (which codes for green fluorescent protein) could easily be detected on LB agar, since its colonies fluoresced green under ultraviolet irradiation at 254 nm.

Plasmid pNF142::Tn*Mod*-OTc was conjugatively transferred from strain *P. putida* KT2442(pNF142::Tn*Mod*-OTc) to auxotrophic strain *P. putida* BS394, which, as a result, acquired tetracycline resistance and the ability to grow on naphthalene.

The samples of plasmid DNA isolated from *P. putida* KT2442(pNF142::Tn*Mod*-OTc) (clone 5) and *P. putida* 142NF(pNF142) were subjected to an analysis using the restriction endonuclease *Eco*R1. The restriction pattern of plasmid pNF142::Tn*Mod*-OTc differed from that of plasmid pNF142 in that it had an additional *Eco*R1 fragment, indicating that the minitransposon was in fact incorporated into the plasmid.

2. Determination of the specific growth rate of the naphthalene-degrading strains. For this purpose, natural strains *Pseudomonas* sp. 142NF(pNF142) and *P. putida* G7(NAH7) and transconjugant strains *P. putida* BS394(pNF142::Tn*Mod*-OTc), KT2442(pNF142::Tn*Mod*-OTc) (two clones, 5 and 8), and KT2442(pBS216) (plasmid pB216 is similar to the widely known natural plasmid pDTG1 [4]) were grown on naphthalene in the liquid Evans medium. As is evident from Fig. 2, the μ_{max} values of strains BS394(pNF142::Tn*Mod*-OTc) and *P. putida* KT2442(pNF142::Tn*Mod*-OTc) (clone 8) were close to that of natural isolate *Pseudomonas* sp. 142NF(pNF142), whereas the μ_{max} values of transconjugant strain *P. putida* KT2442(pNF142::Tn*Mod*-OTc) (clone 5) was almost two times higher (0.75 as compared to 0.46 h^{-1}). For further analysis, we chose transconjugant strain *P. putida* KT2442(pNF142::Tn*Mod*-OTc) (clone 5).

