Salix purpurea and Eleocharis obtusa Rhizospheres Harbor a Diverse Rhizospheric Bacterial Community Characterized by Hydrocarbons Degradation Potentials and Plant Growth-Promoting Properties

Fahad Alotaibi 1,2, Soon-Jae Lee 1,†, Marc St-Arnaud 1 © and Mohamed Hijri 1,3,⁎

1 Institut de Recherche en Biologie Végétale, Université de Montréal and Jardin Botanique de Montréal, Montréal, QC H1X 2B2, Canada; fanalotaibi@ksu.edu.sa (F.A.); soon-jae.lee@unil.ch (S.-J.L.); marc.st-arnaud@umontreal.ca (M.S.-A.)
2 Department of Soil Science, King Saud University, Riyadh 11564, Saudi Arabia
3 African Genome Center, Mohammed VI Polytechnic University (UM6P), Ben Guerir 43150, Morocco
⁎ Correspondence: mohamed.hijri@umontreal.ca
† Present address: Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland.

Abstract: Phytoremediation, a method for phytomanagement using the plant holobiont to clean up polluted soils, is particularly effective for degrading organic pollutants. However, the respective contributions of host plants and their associated microbiota within the holobiont to the efficiency of phytoremediation is poorly understood. The identification of plant-associated bacteria capable of efficiently utilizing these compounds as a carbon source while stimulating plant-growth is a keystone for phytomanagement engineering. In this study, we sampled the rhizosphere and the surrounding bulk soil of Salix purpurea and Eleocharis obtusa from the site of a former petrochemical plant in Varennes, QC, Canada. Our objectives were to: (i) isolate and identify indigenous bacteria inhabiting these biotopes; (ii) assess the ability of isolated bacteria to utilize alkanes and polycyclic aromatic hydrocarbons (PAHS) as the sole carbon source, and (iii) determine the plant growth-promoting (PGP) potential of the isolates using five key traits. A total of 438 morphologically different bacterial isolates were obtained, purified, preserved and identified through PCR and 16S rRNA gene sequencing. Identified isolates represent 62 genera. Approximately, 32% of bacterial isolates were able to utilize all five different hydrocarbons compounds. Additionally, 5% of tested isolates belonging to genera Pseudomonas, Acinetobacter, Serratia, Klebsiella, Microbacterium, Bacillus and Stenotrophomonas possessed all five of the tested PGP functional traits. This culture collection of diverse, petroleum-hydrocarbon degrading bacteria, with multiple PGP traits, represents a valuable resource for future use in environmental bio- and phyto-technology applications.

Keywords: phytoremediation; petroleum hydrocarbon-degrading bacteria; Salix; plant growth promoting rhizobacteria; Eleocharis; alkanes; polycyclic aromatic hydrocarbons

1. Introduction

Industrial activities such as mining for minerals, oil and gas extraction, inorganic fertilizer-based agriculture, and industrial waste disposal, are all associated with environmental contamination risks which represent a global challenge [1]. Among pollutants, petroleum hydrocarbons (PHCs) are of great concern and can pose a high risk in oil spills, and environmental contamination of aquatic and terrestrial ecosystems. PHCs, like crude oil, are heterogeneous organic mixtures composed of carbon and hydrogen atoms and are broadly classified into two major fractions: (1) aliphatic hydrocarbons, like alkenes, alkynes, or alkanes, and (2) aromatic hydrocarbons, including mono-aromatic (i.e., benzene, toluene, phenol, etc.), and polycyclic aromatic hydrocarbons (PAHs) [2,3]. The main
sources of PHCs contamination in the environment are mostly anthropogenic, and include accidental release (i.e., diesel, solvent), and industrial activities (i.e., production of electricity, petrochemical activities) [4]. Environmental contamination with PHCs products has adversely affected various ecosystems, including soils, causing damage to natural habitats with serious economic consequences [5].

Concerns regarding soil pollution with PHCs have initiated the development of several remediation technologies, including biological, chemical and physical methods [2,6]. A promising biological technology for the removal of PHCs from soil is phytoremediation: an eco-friendly, green, solar-driven, and low carbon footprint approach that utilizes plants and their root-associated rhizospheric and endophytic microbiomes to clean-up PHC-contaminated soils [7,8]. Phytoremediation has proven its ability in remediating moderately polluted soils. However, phytoremediation has unreliable effectiveness at high levels of contaminants because of the reduced growth of introduced plants in these conditions. This reduction in plant growth may be partially due to variation in the association between plants and their resident microbiomes [9–13].

