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Bacteria-plant interactions synergistically enhance biodegradation of diesel fuel hydrocarbons

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The biotechnological application of microorganisms for rhizoremediation of contaminated sites requires the development of plant-microbe symbionts capable of plant growth promotion and hydrocarbon degradation. Here, we present a study aimed at isolating single microbial strains that are capable of promoting plant growth as well as rhizoremediation of diesel fuel hydrocarbons. Through genomic analyses and greenhouse-based experiments, we examined the synergistic interactions of *Medicago sativa* L. and *Paraburkholderia tropica* WTPI1 for enhanced rhizoremediation of diesel fuel-contaminated soils. Plant growth-based experiments confirmed that the inoculation of *M. sativa* with *P. tropica* led to a 99% increase in plant biomass. Furthermore, organic geochemical analysis revealed that 96% of all the distinctive diesel fuel hydrocarbons, including C_{10}–C_{25} *n*-alkanes, branched alkanes, cycloalkanes and aromatic hydrocarbons were degraded in the *M. sativa* + *P. tropica* treatment. These results will prove beneficial for biotechnological application of *P. tropica* WTPI1 for plant growth promotion and most importantly for environmental remediation of organic pollutants.

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The quest for energy to meet the growing global demand has depended largely on petroleum and other fossil fuels. With the ever-growing human population and industrialization, there has been an increase in the release of organic pollutants to the environment. Of these, petroleum spillage, either through human error or equipment failure, has plagued the environment for some decades[1]. While large-scale marine spills often make headlines, the majority of petroleum spills occur on land, with significant health and ecological impacts[2]. Remediation of petroleum-contaminated sites is essential to mitigating the human and ecological risks. Remediation strategies are classified as either ex situ or in situ[3]. Ex situ techniques involve the excavation and relocation of contaminants for off-site treatments and, as a result, are expensive and environmentally unfriendly. In contrast, in situ remediation involves the on-site treatment of contaminants, which is typically more eco-friendly and 50–80% cheaper than traditional methods such as excavation and landfill incineration[4]. A cost-effective in situ remediation strategy that has attracted much attention in recent decades is phytoremediation.

Phytoremediation is the use of plants to clean contaminated sites. This technique exploits physical, biochemical, biological, chemical, and microbiological processes to remediate the toxic effects of pollutants[5,6]. Although various aspects of phytoremediation involve the plant and its microbiota, rhizoremediation stands out as an integrated plant-microbe endeavor[7]. Rhizoremediation is the degradation of pollutants in the rhizosphere (the area surrounding the plant roots) through microbial activity. This strategy is based on carefully selected plants with fibrous roots that serve as a natural host for hydrocarbon-degrading microbes[8]. The extensive rooting system also serves as a natural venting mechanism, thus enabling aerobic biodegradation of organic pollutants. During the rhizoremediation process, the plant roots secrete exudates such as sugars and amino acids that stimulate the growth and activity of rhizospheric microbes[9]. The slow metabolic activity of the indigenous microorganisms, however, leads to relatively long remediation times, thereby limiting the effectiveness of this approach. To address this shortfall, there is growing interest in the isolation of microorganisms usable as inocula for enhanced contaminant degradation. The inoculation of pre-cultivated microbes in order to speed up biodegradation processes is referred to as bioaugmentation.

Recent studies have shown that rhizoremediation involving bioaugmentation with pre-grown microbial cultures is the most effective soil and water clean-up technique for sites contaminated with organic pollutants[10]. In addition to their hydrocarbon-degrading capacities, some of the inoculated microbes can potentially enhance the growth of host plants through processes such as nitrogen fixation, and phosphate and potassium solubilization[11]. In turn, the root exudates released by the host plants provide a constant flow of nutrients to the associated bacteria, enabling continuous biodegradation of contaminants. This synergistic relationship has been described as the ecological driver of rhizoremediation[12]. When carefully applied, bioaugmentation offers great potential for effective reclamation of hydrocarbon contaminated sites. While a number of studies have been carried out on the use of microbial consortia for bioremediation[13,14], very few studies have been performed with single bacterial isolates. This is a major challenge to the adoption of microbially-enhanced rhizoremediation, since microbial consortia are often difficult to replicate or commercialize. In addition, it has been shown that strain selection, among other factors, can lead to the failure of bioaugmentation as a remediation strategy[10]. Hence, there is an urgent need to isolate single culturable strains suited to environmental biotechnological applications.

This study was aimed at isolating bacterial species from hydrocarbon-polluted sites that are capable of promoting both plant growth and the rhizodegradation of diesel fuel hydrocarbons. *Medicago sativa*, also known as alfalfa or lucerne, is a perennial leguminous plant that is widely grown primarily for hay, pasturage, and silage. Previous studies have shown that *M. sativa* is tolerant to diesel fuel toxicity[15,16]. *Paraburkholderia tropica* was first isolated from the sugarcane plant[17], and a recent study has shown that it can colonize barley roots and potentially promote plant growth[18]. However, while plant growth-promoting bacteria are frequently used in agriculture, their use for environmental remediation represents a huge untapped potential[7,19]. The novel strain used in this study was isolated from a crude oil tar pit. To the best of our knowledge, this study is the first attempt to examine the synergistic interactions of *M. sativa* L. and *P. tropica* WTPI1 for enhanced rhizoremediation of diesel fuel-contaminated soils. In addition, this study revealed that *P. tropica* WTPI1 thrived in the presence of indigenous microbes. This is vital since the survival and viability of inoculated species is a major factor limiting the success of many bioaugmentation trials[20,21]. By combining genome studies of *P. tropica* with pot-based experiments, we demonstrate that synergistic interactions between *M. sativa* and *P. tropica* significantly promotes rhizodegradation of petroleum hydrocarbons.

Results

Bacteria genomes. The sequencing of the three genomes resulted in a total of 4,228,774 gene sequences. After processing, a total of 3,450,275 paired-end reads and 356,023 unpaired reads were retained and assembled. Assembly resulted in 575 scaffolds (Supplementary Table 1). The average sequencing depth was approximately 30×. The three genomes were taxonomically classified as *Acidocella facilis* (ANI: 97.96; AF: 0.90), *Burkholderia* sp. (ANI: 95.03; AF: 0.84) and *P. tropica* (ANI: 99.03; AF: 0.91).

Genome analysis of plant growth promotion, chemotaxis, motility, and root colonization. Functional analysis of the three genomes revealed that *P. tropica* has the greatest potential for plant growth promotion, with 36, 31, 4, 2, and 2 coding DNA sequences (CDSs) involved in nitrogen fixation, phosphate solubilization, pyrroloquinoline-quinone synthesis, siderophore transport, and indoleacetic acid synthesis, respectively (Supplementary Table 2). Key genes involved in these processes include the *nifAUQ, fixABJL, acpP, otsB, gph, plc, pqqBCDE, entS*, and *iaaH* genes (Table 1). In comparison, the genomes of the other bacterial isolates had far fewer (24–44) CDSs involved in plant growth promotion, with *nifAQ, phoD, entS*, and *iaaH* genes missing in the genome of *A. facilis* (Supplementary Table 2).

Genome analysis of hydrocarbon-degrading potential. Functional analysis revealed that *P. tropica* contains genes involved in hydrocarbon degradation (Table 2). While the three isolates are potentially able to degrade petroleum hydrocarbons, there were some specific differences in their metabolic capabilities. For example, the genomes of *P. tropica* and *Burkholderia* sp. encode for greater numbers of enzymes involved in the degradation of *n*-alkanes (especially long-chain *n*-alkanes) and cycloalkanes than the *Acidocella* genome (Supplementary Table 3). This is crucial for diesel fuel degradation since the chemical composition of diesel fuel is 75% saturated hydrocarbons (predominantly long-chain *n*-alkanes and cycloalkanes)[22,23].

The degradation of aromatic hydrocarbons proceeds through initial activation by monooxygenases and dioxygenases[24,25], followed by ring cleavage of the resulting catechols[26–28]. Genes encoding for monooxygenases and dehydrogenases were more

Table 1 Key genes in *Paraburkholderia tropica* WTPI1 genome that are putatively involved in plant growth promotion, bacterial chemotaxis, motility, root modulation, and colonization (See Supplementary Table 2 for more details).