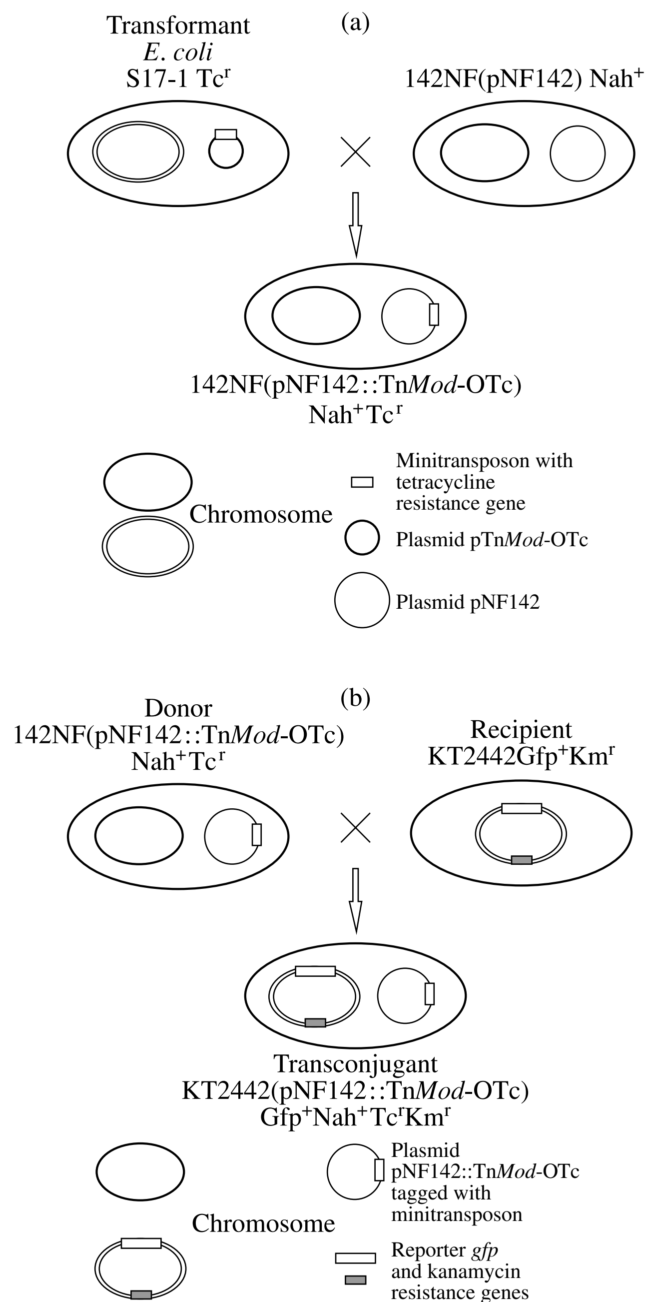

Fig. 1. (a) Construction of naphthalene biodegradation plasmid pNF142 marked by minitransposon Tn*Mod*-OTc and (b) derivation of genetically marked strain *P. putida* KT2442(pNF142::Tn*Mod*-OTc).

3. Stability of the degradation traits controlled by plasmid pNF142::Tn*Mod*-OTc. The study of transconjugant strains *P. putida* BS394 (pNF142::Tn*Mod*-OTc) and *P. putida* KT2442 (pNF142::Tn*Mod*-OTc) showed that they retained their ability to grow on naphthalene, 2-methylnaphthalene,

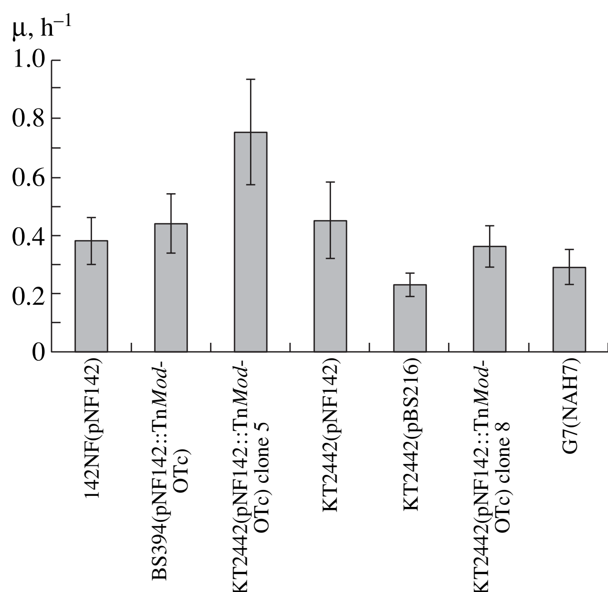

Fig. 2. The maximal specific growth rates, μ, of various naphthalene-degrading strains.

salicylate, and in the presence of tetracycline after 10 successive transfers (at 1-day intervals) into a fresh LB medium (80 generations), i.e., under nonselective conditions.

4. Monitoring of the genetically tagged, plasmid-containing, naphthalene-degrading strains in soil. The fate of the genetically tagged, naphthalene-degrading strains introduced into soil and the horizontal transfer of the labeled biodegradation plasmid could be monitored due to the presence of the *gfp* and the kanamycin and rifampicin reporter genes in the chromosome of *P. putida* KT2442 (Fig. 1b), as well as the presence of the naphthalene biodegradation and the tetracycline resistance genes in plasmid transposon Tn*Mod*-OTc.

As is evident from Fig. 3, the presence of naphthalene in the soil augmented the number of indigenous naphthalene degraders from 10^3 to 10^6 CFU/g of soil. In this case, 20 days after the introduction of marked strain KT2442(pNF142::Tn*Mod*-OTc), the number of cells had increased from 10^5 to 6×10^6 CFU/g of soil, amounting to 90% of the total population of naphthalene degraders.

In that time, the concentration of naphthalene in the experimental soil block, into which laboratory naphthalene-degrading strain KT2442(pNF142::Tn*Mod*-OTc) had been introduced, decreased from 2.0 to 0.2 mg/g of soil (Fig. 3b), whereas the concentration of naphthalene in the control soil block, which contained only indigenous naphthalene degraders, decreased to 0.6 mg/g of soil over the same time period (20 days) (Fig. 3a).