Over the last decade, most of the research efforts aimed at enhancing the efficiency of phytoremediation of PHCs focused on using plant species that can tolerate high levels of PHCs, such as *Salix* spp. [2,8,10,11,14]. *Salix* spp. (willows), which have been shown to be effective in decontaminating soils polluted with organic compounds, such as PHCs, and trace metals. Willows have several characteristics that may facilitate phytoremediation, including their ease of propagation, fast and perennial growth patterns, high-biomass production, high-contaminants tolerance, and massive deep-root systems [2,15–17]. Additionally, several recent studies have shown that *Salix* spp. can recruit certain microbial taxa that could help the plant to cope with PHCs contamination stress and accelerate the biodegradation process [10,18–20].

More recently, a promising strategy that includes the screening and identification of native plants growing spontaneously on PHCs-contaminated soils has been adopted [21–24]. This is the reason we chose *Eleocharis obtusa* (Willd), which dominated the vegetation at the site of study [21]. *Eleocharis* spp. are ubiquitous plants distributed across Canada and United States, where they grow in wetlands. These plants are not used in phytoremediation. Pérez-Jaramillo et al. [25] proposed a “back to the roots” frame that involves the survey of native plants, and their associated microbiomes, in their native habitats, with the goal of restoring plant-microbial associations that may have been diluted during plants domestication [25]. Native plants are more genetically diverse and more adapted to wide-ranging climatic conditions compared to other plant species currently chosen for the phytoremediation of PHCs [26]. Additionally, native plants have been shown to develop more close relationships with local rhizosphere microbiota than introduced plants [11,27], thus making native plants ideal models to study how microbiomes respond to environmental pollutions and explore their future use in the phytoremediation of PHCs.

The rhizomicrobiome, a subset of the plant holobiont, refers to the soil microbiomes associated with a plant’s roots. The rhizomicrobiome contributes to the functioning of plants including through the removal and degradation of PHCs compounds in contaminated soils [28,29]. Plants growth under stress such as PHCs contamination is expected to be lower than it would be under optimal conditions [30]. However, exploiting the potential of plant growth-promoting rhizobacteria (PGPR) in phytoremediation of PHC-contaminated soils holds great promise as it has recently been demonstrated [31–33]. PGPR are soil microbes within the rhizomicrobiome with phenotypes that benefit plant growth [28]. Therefore, plant growth may be positively stimulated by the presence of rhizobacteria with plant growth-promoting (PGP) traits, which alleviate stresses in plants via several mechanisms including: reducing soil nutrient deficiencies (fixing nitrogen, solubilizing phosphorus and enhancing iron uptake), synthesizing plant growth hormones, reduction in ethylene production via 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, as well as [30,34] degrading a broad range of PHCs compounds [2].
It is well documented that some rhizospheric bacteria have beneficial effects on their host in natural and anthropized terrestrial ecosystems. However, the role of rhizobacteria in association with plants that spontaneously grow in heavily PHC-polluted areas is not widely explored. However, some reports documented the influence of pollutants on microbial community structures [35,36]. The aim of this study was to isolate and characterize the PGPR and hydrocarbon-degraders associated with Salix purpurea and Eleocharis obtusa plants growing in a long-term petroleum hydrocarbon-polluted petrochemical site. We hypothesized that the rhizosphere of S. purpurea and E. obtusa plants growing in soils chronically contaminated with PHCs would harbor diverse bacterial communities with multiple key species having hydrocarbon degrading potential and PGP traits.

To address our hypothesis, a structurally and functionally diverse collection of PGPR and degradative bacteria were isolated from the rhizosphere of Salix and Eleocharis plants collected in the contaminated site. The cultured bacteria were all assessed for their abilities to grow in the presence of alkanes and polycyclic aromatic hydrocarbons as the sole carbon source, as well as for their PGP traits.

2. Results
2.1. Isolation and Characterization of Bacteria

Four hundred and thirty-eight morphologically distinct bacterial isolates were initially selected, purified and preserved in −80 °C. Bacterial isolates were identified based on the Sanger sequencing of their 16S rRNA gene. Among the 438 isolates identified, 146 bacterial isolates were recovered from S. purpurea rhizosphere, 146 isolates from E. obtusa rhizosphere and 146 isolates from bulk soil, as are shown in Supplementary Tables S1–S3, respectively.