Genes	CDSs	Active peptide/enzyme	Function
Plant growth promotion			
nifA	2	Nif-specific regulatory protein	Nitrogen fixation
nifUQ	2	Nitrogen fixation proteins NifU, NifQ	Nitrogen fixation
fixAB	10	Electron transfer flavoprotein	Nitrogen fixation
fixJL	22	Two-component system, LuxR family	Nitrogen fixation
acpP	4	Acyl carrier protein	Phosphate solubilization
otsB	3	Trehalose 6-phosphate phosphatase	Phosphate solubilization
gph	6	Phosphoglycolate phosphatase	Phosphate solubilization
plc	9	Phospholipase C	Phosphate solubilization
pqqBDE	3	Pyrroloquinoline-quinone biosynthesis proteins B, D, E	PQQ synthesis
pqqC	1	Pyrroloquinoline-quinone synthase	PQQ synthesis
entS	2	MFS transporter, ENTS family, enterobactin exporter	Siderophore transport
iaaH	2	Indoleacetamide hydrolase	IAA synthesis
Bacterial chemotaxis, motility, and root colonization			
cheABCDVWXYZ	20	Two-component chemotaxis protein	Bacterial chemotaxis
wspBDEF	4	Two-component chemotaxis protein	Bacterial chemotaxis
mcp, tsr, tar, trg, tap, wspA	47	Methyl-accepting chemotaxis protein	Bacterial chemotaxis
flg, flh, fli	52	Flagellar proteins	Bacterial motility
nodD	2	Nod-box dependent transcriptional activator	Root nodulation
tadB	2	Tight adherence protein B	Plant colonization
tadC	2	Tight adherence protein C	Plant colonization

PQQ pyrroloquinoline quinone, *IAA* indole-3-acetic acid.

Table 2 Key genes in the *Paraburkholderia tropica* WTPI1 genome that encode for hydrocarbon-degrading enzymes.

Genes	CDSs	Active peptide/enzyme	Function
Hydrocarbon degradation			
ladA	6	Long-chain alkane monooxygenase	*n*-Alkane degradation
alkR	2	Alkane utilization regulator	Activates AlkM expression
adhP	4	Alcohol dehydrogenase (propanol-preferring)	*n*-Alkane degradation
cpnA	2	Cyclopentanol dehydrogenase	Cycloalkane activation
chnB	2	Cyclohexanone monooxygenase	Cycloalkane activation
gnl	5	Gluconolactonase	Ring cleavage of cycloalkanes
xylC	1	Benzaldehyde dehydrogenase	Toluene/xylene degradation
benABC	6	Benzoate/toluate 1,2-dioxygenase	Benzoate degradation
benD	1	Dihydroxycyclohexadiene carboxylate dehydrogenase	Decarboxylation of benzoates
pcaGH	2	Protocatechuate 3,4-dioxygenase	Ring cleavage of benzoates
etbD	1	2-Hydroxy-6-oxo-octa-2,4-dienoate hydrolase	Ethylbenzene degradation
catA	2	Catechol 1,2-dioxygenase	Ortho-cleavage of catechol
dmpB	1	Catechol 2,3-dioxygenase	Meta-cleavage of catechol
catB	2	Muconate cycloisomerase	Catechol degradation
catC	2	Muconolactone D-isomerase	Catechol degradation
pcaDL	8	3-Oxoadipate enol-lactonase	Catechol degradation

CDSs coding DNA sequences.

abundant in the *P. tropica*, genome, while genes encoding for ring-activating dioxygenases (such as benzoate/toluate 1,2-dioxygenase) were more abundant in the genome of *A. facilis* (Supplementary Table 3). In addition, DNA sequences putatively encoding for ortho-cleavage of catechol (*catABC* and *pcaDL* genes) were more abundant in the *P. tropica* and *Burkholderia* sp. genomes than in the genomes of *A. facilis* (Supplementary Table 3).

Effect of bacterial inoculation on plant growth and biomass production. The inoculation of *M. sativa* with *P. tropica* resulted in increased growth rate and biomass production (Fig. 1). Analysis of variance (ANOVA) revealed that the mean dry biomass (±standard error, SE) produced by plants inoculated with *P. tropica* (6.74 ± 0.06 g) was significantly higher than, and different ($p < 0.001$) from that of the uninoculated plants (3.38 ± 0.07 g) (Fig. 1b). Statistical analysis of growth, in terms of mean shoot height per time, revealed that the inoculated *M. sativa* plants exhibited greater relative growth rate (0.109 ± 0.002 cm/day at the point of inflection) than the uninoculated plants (0.092 ± 0.004 cm/day) (Fig. 1c).

Organic geochemical analysis of residual total petroleum hydrocarbons. Gas chromatography–mass spectrometry (GC–MS) analysis of residual hydrocarbons revealed that inoculation with *P. tropica* significantly enhanced the remediation of the diesel fuel-contaminated soils (Fig. 2). Nearly all the distinctive diesel fuel hydrocarbons, including C_{10}–C_{25} *n*-alkanes, branched alkanes, cyclic alkanes and aromatic hydrocarbons (approximately 96%) were degraded in the planted and

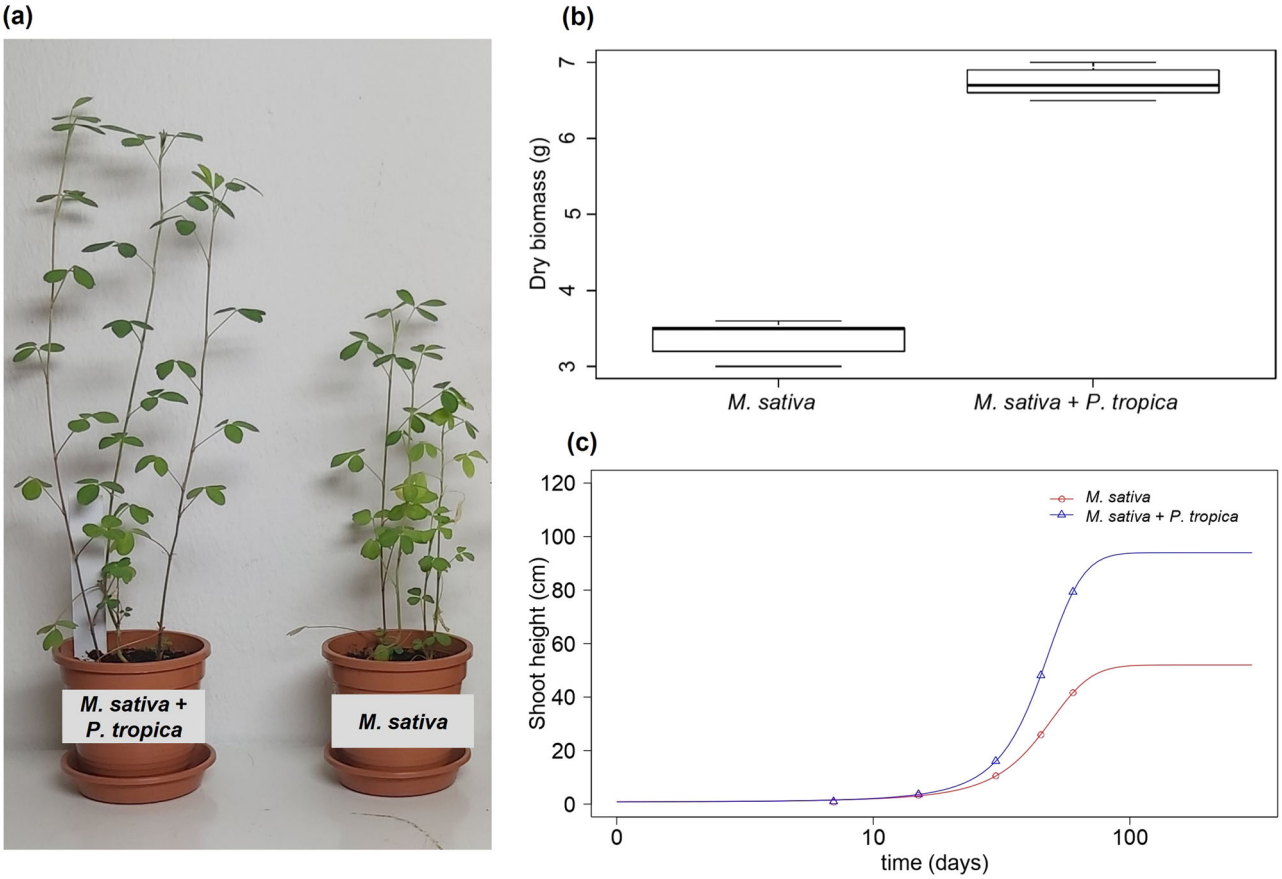

Fig. 1 Effect of bacteria inoculation on plant growth. a Enhancement of growth and biomass production of *M. sativa* plant by *P. tropica* inoculation. **b** Boxplot showing significant differences in dry biomass of inoculated and uninoculated plants. **c** 3-parameter logistic model showing differential growth rates (cm/day) of *M. sativa* inoculated with *P. tropica* versus uninoculated control.