Analysis of the naphthalene-degrading microorganisms isolated from the experimental soil showed that plasmid pNF142::Tn*Mod*-OTc was transferred to indigenous soil bacteria at a rate of 2×10^{-7} to 4×10^{-6} per donor cell over a period of 12 days. Three of the four transconjugant clones isolated from the soil were found to be fluorescent bacteria of the genus *Pseudomonas*, as is evident from their specific fluorescence on a King B medium. All the isolated transconjugant clones were able to grow on naphthalene, 2-methylnaphthalene, salicylate, and in the presence of tetracycline, but, unlike strain KT2442, they were unable to

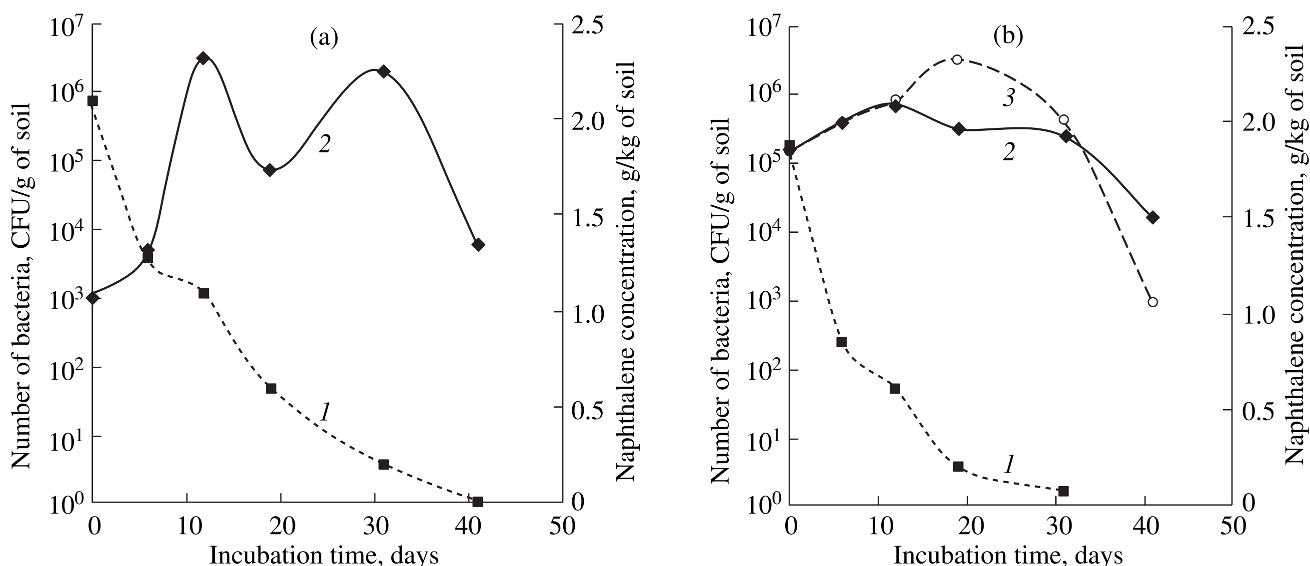

Fig. 3. (*1*) Decline in the concentration of naphthalene in soil and the dynamics of the population of (*2*) indigenous naphthalene degraders and (*3*) introduced naphthalene-degrading strain KT2442(pNF142::Tn*Mod*-OTc) in (a) control nonsterile soil and (b) nonsterile soil inoculated with KT2442(pNF142::Tn*Mod*-OTc).

grow in the presence of kanamycin. The specific growth rates of the isolated transconjugant clones were close to that of the natural isolate *Pseudomonas* sp. 142NF(pNF142).

DISCUSSION

The contamination of the environment with xenobiotics results in a high selective pressure, which may modify the degradative capacity of existing bacterial strains or give rise to new degraders. The genes responsible for biodegradation can propagate within microbial communities due to mobile genetic elements, such as plasmids and transposons. The horizontal transfer of genes and plasmids is often studied with the use of the methods of strain crossing and a detected and transposon-mediated mutagenesis of chromosomal and extrachromosomal DNA [3]. The insertion of transposons may interfere with the normal functioning of plasmid genes, which poses the problem of the stability of genetically labeled plasmids and the expression of plasmid genes.

The naphthalene-degrading strain that was constructed in this study contained a biodegradation plasmid marked by the tetracycline resistance and the naphthalene biodegradation genes. In addition, the chromosome of this strain was marked by the kanamycin resistance and *gfp* reporter genes. Thus, it was possible to monitor the fate of the marked strain and the horizontal transfer of the plasmid in soil.

