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Supplementary Materials

Evaluation of the Effectiveness of Bioaugmentation-Assisted Phytoremediation of Soils Contaminated with Petroleum Hydrocarbons Using *Echinacea purpurea*

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Construction of B1 and B2 microbial consortia

The bacterial and fungal strains used in study came from the hydrocarbon-degrading microbial collection of the Department of Microbiology (at the Oil and Gas Institute—National Research Institute, Poland). Strain diagnostic features were determined based on microscopic observations, morphology, growth on the selective agar media and biochemical profile (API tests, bioMerieux). The hydrocarbon-metabolising capabilities of all microbial strains were examined based on growth on the mineral medium supplemented with the compounds tested, as described in our previous work and according to the methods of Wrenn and Venosa. Each bacterial strain was grown individually in a nutrient broth supplemented with sodium acetate (0.2%, *w*/*v*), while each fungal strain was grown in a Czapek–Dox broth (both BD Difco™) and incubated at room temperature with shaking at 150 rpm for 24–72 h to obtain density $5*10^8$–$1*10^9$ cfu/mL. The biopreparations used in this study were constructed by mixing equal volumes of each strain. Then, the obtained mixed culture was kept in a nutrient broth supplemented with sodium acetate (0.2%, *w*/*v*) and sucrose and occasionally examined for the presence of all introduced strains. Table S1 presents the putative genes involved in petroleum hydrocarbon degradation found in genomes of the closest relatives of all bacterial strains used in this study

Table S1 Identification of bacterial strains used in B1 and B2 microbial consortia

Strain	The Closest Relative Based on 16S rRNA Accession Number (% of Identity) *	The Closest Relative for which the Genome Sequence is Available in NCBI GenBank, Accession Number (% of Identity) *	Putative Gene Encoding for the Enzymes Catalysing the Breakdown of Hydrocarbons
Dietzia sp. IN118	Dietzia sp. LJ3 MG049763 99.93%	Dietzia kunjamensis 313 CP099712 99.78%	Alkane 1-monooxygenase, aromatic ring-hydroxylating dioxygenase subunit alpha
Gordonia sp.IN101	Gordonia sp. Tm-B24 MT533993 99.63%	Gordonia terrae RL-JC02 CP049836 99.63%	Alkane 1-monooxygenase, pentachlorophenol monooxygenase, naphthalene 1,2-dioxygenase subunit alpha (2 copies), 2,3-dihydroxybiphenyl 1,2-dioxygenase (2 copies)
Mycolicibacterium frederiksbergense IN53	Mycolicibacterium frederiksbergense DSM 44346 (typical strain) NR_025393.1 99.58%	Mycolicibacterium frederiksbergense LB 501T 99.58%	Alkane 1-monooxygenase (2 copies), pentachlorophenol monooxygenase, naphthalene 1,2-dioxygenase subunit alpha (2 copies), 2,3-dihydroxybiphenyl 1,2-dioxygenase (2 copies)
Rhodococcus erythropolis IN119	Rhodococcus erythropolis KD-1 CP050124 99.42%	Rhodococcus erythropolis KD-1 CP050124 99.42%	Alkane 1-monooxygenase (5 copies), pentachlorophenol monooxygenase, cyclohexanone monooxygenase (2 copies), biphenyl 2,3-dioxygenase (2 copies), 2,3-dihydroxybiphenyl 1,2-dioxygenase (2 copies)
Rhodococcus globerulus IN113	Rhodococcus globerulus D757 CP079698 99.86%	Rhodococcus globerulus D757 CP079698 99.86%	Alkane 1-monooxygenase (3 copies), 2,3-dihydroxybiphenyl 1,2-dioxygenase (2 copies), aromatic ring-hydroxylating dioxygenase subunit alpha (7 copies)
Raoultella sp. IN109	Raoultella planticola SCLZS62 CP082168 99.08%	Raoultella planticola SCLZS62 CP082168 99.08%	aromatic ring-hydroxylating dioxygenase subunit alpha (2 copies)

Identification of Fungal Strains by MALDI-TOF MS

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify specific fungal strains and was performed at the Jagiellonian Centre of Innovation (Kraków, Poland). A colony from a fresh overnight culture was used for sample preparation to obtain the mass spectrometer measurement. The material was suspended in 300 µL double-distilled water, following which, 900 µL ethanol was added, and the components were mixed well; thereafter, the mixture was centrifuged (2 min, 9000× *g*), and the supernatant was removed. For sample extraction, 40 µL of formic acid (70% in water) was added to the fungal pellet, following which, the components were mixed thoroughly, and 40 µL of acetonitrile was added. After performing centrifugation at 9000× *g* for 2 min, 1 µL of the supernatant containing the fungal extract was transferred to a well of the 96-well MALDI Biotarget plate (Bruker Daltonics, Bremen, Germany) and was allowed to dry at room temperature. Subsequently, the sample was overlaid with 2 µL of the MALDI matrix solution (*α*-cyano-4-hydroxy-cinnamic acid) and air-dried again. The measurements were performed using the MALDI BioTyper 2.0 Microflex LT system (Bruker), using the manufacturer's recommended settings. In order to perform microorganism identification, the raw protein spectral data were imported into the MALDI BioTyper 2.0 software (Bruker) and were analysed via standard pattern matching (with default parameter settings) against the spectral data in the BioTyper database (Bruker). Parameters such as the mass-to-charge ratio and peak intensity were considered to produce a matching score, which was subsequently used to rank the results. In these studies, the identification criteria were as follows: a score of 2.000 and above was considered sufficient

for correct species identification; score values of 1.700 to 1.999 confirmed genus identification; and scores lower than 1.700 indicated no identification.