inoculated soils (Fig. 2a). Similar results were obtained with the replicates (Supplementary Fig. 1). The mean total petroleum hydrocarbon (± SE) at the beginning of the experiment (T0) was 4.13 ± 0.04 g/kg. At the end of the experimental period, the greatest decrease in residual total petroleum hydrocarbons was observed in the "planted and inoculated soils" (Soil + *M. sativa* + *P. tropica*), while the least decrease occurred in the "unplanted and uninoculated soils" (Soil at T60) (Fig. 2b). This is an indication of greatest biodegradation in the "Soil + *M. sativa* + *P. tropica*" treatment, a claim further supported by the huge unresolved complex mixture (UCM) observed under this treatment (Fig. 2c). Preparation blanks ruled out possible cross contamination of hydrocarbons among the samples (Supplementary Fig. 2).

Biodegradation parameters. The lowest values of Pr/nC_{17}, Ph/nC_{18}, *nor*-Pr/nC_{16} and unresolved complex mixture/total petroleum hydrocarbons (UCM/TPH) were observed in the aged initial contaminated soil (Soil at T0) (Table 3), followed by the unplanted and uninoculated contaminated soil at the end of the experimental period (Soil at T60). The highest values of these ratios, indicating most intense biodegradation, were found in the planted and inoculated soils (i.e., "Soil + *M. sativa* + *P. tropica*"). The Pr/nC_{17}, Ph/nC_{18}, *nor*-Pr/nC_{16} and UCM/TPH ratios for the treatment "Soil + *P. tropica*" were higher than that of "Soil + *M. sativa*" treatment (Table 3). This is an indication that the inoculation of diesel fuel-contaminated soils with *P. tropica* alone resulted in greater hydrocarbon degradation than simple phytoremediation using *M. sativa*. This is also in agreement with the results of total residual hydrocarbon measurements (Fig. 2b).

Tukey's pairwise comparisons showed that the mean total petroleum hydrocarbons (g/kg) in the soils at the end of the experiment (Soil at T60) were significantly different from the initial mean concentration (Soil at T0), indicating that biodegradation occurred in the treatments (Supplementary Table 4). It also revealed that the residual hydrocarbon concentrations differed significantly between each treatment (Fig. 2b; Supplementary Table 4). The greatest percentage of biodegraded hydrocarbons (96%) was observed in the planted and inoculated samples (Soil + *M. sativa* + *P. tropica*) as a result of the synergistic interactions of *M. sativa* and *P. tropica*.

Biodegradation of polycyclic aromatic hydrocarbons. Molecular analysis of residual polycyclic aromatic hydrocarbons such as alkylnaphthalenes and alkylphenanthrenes revealed that the combined application of *M. sativa* and *P. tropica* resulted in complete removal of these otherwise recalcitrant hydrocarbons (Fig. 3). When compared to other treatments, "*M. sativa* + *P. tropica*" appeared to be the most effective approach for biodegradation of these organic pollutants (Supplementary Fig. 3).

16S rRNA analysis of the residual soils. The relative abundances of *Paraburkholderia* in the uninoculated soils (both planted and unplanted) were less than 1%. In contrast, the relative abundance of *Paraburkholderia* in the inoculated soil was approximately 5%, making it the fourth most-abundant bacterial genera in the inoculated soils (Supplementary Fig. 4).

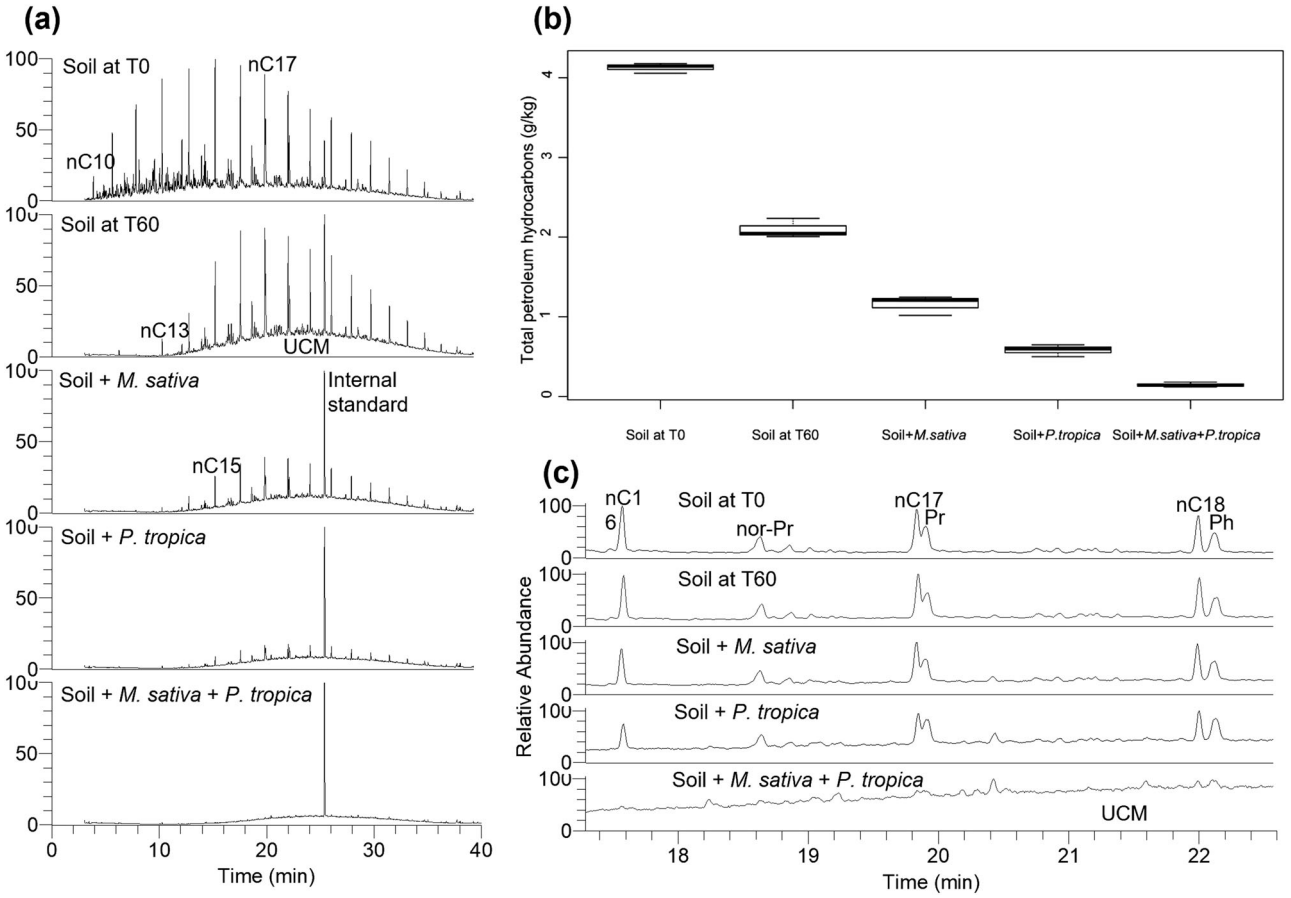

Fig. 2 Enhanced degradation of diesel fuel hydrocarbons resulting from bacteria inoculation. a GC–MS chromatograms (total ion current) showing the initial total hydrocarbons in the contaminated soils and the residual hydrocarbons after 60 days under different treatments. The chromatograms are normalized to sample weight and can be directly compared with reference to the internal standard peak (the same amount was added to all samples). **b** Boxplot showing the mean values of residual total petroleum hydrocarbons for the different treatments. **c** Partial total ion chromatograms showing preferential biodegradation of nC_{16}, nC_{17} and nC_{18} relative to nor-pristane (nor-Pr), pristane (Pr), and phytane (Ph) with increasing biodegradation among the different treatments. "Soil at T0": contaminated soils at the beginning of experiment; "Soil at T60": unplanted and uninoculated (control) soils after 60 days; "Soil + *M. sativa*": soils planted with *M. sativa*; "Soil + *P. tropica*": soils inoculated with *P. tropica*; "Soil + *M. sativa* + *P. tropica*": soils planted with *M. sativa* and inoculated with *P. tropica*; *UCM* unresolved complex mixture.