It is known that biodegradation plasmids are typically large [6] and unstable in new hosts [18]. In contrast, marked plasmid pNF142::Tn*Mod*-OTc is stable in *P. putida* KT2442 and *P. putida* BS394 cells cultivated under nonselective conditions.

Transconjugant strain KT2442(pNF142::Tn*Mod*-OTc) (clone 5) exhibited a higher specific growth rate on naphthalene (Fig. 2) than natural strains 142NF(pNF142) and G7(NAH7), indicating that the incorporation of minitransposon Tn*Mod*-OTc influenced neither the expression of the naphthalene catabolism genes on plasmid pNF142 nor the key genes *rep* and *par*, which are responsible for the maintenance of plasmid pNF142::Tn*Mod*-OTc and the partition of its copies between daughter cells. It should be noted that the specific growth rate of transconjugant strain KT2442(pBS216) was minimal (0.24 h^{-1}).

The horizontal transfer of plasmids marked by antibiotic and heavy metal resistance has been thoroughly studied [19], whereas less is known about the transfer of catabolic plasmids [4, 20–23]. In particular, Herrick *et al.* [23] reported the possibility of the horizontal transfer of naphthalene biodegradation plasmids in soil, but did not provide direct evidence for this assertion. Similarly, Ramos-Gonzalez *et al.* [3] suggested the possibility of the interspecies transfer of the toluene and ethylbenzoate biodegradation plasmid pWWO-EB62 from *P. putida* EEZ15 to *P. putida* in sterile soil. Under the same conditions, plasmid pWWO-EB62K (labeled by minitransposon Tn_5) could not to be transferred to *Erwinia chrysanthemi.*

The labeled naphthalene biodegradation plasmid, pNF142::Tn*Mod*-OTc, constructed in this study allows the direct selection of transconjugants in various model systems, since these transconjugants can easily be distinguished from natural naphthalene degraders by their tetracycline resistance trait.

It is known that a population of tagged laboratory strains introduced into nonsterile soil rapidly decreases [21]. In our experiments, the population of tagged plasmid-containing strain *P. putida* KT2442(pNF142::Tn*Mod*-OTc) when introduced into the model soil system comprised 10^3 cells/g of soil after 40 days of incubation. The introduction of this strain into nonsterile soil made it possible to study the survival and competitiveness of this strain with respect to the indigenous microorganisms present, to monitor the horizontal transfer of the naphthalene biodegradation plasmid in the soil, and to determine the range of possible hosts of this plasmid.

The transfer of plasmid DNA is a naturally occurring process, which gives rise to new bacterium–plasmid combinations. Some of these combinations may prove to be efficient xenobiotic degraders. In other words, the degradation potential of soil microorganisms can be increased not only by the introduction of artificial bacterial degraders but also by the horizontal transfer of biodegradation plasmids.

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REFERENCES

1. Cerniglia, C.E., Biodegradation of Polycyclic Aromatic Hydrocarbons, *Curr. Opin. Biotechnol.*, 1993, vol. 4, pp. 331–338.
2. Pieper, D.H. and Reineke, W., Engineering Bacteria for Bioremediation, *Curr. Opin. Biotechnol.*, 2000, vol. 11, pp. 262–270.
3. Ramos-Gonzalez, M.-I., Duque, E., and Ramos, J.L., Conjugational Transfer of Recombinant DNA in Cultures and in Soils: Host Range of *Pseudomonas putida* TOL Plasmids, *Appl. Environ. Microbiol.*, 1991, vol. 57, no. 10, pp. 3020–3027.
4. Stuart-Keil, K.G., Hohnstock, A.M., Drees, K.P., Herrick, J.B., and Madsen, E.L., Plasmids Responsible for Horizontal Transfer of Naphthalene Catabolism Genes between Bacteria at a Coal Tar–Contaminated Site Are Homologous to pDTG1 from *Pseudomonas putida* NCIB 9816-4, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 10, pp. 3633–3640.