Bacterial isolates were classified into 62 genera, belonging to the phyla Actinobacteria, Bacteroidetes, Firmicutes and the Alpha, Beta and Gamma-subgroups of Proteobacteria. Interestingly, approximately 5% of the total sequences were not assigned to any known bacteria (Table 1).

Bacteria from the subphylum Gammaproteobacteria dominated most of the isolates selected, which included 12 genera representing 37.5% of the total sequences. Within this subphylum, the most abundant genera were Pseudomonas (14.3%), Klebsiella (5%), Acinetobacter (4%), Pseudoxanthomonas (3.8%), Enterobacter (3%), Stenotrophomonas (2.7%), Rheinheimera (1.3%) and Serratia (1.1%) (Table 1).

The phylum Actinobacteria corresponded to 29% of the bacterial isolates, and was represented by 18 genera. The most abundant genus were Streptomyces (7.3%), Microbacterium (5.2%), Arthrobacter (4.5%), Rhodococcus (3%), Nocardoides (1.8%), Mycobacterium (1.3%) and Gordonia (1%) (Table 1). The third most predominant phylum was Firmicutes representing 11% of the total bacterial collection, with Bacillus (9%) and Exiguobacterium (1%) as the dominant genera (Table 1).

Bacteria from the subphylum Betaproteobacteria correspond to 5% of the total isolates, including seven genera. The two predominant genera were Variovorax (3%) and Massilia (1%) (Table 1). The sub phylum Alphaproteobacteria correspond to 5% of the total isolates, including 11 genera, with Rhizobium (1.1%) as the dominant genus (Table 1). The phylum Bacteroidetes represents 3.5% of the entire bacterial collection (Table 1).

The E. obtusa rhizosphere was dominated by Gammaproteobacteria, followed by Actinobacteria and Alpha- and Betaproteobacteria, Bacteroidetes, Firmicutes and unidentified bacteria (Figure 1). The S. purpurea rhizosphere was dominated by Actinobacteria, followed by Gammaproteobacteria, Firmicutes, Alpha- and Betaproteobacteria (Figure 1). In the polluted bulk soil, Gammaproteobacteria was the predominant phyla, followed by Actinobacteria, Alphaproteobacteria, Bacteroidetes, Firmicutes and unidentified bacteria (Figure 1).
Table 1. Number of isolates belonging to each bacterial genus associated with *S. purpurea* rhizosphere, *E. obtusa* rhizosphere and bulk soil samples.