Table 3 Biodegradation parameters (ratios) for the different treatments.

Biodegradation parameters

Treatment	Pr/nC_{17}	Ph/nC_{18}	*nor*-Pr/nC_{16}	UCM/TPH
Soil at T0	0.75	0.72	0.38	0.55
Soil at T60	0.75	0.73	0.39	0.69
Soil + *M. sativa*	0.78	0.75	0.48	0.72
Soil + *P. tropica*	1.22	1.09	0.70	0.75
Soil + *M. sativa* + *P. tropica*	1.75	1.67	1.82	0.85

Discussion

The results of whole genome analysis revealed that *P. tropica* WTPI1 is a potential plant growth-promoting bacterium (Table 1). Functional analysis of the genomes of the three isolates revealed that *P. tropica* has the highest potential for plant growth promotion. For example, while *A. facilis has* 11 coding DNA sequences (CDSs) putatively involved in nitrogen metabolism (Supplementary Table 2), *P. tropica* showed 36 such CDSs (Supplementary Table 2). Similarly, *P. tropica* isolates possess the highest number of genes encoding phosphatases, siderophore exporter and indoleacetic acid synthase. Previous studies have shown that these enzymes and active peptides are vital for phosphate solubilization[29], photosynthesis[30], phytotoxicity tolerance[31,32], and cellular membranes' integrity, fluidity and permeability[33,34]. The potential of other *Paraburkholderia* species to enhance plant growth through these processes has also been reported recently[35,36].

The genome of *P. tropica* contained other important genes involved in chemotaxis, motility, and root colonization. These processes are highly important for the inoculated isolate to prosper in the rhizosphere. Chemotaxis proteins identified in the genome include 24 genes encoding two-component systems (*cheABCDVWXYZ* and *wspBDEF* genes) and 47 methyl-accepting chemotaxis proteins. This is about 2–4 times the number present in the genomes of the other bacterial species studied (Supplementary Table 2). We also identified 52 flagellar biosynthesis proteins belonging to the *flg, flh* and *fli* genes. These genes are crucial for bacterial motility. For example, it has been reported that the deletion of genes involved in motility in the endophyte *Azoarcus* sp. prevented their twitching, motility and colonization of rice plants[37]. The *nod* and *tad* genes are vital for

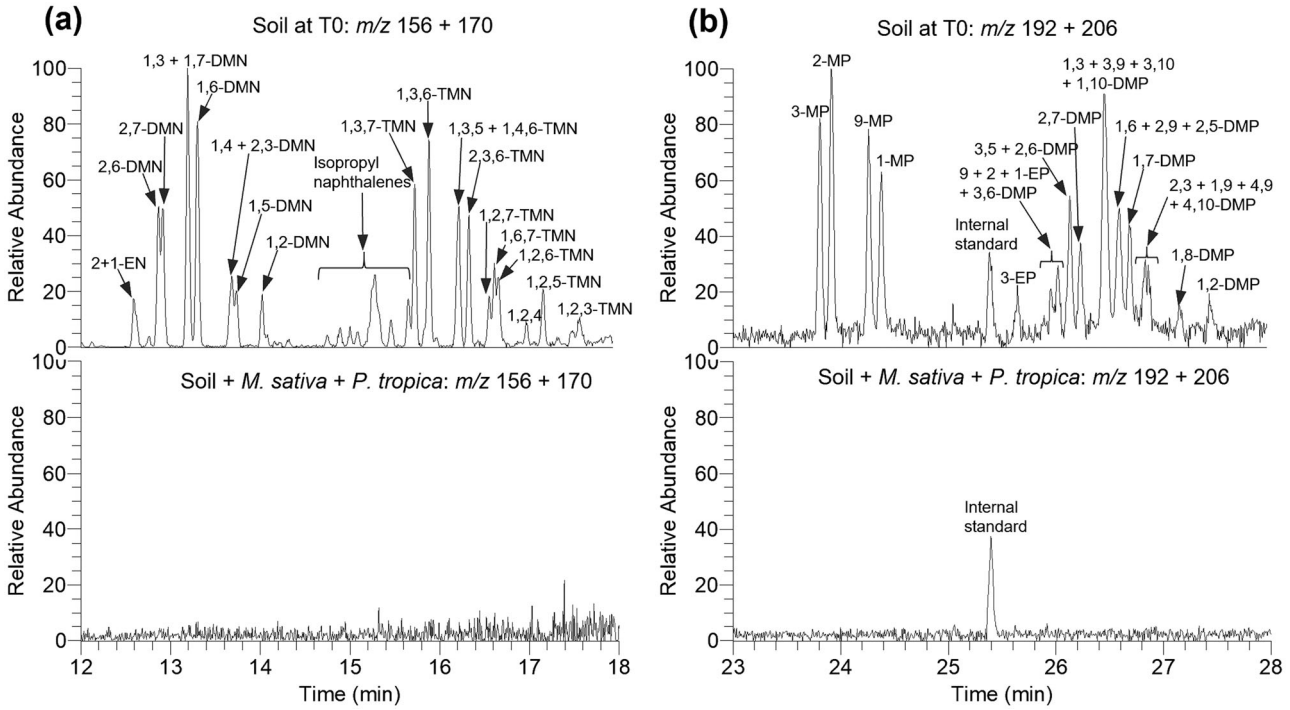

Fig. 3 Biodegradation of polycyclic aromatic hydrocarbons. a Partial m/z 156 + 170 and **b** *m/z* 192 + 206 mass chromatograms of contaminated "soil at T0" and "planted and inoculated soil" at the end of the experiment, showing effective biodegradation of alkylnaphthalenes and alkylphenanthrenes by the combined actions of *M. sativa* and *P. tropica*. EN ethylnaphthalene, DMN dimethylnaphthalene, MP methylphenanthrene, EP ethylphenanthrene, DMP dimethylphenanthrene. Numbers denote positions of alkylation.

root nodulation and plant colonization. The *nodD* gene has previously been associated with root nodulation in *Rhizobium*[38]. Similarly, previous in silico and experimental studies of other plant growth-promoting bacteria have linked the tight adherence (*tad*) systems to plant attachment and colonization[39–41]. The higher number of genes encoding for plant growth-promoting processes, and for chemotaxis, motility, and root colonization in *P. tropica* than in the other bacteria strongly suggests a possible advantage in rhizoremediation.

Diesel fuel is phytotoxic to most of the plant species and typically has negative effects on plant growth and biomass production[15]. The inoculation of *M. sativa* with *P. tropica* in this study resulted in increased growth rate and biomass production of *M. sativa*. The mean dry biomass of inoculated plants was more than twice that of the uninoculated plants (Fig. 1). Additionally, during the 60-day experimental period, the inoculated plants attained an average height of 80 cm, in contrast to approximately 42 cm for the uninoculated plants. Biomass analysis revealed that the inoculation of *M. sativa* with *P. tropica* led to a 99% increase in plant biomass (Fig. 1). These results harmonize with the plant growth-promoting potential of *P. tropica* observed in a previous study[18] and further revealed by the genome analysis data in this study.