5. Sayler, G.S., Hooper, S.W., Layton, A.C., and Henry, King, J.M., Catabolic Plasmids of Environmental and Ecological Significance, *Microbiol. Ecol.*, 1990, vol. 19, pp. 1–20.
6. Boronin, A.M., Diversity of *Pseudomonas* Plasmids: To What Extent?, *FEMS Microbiol. Lett.*, 1992, vol. 79, nos. 1–3, pp. 461–468.
7. Stotzky, G. and Babich, H., Survival of, and Genetic Transfer by Genetically Engineered Bacteria in Natural Environments, *Adv. Appl. Microbiol.*, 1986, vol. 31, pp. 93–137.
8. Top, E., Springael, D., and Boon, N., Catabolic Mobile Genetic Elements and Their Potential Use in Bioaugmentation of Polluted Soils and Waters, *FEMS Microbiol. Ecol.*, 2002, vol. 42, pp. 199–208.
9. Dennis, J.J. and Zylstra, G.J., Plasposons: Modular Self-Cloning Minitransposon Derivatives for Rapid Genetic Analysis of Gram-Negative Bacterial Genomes, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 7, pp. 2710–2715.
10. Bagdasarian, M., Lurz, R., Ruchert, B., Franklin, F.C.H., Bagdasarian, M.M., Frey, J., and Timmis, K.N., Specific-Purpose Plasmid Cloning Vectors: II. Broad-Host-Range, High-Copy-Number, RSF1010-Derived Vectors for Gene Cloning in *Pseudomonas, Gene*, 1981, vol. 16, nos. 1–3, pp. 237–247.
11. Dunn, N.W. and Gunsalus, I.C., Transmissible Plasmid Coding for Early Enzymes of Naphthalene Oxidation in *Pseudomonas putida, J. Bacteriol.*, 1973, vol. 114, pp. 974–979.
12. Carhart, G. and Hegeman, G., Improved Method of Selection for Mutants of *Pseudomonas putida, Appl. Microbiol.*, 1975, vol. 30, no. 6, p. 1046.
13. 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.
14. Evans, C.G.T., Herbert, D., and Tempest, D.B., The Continuous Cultivation of Microorganisms: 2. Construction of a Chemostat, *Methods Microbiol*, 1970, vol. 2, pp. 277–327.
15. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Lab., 1989.
16. Birnboim, H.C. and Doly, J.A., A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmids DNA, *Nucleic Acids Res.*, 1979, vol. 7, no. 6, p. 1513.
17. James, F. and Roos, M., MINUIT, a System for Function Minimization and Analysis of the Parameters Errors and Correlation, *Comput. Phys. Commun.*, 1975, vol. 10, pp. 343–367.
18. Filonov, A.E. and Boronin, A.M., Plasmid Stability and the Competitiveness of Plasmid-containing and Plasmidless Strains during Continuous Cultivation, *Antibiot. Khimioter.*, 1990, vol. 35, no. 5, pp. 46–50.
19. Gorb, T.E. and Tovkach, F.I., Method for Studying the Horizontal Transfer of Plasmids in *Erwinia carotovora, Mikrobiol. Zh.* (Kiev), 2002, vol. 64, no. 3, pp. 20–26.
20. De Rore, H., Demolder, K., de Wilde, K., Top, E., Houwen, F., and Verstraete, W., Transfer of the Catabolic Plasmid RP4::Tn4377 to Indigenous Soil Bacteria and Its Effect on Respiration and Biphenyl Breakdown, *FEMS Microbiol. Ecol.*, 1994, vol. 15, pp. 71–78.
21. Newby, D.T., Gentry, T.J., and Pepper, I.L., Comparison of 2.4-Dichlorophenoxyacetic Acid Degradation and Plasmid Transfer in Soil Resulting from Bioaugmentation with Two Different pJP4 Donors, *Appl. Environ. Microbiol.*, 2000, vol. 66, no. 8, pp. 3399–3407.
22. Brokamp, A.F. and Schmidt, R.J., Survival of *Alcaligenes xylosoxidans* Degrading 2.2-Dichloropropionate and Horizontal Transfer of Its Halidohydrolase Gene in a Soil Microcosm, *Curr. Microbiol.*, 1991, vol. 22, pp. 299–306.
23. Herrick, J.B., Stuart-Keil, K.G., Ghiorse, W.C., and Madsen, E.L., Natural Horizontal Transfer of a Naphthalene Dioxygenase Gene between Bacteria Native to a Coal Tar–Contaminated Field Site, *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 6, pp. 2330–2337.