| Serial # | Genus \(^1\) | *Eleocharis* Rhizosphere \(^2\) | Bulk Soil \(^2\) | *Salix* Rhizosphere \(^2\) |
|----------|---------------|-----------------------------|----------------|-----------------------------|
| 1        | Acidovorax    | 1                          | 0              | 0                           |
| 2        | Acinetobacter | 3                          | 15             | 0                           |
| 3        | Aeromonas     | 4                          | 0              | 0                           |
| 4        | Agrococcus    | 0                          | 1              | 0                           |
| 5        | Agromyces     | 2                          | 3              | 0                           |
| 6        | Amycolatepsis | 0                          | 0              | 2                           |
| 7        | Arcylobacter  | 1                          | 0              | 0                           |
| 8        | Arthrobacter  | 0                          | 0              | 20                          |
| 9        | Azorhizobium  | 2                          | 0              | 0                           |
| 10       | Bacillus      | 8                          | 16             | 17                          |
| 11       | Bosea         | 2                          | 0              | 0                           |
| 12       | Brevisacillus | 0                          | 1              | 0                           |
| 13       | Brevedimonas  | 0                          | 2              | 0                           |
| 14       | Caulobacter   | 0                          | 0              | 1                           |
| 15       | Chitinimonas  | 2                          | 0              | 9                           |
| 16       | Chryseobacterium | 2                  | 2              | 0                           |
| 17       | Citrobacter   | 0                          | 2              | 1                           |
| 18       | Comamonas     | 1                          | 0              | 0                           |
| 19       | Delfia        | 3                          | 0              | 0                           |
| 20       | Diella        | 1                          | 3              | 0                           |
| 21       | Empedobacter  | 1                          | 2              | 0                           |
| 22       | Enterobacter  | 1                          | 3              | 3                           |
| 23       | Exiguobacterium | 2                     | 2              | 0                           |
| 24       | Flavilimibacter| 0                          | 1              | 0                           |
| 25       | Flavobacteriaceae | 1                        | 0              | 0                           |
| 26       | Geogenia      | 0                          | 1              | 0                           |
| 27       | Gordonia      | 1                          | 1              | 3                           |
| 28       | Hydrogenophaga| 0                          | 1              | 0                           |
| 29       | Klebsiella    | 11                         | 7              | 3                           |
| 30       | Luteibacter   | 0                          | 1              | 0                           |
| 31       | Lysinibacillus| 0                          | 0              | 1                           |
| 32       | Lysinimonas   | 2                          | 0              | 0                           |
| 33       | Massilia      | 0                          | 4              | 1                           |
| 34       | Mesorhizobium | 0                          | 0              | 1                           |
| 35       | Microbacterium| 13                         | 9              | 1                           |
| 36       | Micromonospora| 0                          | 0              | 2                           |
| 37       | Mycobacterium | 1                          | 0              | 0                           |
| 38       | Mycolicibacter| 2                          | 3              | 2                           |
| 39       | Myroides      | 3                          | 2              | 0                           |
| 40       | Nocardia      | 0                          | 2              | 0                           |
| 41       | Nocardoides   | 0                          | 0              | 9                           |
| 42       | Paenarthrobacter| 0                   | 0              | 2                           |
| 43       | Paenibacillus | 0                          | 0              | 1                           |
| 44       | Pantoa        | 1                          | 1              | 1                           |
| 45       | Phyicoccus    | 0                          | 0              | 2                           |
| 46       | Pseudarthrobacter| 0                   | 0              | 2                           |
| 47       | Pseudomonas   | 36                         | 11             | 15                          |
| 48       | Pseudoxanthomonas | 5                     | 12             | 0                           |
| 49       | Raoultella    | 0                          | 0              | 6                           |
| 50       | Rheinheimera  | 1                          | 2              | 0                           |
| 51       | Rhizobium     | 4                          | 1              | 0                           |
| 52       | Rhodococcus   | 2                          | 6              | 2                           |
Table 1. Cont.

| Serial # | Genus ¹ | "Eleocharis Rhizosphere" ² | Bulk Soil ² | "Salix Rhizosphere" ² |
|----------|---------|---------------------------|-------------|----------------------|
| 53       | *Rhodospirillum* | 1                         | 0           | 0                     |
| 54       | *Serratia* | 5                         | 0           | 0                     |
| 55       | *Sphingobacterium* | 0                         | 2           | 0                     |
| 56       | *Sphingobium* | 0                         | 1           | 0                     |
| 57       | *Sphingomonas* | 1                         | 1           | 1                     |
| 58       | *Sphingopyxis* | 2                         | 1           | 0                     |
| 59       | *Staphylococcus* | 0                         | 1           | 1                     |
| 60       | *Stenotrophomonas* | 3                         | 8           | 1                     |
| 61       | *Streptomyces* | 1                         | 0           | 32                    |
| 62       | *Unidentified bacteria* | 7                         | 14          | 0                     |
| 63       | *Variovorax* | 1                         | 0           | 12                    |

¹ Closest identity at the genus level of our sequences using BLAST in the Genbank database. ² Number of bacterial isolates obtained per genus.

Figure 1. Taxonomic breakdown and relative abundance of bacterial isolates at the phylum level.

Dominant families within the *E. obtusa* rhizosphere were Xanthobacteraceae, Comamonadaceae, Microbacteriaceae, Flavobacteriaceae, Bacillaceae, Xanthomonadaceae, and Enterobacteriaceae (Figure 2). *S. purpurea* rhizosphere was dominated by Enterobacteriaceae, Micrococccaceae, Nocardioidaceae, Nocardiaceae, and Bacillaceae (Figure 2). Several families were predominant in the bulk soil including Sphingomonadaceae, Flavobacteriaceae, Enterobacteriaceae, Microbacteriaceae, Bacillaceae, and Xanthomonadaceae (Figure 2). Notably, several families with known petroleum hydrocarbons degradation capabilities were present in all three environmental habitats, including Comamonadaceae, Enterobacteriaceae, Microbacteriaceae, Sphingomonadaceae, Bacillaceae, Xanthomonadaceae, and Nocardiaceae (Figure 2). Notably, 11 bacterial genera were shared between the *S. purpurea* rhizosphere, *E. obtusa* rhizosphere and the bulk soil (Figure 3).
Figure 2. Taxonomic breakdown and relative abundance of bacterial isolates at the family level.