Rhizoremediation of petroleum-contaminated soils also depends on the ability of the inoculated bacteria to degrade hydrocarbons. The examination of the three genomes revealed that all of them are potentially able to utilize aliphatic and aromatic hydrocarbons as carbon and energy sources. Key hydrocarbon-degrading enzymes present in the genome of *P. tropica* include long-chain alkane monooxygenase, cyclohexanone monooxygenase, benzoate/toluate 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase (Table 2). Among the three genomes studied, *P. tropica* possesses more genes potentially involved in long-chain alkane (*ladA* and *alkR* genes) and cycloalkane (*cpnA, chnB*, and *gnl* genes) degradation. Since diesel fuels and similar low-volatile petroleum distillates are composed predominantly of aliphatic hydrocarbons (approximately 75%)[22,23], the presence of more high-molecular weight aliphatic hydrocarbon-degrading genes in *P. tropica* than in other species suggests stronger potential for the biodegradation of diesel spills. The presence of ring-cleavage dioxygenase such as catechol 1,2-dioxygenase and catechol 2,3-dioxygenase in the genome of *P. tropica* also indicates its potential to metabolize intermediate products of aromatic hydrocarbon degradation. In a recent study, a particular strain of *Paraburkholderia aromaticivorans* isolated from a petroleum-contaminated soil was found to be capable of degrading naphthalene and BTEX (benzene, toluene, ethylbenzene and xylene)[42]. In addition, the *Paraburkholderia* isolate exhibited a higher growth rate at neutral pH (as determined by OD_{600} values) than either the *A. facilis* WTPI2 or the *Burkholderia* sp. WTPI3 isolates (Supplementary Fig. 5), indicating potential wider application of *P. tropica* WTPI1 for rhizoremediation than the other strains. Conversely, the adaptability of *Acidocella* to acidic conditions can be exploited for the remediation of sites contaminated with metals and organic pollutants such as mining sites[43–45].

Geochemical analysis revealed that natural attenuation led to only 49% degradation of the total petroleum hydrocarbons (Fig. 2). In comparison, *M. sativa* alone, *P. tropica* alone, and *M. sativa* + *P. tropica* treatments resulted in 72%, 86%, and 96% degradation, respectively. Biodegradation results from the activity of microorganisms as they utilize petroleum hydrocarbons as their carbon and energy source. Often, the first indication of petroleum biodegradation is the selective removal of C_6–C_{12} *n*-alkanes (Fig. 2a). Biodegradation parameters revealed that the removal of petroleum hydrocarbons in the different treatments came from biodegradation. These parameters (Pr/nC_{17}, Ph/nC_{18}, *nor*-Pr/nC_{16}, and UCM/

TPH) followed the expected trends, with *n*-alkanes being more biodegradable than branched alkanes, and the UCM more recalcitrant than TPH. *n*-Alkanes (nC_{16}, nC_{17}, and nC_{18}) are easily activated by monooxygenases and as a result degrade at faster rates than multi-methylated alkanes (*nor*-Pr, Pr, and Ph)[24]. This is due to the complex branching, which hinders terminal carbon activation by oxygenating enzymes[24]. As biodegradation proceeds, the abundances of major resolved compounds diminish, resulting in the more prominent chromatographic baseline hump, termed the unresolved complex mixture (UCM) (Fig. 2c). The UCM is comprised of bioresistant compounds (including polar compounds) that are unresolvable by gas chromatography[24]. The results indicate that biodegradation occurred in the different treatments and at significantly varying degrees (Table 3; Supplementary Table 4). The highest degree of biodegradation among the different treatments occurred in the "planted and inoculated" treatment, with minimal biodegradation occurring in the "unplanted and uninoculated" (control) soils.

Although the chemical composition of diesel fuel is predominantly *n*-alkanes, branched alkanes and cycloalkanes, it also contains approximately 25% aromatic hydrocarbons such as alkylbenzenes, naphthalene, alkylnaphthalenes, phenanthrene, and alkylphenanthrenes[23]. Molecular analysis of residual polycyclic aromatic hydrocarbons revealed that the *M. sativa* + *P. tropica* treatment led to an almost complete degradation of these contaminants (Fig. 3). These results are of great relevance considering the toxicity of these compounds. Naphthalene and phenanthrene are among the United States Environmental Protection Agency's 16 priority polycyclic aromatic hydrocarbons[46]. Naphthalene is also classified as a group 2b carcinogen. In 2019, the European Chemical Agency added phenanthrene to the candidate list of substances of very high concern due to its very persistent, very bioaccumulative (vPvB) nature[47,48].

Finally, the relative abundances of *Paraburkholderia* in the residual uninoculated and inoculated soils were <1% and 5% respectively. Although semi-quantitative, this method enabled us to assess the potential of the inoculated microbes to prosper in the contaminated soil. Our results revealed that *Paraburkholderia* thrived in the soils and were evidently responsible for the observed growth promotion of *M. sativa* and associated diesel fuel degradation. While many plant growth-promoting rhizobacteria have been experimented as inoculants for agricultural purposes, a major setback has always been the failure of inoculated microorganisms to effectively thrive against indigenous microbes[20,21,49]. Therefore, owing to the complexity of soils and environmental variables, the viability of *P. tropica* WTPI1 may vary under different conditions. Nonetheless, the results of this study will prove beneficial for environmental remediation purposes and for other potential biotechnological applications (such as crop yield enhancement) in agriculture.

Materials and methods

Soil sampling. A topsoil sample (10 g) was taken from a heavily polluted site in Wietze (52° 39′ 0″ N, 09°50′ 0″ E), Germany in November 2019 and transported to the laboratory on ice. Wietze is a site of historical petroleum production beginning in 1859[50]. Between 1900 and 1920, about 80% of German crude oil was produced in Wietze. The former oil field still contains petroleum seepages (Supplementary Fig. 6) amidst a forested environment. In a previous study, we examined the microbial community compositions of the contaminated oil field[51].

Enrichment cultures, isolation of single bacterial isolates, and growth conditions. Approximately 1 g of the crude oil-polluted soil was added to an Erlenmeyer flask (300 mL) containing 100 mL of liquid mineral medium (MM) composed of KH_2PO_4 (0.5 g/L), NaCl (0.5 g/L), NH_4Cl (0.5 g/L). Sterile-filtered trace elements (1 mL/L)[52], vitamin solution (1 mL/L)[52] and $MgSO_4.7H_2O$ (5 mL of a 100 mg/mL solution) were added to the MM, post-MM autoclaving. The pH was adjusted to 7.0. One milliliter of sterile-filtered diesel fuel was added to the flask as the sole carbon and energy source. The culture was grown at 30 °C with shaking at 110 rpm (INFORS HT shaker, model CH-4103, Infors AG, Bottmingen, Switzerland) and subcultured after every five days. After three successive subculturing, the cells were plated on agar plates, after which diesel fuel was added to the plates using an airbrush. The plates were incubated at 30 °C. After 48 h, single colonies were transferred into separate flasks containing 100 mL liquid MM and 1 mL diesel fuel, and grown for five days. During the five-day period, bacterial growth in terms of optical density (OD_{600}) was monitored every 12 h using the UV/Visible spectrophotometer (Ultrospec 3000, Model 80-2106-20, Pharmacia Biotech, Cambridge England). Based on the OD_{600} values, three representative isolates were selected for whole genome studies.

DNA extraction. Microbial cells from approximately 30 mL of the cultures containing single isolates were harvested by centrifugation at 4000 × *g* for 10 min. DNA from the cell pellets were extracted using a MasterPure™ DNA Extraction kit (Epicentre®, Madison, USA) according to the manufacturer's protocol. The DNA was used for Illumina-based whole genome sequencing. In addition, microbial cells from 30 mL of the cultures were harvested for Nanopore-based whole genome sequencing. Similarly, at the end of the experimental period, DNA from 100 mg of the inoculated and uninoculated residual soil samples were extracted using the PowerSoil® DNA Extraction kit (Qiagen, Hilden, Germany).

Genome sequencing and assembly. Genomes were sequenced at the Göttingen Genomics Laboratory, Germany. Short-reads were generated using a MiSeq sequencer and v3 chemistry (Illumina, San Diego, CA, USA), while long-reads were sequenced using a MinIon (Oxford Nanopore Technologies; Oxford, England). Reads were quality filtered with fastp version 0.20.1[53]. The leading 15cp were truncated from forward and reverse reads. Read shorter than 30 bp were removed. Adapter sequences were trimmed. Nanopore data were processed with porechop version 0.2.4[54]. Default parameters were used for all software unless otherwise specified. The quality of the processing was confirmed using FastQC version 0.91. Reads were assembled using Unicycler version 0.4.8[55]. Contigs shorter than 500 bp were removed. Coverage information for each scaffold was determined using Bowtie 2 version 2.4.2[56] and SAMtools version 1.11[57]. Genomes were classified taxonomically using GTDB-Tk version 1.0.2 and the Genome Taxonomy Database (release 86)[58,59].