Figure 3. Identity of bacterial isolates at the genus level shared among the three environmental niches.
2.2. Petroleum-Hydrocarbon Degradation Potential

All bacterial isolates were assessed for their ability to degrade various alkanes (n-hexadecane and dodecane) and polycyclic aromatic hydrocarbons (PAHs) compounds (naphthalene, phenanthrene and pyrene). Our results indicate that 144 bacterial strains out of the total 438 isolates were able to utilize all 5-hydrocarbon compounds under investigation. Focusing on bacterial isolates able to degrade PAHs, 283 bacterial strains (64%) were able to utilize naphthalene (2-rings PAH-compound) and 275 bacterial strains (62%) were able to utilize phenanthrene (3-rings PAH-compound) (Figure 4). Additionally, 229 bacterial strains (52%) were able to utilize pyrene (4-rings PAH-compound) as a sole carbon source (Figure 4).

Regarding n-alkanes degrading bacteria, our results show that 254 bacterial strains (57%) were able to utilize dodecane (12-carbon compound) and 263 bacterial strains (60%) were able to utilize n-hexadecane (16-carbon compound) as sole carbon source (Figure 4).

About 32% of isolates were able to utilize all 5-hydrocarbon compounds tested in this study (Figure 5). Of those, 16% belonged to the family Micrococcaceae, 15% to Pseudomonadaceae, 13% to Actinomycetaceae, 10% to Enterobacteriaceae, 7% to Xanthomonadaceae, 5% to Moraxellaceae, and 4% to Microbacteriaceae (Figure 5).
Figure 5. Bars indicate the relative abundance of genera among isolates, which are able to degrade five and four different petroleum hydrocarbons compounds (PHC) in vitro.

Isolates that were able to utilize four hydrocarbon compounds represented 41% of total bacterial collection, and 26% of them belonged to the family Pseudomonadaceae followed by Comamonadaceae (10%), Bacillaceae (8%), Actinomycetaceae (7%), Yersiniaceae (5%), Microbacteriaceae (4%), Micrococccaceae (4%), Moraxellaceae (4%) and Sphingomonadaceae (4%) (Figure 5).

2.3. Plant Growth-Promoting Traits

Our 438 bacterial isolates were screened for traits that are commonly associated with plant growth-promoting (PGP) abilities. Our results show that only 22 (5%) bacterial isolates were positive for all five PGP traits (Table 2 and Figure 6). Results also show that 267 isolates (60%) were able to grow on a DF-minimal salt medium with ACC as the sole nitrogen source, indicating the presence of ACC deaminase (Figure 6), 249 bacterial isolates (56%) were able to fix nitrogen, 216 bacterial isolates (49%) were able to synthesize siderophores, 191 bacterial isolates (43%) were able to produce IAA and 59 bacterial isolates (13%) could solubilize inorganic phosphorus (Figure 6).
Table 2. List of bacterial isolates that possessed all five-plant growth-promoting traits that were tested in this study.