Sequencing of bacterial 16S rRNA genes from residual soils. Bacterial 16S rRNA genes were amplified using the forward primer S-D-Bact-0341-b-S-17 (5′-CCT ACG GGN GGC WGC AG-3′)[60] and the reverse primer S-D-Bact-0785-a-A-21 (5′-GAC TAC HVG GGT ATC TAA TCC-3′)[60] containing Illumina Nextera adapters for sequencing. The PCR reaction (25 µL) contained 5 µL of five-fold Phusion HF buffer, 200 µM of each of the four deoxynucleoside triphosphates, 4 µM of each primer, 1 U of Phusion high fidelity DNA polymerase (Thermo Scientific, Waltham, MA, USA), and approximately 50 ng of the extracted DNA as a template. The negative controls were performed by using the reaction mixture without a template. The following thermal cycling scheme was used: initial denaturation at 98 °C for 30 s, 30 cycles of denaturation at 98 °C for 15 s, annealing at 53 °C for 30 s, followed by extension at 72 °C for 30 s. The final extension was carried out at 72 °C for 2 min. Obtained PCR products per sample were controlled for appropriate size and purified using the MagSi-NGS Plus kit according to the manufacturer's protocol (Steinbrenner Laborsysteme GmbH, Germany). The quantification of the PCR products was performed using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer, as recommended by the manufacturer (Thermo Scientific). The DNA samples were barcoded using the Nextera XT-Index kit (Illumina, San Diego, USA) and the Kapa HIFI Hot Start polymerase (Kapa Biosystems, USA). Sequencing was performed at the Göttingen Genomics Laboratory on an Illumina MiSeq Sequencing platform (paired end 2 × 300 bp) using the MiSeq Reagent kit v3, as recommended by the manufacturer (Illumina). All bacterial samples were sequenced on the same MiSeq run.

Processing of the 16S rRNA gene data. Trimmomatic version 0.39[61] was initially used to truncate low-quality reads if quality dropped below 12 in a sliding window of 4 bp. Datasets were subsequently processed with Usearch version 11.0.667[62] as described in Wemheuer, Berkelmann[63]. In brief, paired end reads were merged and quality filtered. Filtering included the removal of low-quality reads (maximum number of expected errors >2 and more than 1 ambitious base, respectively) and those shorter than 200 bp. Processed sequences of all samples were joined, dereplicated and clustered in zero-radius operational taxonomic units (zOTUs) using the UNOISE algorithm implemented in Usearch. A de novo chimera removal was included in the clustering step. Afterward, zOTU sequences were taxonomically classified using the SINTAX algorithm against the SILVA database (SILVA SSURef 138 NR99). All non-bacterial zOTUs were removed based on their taxonomic classification. Subsequently, processed sequences were mapped on final zOTU sequences to calculate the distribution and abundance of each OTU in every sample. Richness and coverage based on the Chao1 richness estimator were estimated in R using the vegan package.

Identification of CDSs involved in plant growth-promoting and hydrocarbon-degrading processes. Coding DNA sequences of enzymes involved in both plant

growth-promoting activities and hydrocarbon degradation were identified in the bacterial genomes by means of annotations with prodigal version 2.6.3[64]. Functional annotation was performed with diamond version v0.9.29[65] and the KEGG database (October release 2018)[66]. The plant growth-promoting enzymes of interest include nif-specific regulatory protein, nitrogen fixation protein, electron transfer flavoprotein, acyl carrier protein, trehalose 6-phosphate phosphatase, phosphoglycolate phosphatase, phospholipase C, pyrroloquinoline-quinone biosynthesis proteins B, D and E, pyrroloquinoline-quinone synthase, enterobactin (siderophore) exporter, and indoleacetamide hydrolase. Additionally, the genes responsible for bacterial chemotaxis, motility, and root colonization were examined. These include the *che, wsp, flg, flh, fli*, and *tad* genes. Similarly, the hydrocarbon-degrading enzymes of interest include long-chain alkane monooxygenase, cyclopentanol dehydrogenase, cyclohexanone monooxygenase, benzoate/toluate 1,2-dioxygenase, benzaldehyde dehydrogenase, dihydroxycyclohexadiene carboxylate dehydrogenase, catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, muconate cycloisomerase, muconolactone D-isomerase, 3-oxoadipate enol-lactonase. On the basis of the following factors, *P. tropica* was selected for the greenhouse-based rhizoremediation study: (1) the differences in the plant growth-promoting potentials of the different isolates as revealed by functional genomics, and (2) the differences in the tolerance of isolates to and utilization by isolates of diesel fuel hydrocarbons, as shown by their growth rates (OD_{600}) in the diesel-containing liquid mineral medium (Supplementary Fig. 5).

Plant growth and bacterial inoculation. The soil used for this experiment was "Primaster turf", which is a mixture of screened sand, soil, and composted organics blended with an NPK fertilizer. The soil textural class was determined as sand (88.6% sand, 6.1% silt and 5.3% clay) with 12.5% organic matter content by loss on ignition, total nitrogen content of 0.15%, and a pH of 7.1. The soil was initially homogenized by sieving using a 2-mm mesh sieve to remove large particles. Diesel fuel-contaminated soil was prepared following the detailed procedure described in Eze, Thiel[67]. Preliminary gas chromatography–mass spectrometry (GC–MS) analysis of the diesel fuel revealed the presence of fatty acid methyl esters (FAMEs) evidently from biodiesel (Supplementary Fig. 7). Therefore, the soil was allowed to age for 7 days, so as to enable the removal of the FAMEs through natural attenuation by indigenous organisms. The resulting total petroleum hydrocarbons in the soil after ageing (designated as time T0) was determined using GC–MS.

The contaminated soil was treated with the following: (1) *M. sativa;* (2) *P. tropica*; (3) *M. sativa* + *P. tropica*. For the planted treatments, viable seeds of *M. sativa* were placed in pots (3 seeds per pot) containing 150 g of the aged diesel fuel-contaminated soils. Since the goal of the study was to assess the effectiveness of each treatment for hydrocarbon degradation, the soil used for the entire experiment was the diesel fuel-contaminated soil described above. Unplanted and uninoculated pots served as the control. The use of a single exposure diesel fuel concentration was necessary to enable manageability of the project, since various treatment conditions were examined. In addition, in a previous bioassay phytotoxicity study, the selected plant (*M. sativa*) demonstrated tolerance to a relatively wide range of diesel fuel concentrations[15]. The microbial consortium used was harvested from the culture by centrifugation at 4000 × *g* for 10 min, washed twice in mineral medium and concentrated to $OD_{600} = 1.650$. The same volume of cells was applied to both the "*P. tropica*" and the "*M. sativa* + *P. tropica*" treatments. For the *M. sativa* + *P. tropica* treatment, the cells (at $OD_{600} = 1.650$) were inoculated to the base of one-week-old *M. sativa* seedlings at the depth of 1.5 cm below-ground level. The whole experiment was performed in triplicate, and pots were watered with 90 mL sterile water every three days for the first two weeks. After that, the planted pots were watered with 90 mL sterile water every two days to compensate for the water needs of *M. sativa* plants. To assess the effect of bacterial inoculation on growth rate, shoot heights (in mean values of plants per pot) attained with time were taken every two weeks. Plant height was measured from the shoot tip to the base of stem[15,68]. After 60 days, each plant was harvested, washed under tap water, oven-dried at 70 °C until constant weights were achieved, and then their dry biomass weights were recorded.

Extraction of residual hydrocarbons. After 60 days, *M. sativa* plants were harvested from the pots. The entire residual soils from each pot were first manually homogenized. For hydrocarbon analyses, 1 g of the ground freeze-dried soils were further homogenized with a small amount of sodium sulfate (Na_2SO_4) and transferred into a Teflon microwave digestion vessel. The samples were solvent extracted twice with fresh 2.5 mL *n*-hexane in a microwave device (Mars Xpress, CEM; 1600W, 100 °C, 20 min). For reference, 2.5 µL diesel fuel (density = 0.82 g/mL) were dissolved in 5 mL *n*-hexane instead of 1 g soil sample. The extracts were combined into 7 mL vials and topped to 5 mL with *n*-hexane. A 1 mL aliquot (20%) of each extract was pipetted into a 2 mL autosampler vial, and 20 µL *n*-icosane D42 [200 mg/L] was added as an internal quantification standard.

Molecular analysis of biodegradation. GC-MS analyses of the samples were performed using a Thermo Scientific Trace 1300 Series GC coupled to a Thermo Scientific Quantum XLS Ultra MS. The GC capillary column was a Phenomenex Zebron ZB–5MS (30 m, 0.1 µm film thickness, inner diameter 0.25 mm). Compounds were transferred splitless to the GC column at an injector temperature of 300 °C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The GC temperature program was as follows: 80 °C (hold 1 min), 80 °C to 310 °C at 5 °C/min (hold 20 min). Electron ionization mass spectra were recorded at 70 eV electron energy in full scan mode (mass range *m/z* 50–600, scan time 0.42 s). Peak areas were integrated using Thermo Xcalibur software version 2.2 (Thermo Fisher Scientific Inc., Waltham, USA).

Biodegradation parameters. Biodegradation is the alteration of petroleum compounds by living organisms, the occurrence and extent of which can be monitored by specific biomarker and non-biomarker analyses[24]. To assess the nature and extent of biodegradation in the different treatments, the ratios of the more refractory isoprenoid hydrocarbons *nor*-pristane (2,6,10-trimethylpentadecane, *nor*-Pr), pristane (2,6,10,14-tetramethylpentadecane, Pr) and phytane (2,6,10,14-tetramethylhexadecane, Ph) versus *n*-hexadecane (nC_{16}), *n*-heptadecane (nC_{17}) and *n*-octadecane (nC_{18}) respectively were calculated. As an additional parameter, the relative abundance of unresolved complex mixture (UCM, often referred to as the "hump", a typical indicator of biodegradation[24]) versus total petroleum hydrocarbons (TPH) was determined.

Statistical analyses. All statistical analysis were performed using R[69]. One-way ANOVA was used to compare the mean dry biomass of *M. sativa* and *M. sativa* + *P. tropica* treatments. The normality and homogeneity of variances were tested by the Shapiro-Wilk's test[70] and the Levene's test[71] respectively. Relative growth rates of plants under different treatments were determined following the method of Eze, George[15]. This method involved the assessment of growth rate in terms of mean shoot height per pot attained with time. This approach eliminates the biases associated with destructive harvesting methods[72]. The logistic model was used for the statistical analysis of growth rates of the single bacteria isolates in diesel fuel-containing mineral medium[73,74] and those of plants in soils[75,76]. Bacteria growth (in terms of OD_{600}) was set at 0 at time t_0. Similarly, plant height at time t_0 was 0. Therefore, the lower asymptote c was fixed at 0 in both cases, resulting in 3-parameter logistic models[73,75,76].

Similarly, comparisons of the initial (T0) and residual (T60) soil hydrocarbon concentrations for all treatments were made using one-way ANOVA, followed by Tukey's all-pairwise comparisons. In all cases, the normality of variances was tested by the Shapiro-Wilk's method[70], and homogeneity of variances was tested using the Levene's test[71]. The significance level (nominally 0.05) was adjusted for multiple comparisons using the Holm method[77,78].

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers JAGIXD000000000, JAGIXE000000000, and JAGIXF000000000. The versions described in this paper are versions JAGIXD010000000, JAGIXE010000000, and JAGIXF010000000. In addition, GC–MS dataset of hydrocarbon biodegradation has been deposited to figshare (https://doi.org/10.6084/m9.figshare.20400984.v1)[79].

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References

1. Bragg, J. R., Prince, R. C., Harner, E. J. & Atlas, R. M. Effectiveness of bioremediation for the Exxon Valdez oil spill. *Nature* **368**, 413–418 (1994).
2. Duffy, J. J., Peake, E. & Mohtadi, M. F. Oil spills on land as potential sources of groundwater contamination. *Environ. Int.* **3**, 107–120 (1980).
3. Azubuike, C. C., Chikere, C. B. & Okpokwasili, G. C. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World J. Microbiol. Biotechnol.* **32**, 180 (2016).
4. USEPA. *Introduction to Phytoremediation.* EPA/600/R-99/107 (United States Environmental Protection Agency, 2000).
5. USEPA. *Brownfields Technology Primer: Selecting and Using Phytoremediation for Site Cleanup*. EPA 542-R-01-006 (United States Environmental Protection Agency, 2001).
6. Meagher, R. B. Phytoremediation of toxic elemental and organic pollutants. *Current Opin. Plant Biol.* **3**, 153–162 (2000).
7. Correa-García, S., Pande, P., Séguin, A., St-Arnaud, M. & Yergeau, E. Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. *Microb. Biotechnol.* **11**, 819–832 (2018).
8. Cheng, L., Zhou, Q. & Yu, B. Responses and roles of roots, microbes, and degrading genes in rhizosphere during phytoremediation of petroleum hydrocarbons contaminated soil. *Int. J. Phytoremediation* **21**, 1161–1169 (2019).

9. Saravanan, A. et al. Rhizoremediation – a promising tool for the removal of soil contaminants: a review. *J. Environ. Chem. Eng.* **8**, 103543 (2020).
10. Tyagi, M., da Fonseca, M. M. R. & de Carvalho, C. C. C. R. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation* **22**, 231–241 (2011).
11. Bevivino, A., Dalmastri, C., Tabacchioni, S. & Chiarini, L. Efficacy of *Burkholderia cepacia* MCI 7 in disease suppression and growth promotion of maize. *Biol. Fertil. Soils* **31**, 225–231 (2000).
12. Rohrbacher, F. & St-Arnaud, M. Root exudation: the ecological driver of hydrocarbon rhizoremediation. *Agronomy* **6**, 19 (2016).
13. Garrido-Sanz, D. et al. Metagenomic insights into the bacterial functions of a diesel-degrading consortium for the rhizoremediation of diesel-polluted soil. *Genes* **10**, 456 (2019).
14. Glick, B. R. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* **21**, 383–393 (2003).
15. Eze, M. O., George, S. C. & Hose, G. C. Dose-response analysis of diesel fuel phytotoxicity on selected plant species. *Chemosphere* **263**, 128382 (2021).
16. Eze, M. O., Hose, G. C. & George, S. C. Assessing the effect of diesel fuel on the seed viability and germination of *Medicago sativa* using the event-time model. *Plants* **9**, 1062 (2020).
17. Silva, P. R. A. D. et al. Draft genome sequence of *Paraburkholderia tropica* Ppe8 strain, a sugarcane endophytic diazotrophic bacterium. *Braz. J. Microbiol.* **49**, 210–211 (2018).
18. García, S. S. et al. *Paraburkholderia tropica* as a plant-growth–promoting bacterium in barley: characterization of tissues colonization by culture-dependent and -independent techniques for use as an agronomic bioinput. *Plant Soil* **451**, 89–106 (2020).
19. Rahman, M. et al. Plant probiotic bacteria *Bacillus* and *Paraburkholderia* improve growth, yield and content of antioxidants in strawberry fruit. *Sci. Rep.* **8**, 2504 (2018).
20. Goldstein, R. M., Mallory, L. M. & Alexander, M. Reasons for possible failure of inoculation to enhance biodegradation. *Appl. Environ. Microbiol.* **50**, 977–983 (1985).
21. van Veen, J. A., van Overbeek, L. S. & van Elsas, J. D. Fate and activity of microorganisms introduced into soil. *Microbiol. Mol. Biol. Rev.* **61**, 121–135 (1997).
22. Eze, M. O. & George, S. C. Ethanol-blended petroleum fuels: implications of co-solvency for phytotechnologies. *RSC Adv.* **10**, 6473–6481 (2020).
23. ATSDR. *Toxicological Profile for Fuel Oils* (U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, United States, 1995).
24. Peters, K. E., Walters, C. C. & Moldowan, J. M. *The Biomarker Guide: Volume 2: Biomarkers and Isotopes in Petroleum Systems and Earth History* (Cambridge University Press, 2004).
25. Bouhajja, E. et al. Identification of novel toluene monooxygenase genes in a hydrocarbon-polluted sediment using sequence- and function-based screening of metagenomic libraries. *Appl. Microbiol. Biotechnol.* **101**, 797–808 (2017).
26. Hamzah, R. Y. & Al-Baharna, B. S. Catechol ring-cleavage in *Pseudomonas cepacia*: the simultaneous induction of ortho and meta pathways. *Appl. Microbiol. Biotechnol.* **41**, 250–256 (1994).
27. Ishida, T., Kita, A., Miki, K., Nozaki, M. & Horiike, K. Structure and reaction mechanism of catechol 2,3-dioxygenase (metapyrocatechase). *Int. Cong. Ser.* **1233**, 213–220 (2002).
28. Atashgahi, S. et al. A benzene-degrading nitrate-reducing microbial consortium displays aerobic and anaerobic benzene degradation pathways. *Sci. Rep.* **8**, 4490 (2018).
29. Duff, S. M. G., Sarath, G. & Plaxton, W. C. The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant.* **90**, 791–800 (1994).
30. Flügel, F. et al. The photorespiratory metabolite 2-phosphoglycolate regulates photosynthesis and starch accumulation in *Arabidopsis*. *Plant Cell* **29**, 2537–2551 (2017).
31. Paul, M. J., Gonzalez-Uriarte, A., Griffiths, C. A. & Hassani-Pak, K. The role of trehalose 6-phosphate in crop yield and resilience. *Plant Physiol.* **177**, 12–23 (2018).
32. Cabot, C. et al. A role for zinc in plant defense against pathogens and herbivores. *Front. Plant Sci.* **10**, 1171 (2019).
33. Rodríguez-Vargas, S., Sánchez-García, A., Martínez-Rivas, J. M., Prieto, J. A. & Randez-Gil, F. Fluidization of membrane lipids enhances the tolerance of *Saccharomyces cerevisiae* to freezing and salt stress. *Appl. Environ. Microbiol.* **73**, 110–116 (2007).
34. Huang, J. et al. Genes of acyl carrier protein family show different expression profiles and overexpression of acyl carrier protein 5 modulates fatty acid composition and enhances salt stress tolerance in *Arabidopsis*. *Front. Plant Sci.* **8**, 987 (2017).
35. Esmaeel, Q. et al. *Paraburkholderia phytofirmans* PsJN-plants interaction: from perception to the induced mechanisms. *Front. Microbiol.* **9**, 2093 (2018).
36. Dias, G. M. et al. Comparative genomics of *Paraburkholderia kururiensis* and its potential in bioremediation, biofertilization, and biocontrol of plant pathogens. *MicrobiologyOpen* **8**, e00801 (2019).
37. Böhm, M., Hurek, T. & Reinhold-Hurek, B. Twitching motility is essential for endophytic rice colonization by the N_2-fixing endophyte *Azoarcus* sp. strain BH72. *Mol. Plant Microbe Interact.* **20**, 526–533 (2007).
38. Brencic, A. & Winans, S. C. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiol. Mol. Biol. Rev.* **69**, 155–194 (2005).
39. Chen, Y. et al. Comparative genomic analysis and phenazine production of *Pseudomonas chlororaphis*, a plant growth-promoting rhizobacterium. *Genom. Data* **4**, 33–42 (2015).
40. Haq, I. U., Graupner, K., Nazir, R. & van Elsas, J. D. The genome of the fungal-interactive soil bacterium *Burkholderia terrae* BS001—a plethora of outstanding interactive capabilities unveiled. *Genome Biol. Evol* **6**, 1652–1668 (2014).
41. Shen, X., Hu, H., Peng, H., Wang, W. & Zhang, X. Comparative genomic analysis of four representative plant growth-promoting rhizobacteria in *Pseudomonas*. *BMC Genom.* **14**, 271 (2013).
42. Lee, Y., Lee, Y. & Jeon, C. O. Biodegradation of naphthalene, BTEX, and aliphatic hydrocarbons by *Paraburkholderia aromaticivorans* BN5 isolated from petroleum-contaminated soil. *Sci. Rep.* **9**, 860 (2019).
43. Gemmell, R. T. & Knowles, C. J. Utilisation of aliphatic compounds by acidophilic heterotrophic bacteria. The potential for bioremediation of acidic wastewaters contaminated with toxic organic compounds and heavy metals. *FEMS Microbiol. Lett.* **192**, 185–190 (2000).
44. Röling, W. F. M., Ortega-Lucach, S., Larter, S. R. & Head, I. M. Acidophilic microbial communities associated with a natural, biodegraded hydrocarbon seepage. *J. Appl. Microbiol.* **101**, 290–299 (2006).
45. Giovanella, P. et al. Metal and organic pollutants bioremediation by extremophile microorganisms. *J. Hazard. Mater.* **382**, 121024 (2020).
46. Bojes, H. K. & Pope, P. G. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. *Regul. Toxicol. Pharmacol.* **47**, 288–295 (2007).
47. ECHA. *The Candidate List of Substances of Very High Concern (SVHCs).* ECHA/PR/19/01 (European Chemical Agency, 2019).
48. Loibner, A. P., Szolar, O. H. J., Braun, R. & Hirmann, D. Toxicity testing of 16 priority polycyclic aromatic hydrocarbons using Lumistox®. *Environ. Toxicol. Chem.* **23**, 557–564 (2004).
49. Bashan, Y., de-Bashan, L. E., Prabhu, S. R. & Hernandez, J.-P. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil* **378**, 1–33 (2014).
50. Craig, J., Gerali, F., MacAulay, F. & Sorkhabi, R. The history of the European oil and gas industry (1600s–2000s). *Geol. Soc. Spec. Publ.* **465**, 1 (2018).
51. Eze, M. O., Hose, G. C., George, S. C. & Daniel, R. Diversity and metagenome analysis of a hydrocarbon-degrading bacterial consortium from asphalt lakes located in Wietze, Germany. *AMB Express* **11**, 89 (2021).
52. Atlas, R. M. *Handbook of Microbiological Media*, 4th edn (CRC Press, Boca Raton, Florida, 2010).
53. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
54. Wick, R. Porechop. Github. https://github.com/rrwick/Porechop (2017).
55. Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput. Biol.* **13**, e1005595 (2017).
56. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).
57. Li, H. et al. The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
58. Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P. & Parks, D. H. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* **36**, 1925–1927 (2019).
59. Parks, D. H. et al Selection of representative genomes for 24,706 bacterial and archaeal species clusters provide a complete genome-based taxonomy. Preprint at *bioRxiv* https://doi.org/10.1101/771964 (2019).
60. Klindworth, A. et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1–e1 (2013).
61. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
62. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010).
63. Wemheuer, F. et al. Agroforestry management systems drive the composition, diversity, and function of fungal and bacterial endophyte communities in *Theobroma cacao* leaves. *Microorganisms* **8**, 405 (2020).
64. Hyatt, D. et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform.* **11**, 119–119 (2010).

65. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12**, 59–60 (2015).
66. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
67. Eze, M. O., Thiel, V., Hose, G. C., George, S. C. & Daniel, R. Enhancing rhizoremediation of petroleum hydrocarbons through bioaugmentation with a plant growth-promoting bacterial consortium. *Chemosphere* **289**, 133143 (2022).
68. Chen, S., Li, J., Fritz, E., Wang, S. & Hüttermann, A. Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. *For. Ecol. Manag.* **168**, 217–230 (2002).
69. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2018).
70. Ghasemi, A. & Zahediasl, S. Normality tests for statistical analysis: a guide for non-statisticians. *Int. J. Endocrinol. Metab.* **10**, 486–489 (2012).
71. Levene, H. Robust tests for equality of variances. In *Contributions to Probability and Statistics; Essays in Honor of Harold Hotelling* (eds. Ingram, O. & Harold, H.) (Stanford University Press, 1960).
72. Hoffmann, W. A. & Poorter, H. Avoiding bias in calculations of relative growth rate. *Ann. Bot.* **90**, 37–42 (2002).
73. Zwietering, M. H., Jongenburger, I., Rombouts, F. M. & van, tRietK. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **56**, 1875 (1990).
74. Wu, B. et al. Establishing and optimizing a bacterial consortia for effective biodegradation of petroleum contaminants: Advancing classical microbiology via experimental and mathematical approach. *Water* **13**, 3311 (2021).
75. Gregorczyk, A. A logistic function—its application to the description and prognosis of plant growth. *Acta Soc. Bot. Pol.* **60**, 67–76 (1991).
76. Szparaga, A. & Kocira, S. Generalized logistic functions in modelling emergence of *Brassica napus* L. *PLOS ONE* **13**, e0201980 (2018).
77. Lee, S. & Lee, D. K. What is the proper way to apply the multiple comparison test? *Korean J. Anesthesiol.* **71**, 353–360 (2018).
78. Holm, S. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65–70 (1979).
79. Eze, M. O., Thiel, V., Hose, G. C., George, S. C. & Daniel, R. GC-MS dataset of hydrocarbon biodegradation. figshare. Dataset. https://doi.org/10.6084/m9.figshare.20400984.v1.

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Competing interests

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