| Serial # | Isolate | Closest NCBI Relative | Environmental Niche | Isolation Medium |
|----------|---------|-----------------------|---------------------|------------------|
| 1        | SB41    | Acinetobacter calcoaceticus | Bulk soil          | B-H_amended diesel |
| 2        | SB55    | Acinetobacter calcoaceticus | Bulk soil          | B-H_amended diesel |
| 3        | SB60    | Acinetobacter sp.        | Bulk soil          | B-H_amended diesel |
| 4        | ET27    | Pseudomonas plecoglossicida | Eleocharis rhizosphere | TSA             |
| 5        | ET43    | Serratia sp.            | Eleocharis rhizosphere | TSA             |
| 6        | ET45    | Pseudomonas fluorescens | Eleocharis rhizosphere | TSA             |
| 7        | ET46    | Serratia sp.            | Eleocharis rhizosphere | TSA             |
| 8        | ET50    | Pseudomonas putida      | Eleocharis rhizosphere | TSA             |
| 9        | ET52    | Serratia sp.            | Eleocharis rhizosphere | TSA             |
| 10       | ET57    | Pseudomonas monteilii   | Eleocharis rhizosphere | TSA             |
| 11       | ET60    | Azomonas macrocytogenes | Eleocharis rhizosphere | TSA             |
| 12       | EB3     | Pseudomonas songnenensis | Eleocharis rhizosphere | B-H_amended diesel |
| 13       | EB31    | Stenotrophomonas pavani | Eleocharis rhizosphere | B-H_amended diesel |
| 14       | WT4     | Pseudomonas mandelii    | Salix rhizosphere  | 1/10TSA          |
| 15       | WT8     | Streptomyces atriruber  | Salix rhizosphere  | 1/10TSA          |
| 16       | WT17    | Pseudomonas kilomensis  | Salix rhizosphere  | 1/10TSA          |
| 17       | WT22    | Pseudomonas frederiksbergensis | Salix rhizosphere | 1/10TSA          |
| 18       | WT32    | Bacillus megaterium     | Salix rhizosphere  | 1/10TSA          |
| 19       | WT50    | Pseudomonas frederiksbergensis | Salix rhizosphere | 1/10TSA          |
| 20       | WT56    | Pseudomonas frederiksbergensis | Salix rhizosphere | 1/10TSA          |
| 21       | WB31    | Pseudomonas putida      | Salix rhizosphere  | B-H_amended diesel |
| 22       | EA21    | Pantoea agglomerans     | Eleocharis rhizosphere | ACCD            |

Figure 6. Bacterial isolates with plant growth-promoting (PGP) properties. (Above) Venn diagram showing the number of isolates that possess one or many of the five PGP traits. (Below) Bar graph indicating the absolute numbers of bacterial isolates having each of the PGP traits under investigation (over 438 isolates tested).
From the 22 isolates (or 5% of all isolates) that were positive for all PGP traits, 45% belonged to the family *Pseudomonadaceae*, 15% to *Moraxellaceae*, 14% to *Yersiniaceae*, 5% to *Microbacteriaceae*, 4% to *Bacillaceae*, 3% to *Actinomycetaceae* and 3% to *Xanthomonadaceae* (Figure 7).

Isolates that were positive for at least four PGP traits represented (12%) of total bacterial collection, with *Pseudomonadaceae* being the predominant family being (33%) followed by *Enterobacteriaceae* (32%), *Moraxellaceae* (12%), *Comamonadaceae* (5%) and *Microbacteriaceae* (4%) (Figure 7).

### 3. Discussion

The study of rhizosphere microbial communities associated with plants growing in long-term PHC-contaminated soil represents an opportunity for phytoremediation research. Several reports described the microbial community structures, diversities and functions in the rhizosphere of planted *Salix* trees as well as in ruderal plants growing spontaneously in soils highly contaminated with PHCs, using different sequencing techniques including cloning [37,38], next generation-targeted amplicon sequencing [10,11,20] and metatranscriptomics [14,39]. This study used a conventional microbiological approach to isolate, identify and characterize bacteria with multiple petroleum hydrocarbon-degrading capacities and plant growth-promoting capabilities to generate a bacterial culture collection for future use as a source of bacterial inoculants to enhance phytoremediation of PHCs-contaminated soils.

High concentrations of PHCs cause phytotoxic effects on plants growing in contaminated soils [23,40]. For example, the growth rates of corn and red bean plants were reduced at 10000 mg/kg of crude oil [40]. Similarly, Chaîneau et al. [41] reported a stunted plant...
growth and inhibitory effects on the seed germination of several plants such as *Helianthus annuus*, *Zea mays*, *Lactuca sativa*, *Phaseolus vulgaris*, *Triticum* sp. and *Trifolium* sp. when exposed to high concentrations of fuel oil ranging from 3000 to 12,000 mg/kg. However, despite the devastating effects of PHCs on plant growth, recent studies have reported that several spontaneously growing herbaceous plants were found to flourish in highly contaminated soils near abandoned oil wells where the concentrations of PHCs could reach up to 45,000 mg/kg [22,42]. Similarly, Desjardins et al. [21] reported three indigenous plant species (*Alisma triviale*, *Eleocharis obtuse* and *Panicum capillare*) that grow spontaneously in highly petroleum-contaminated decantation basins of a former petrochemical plant in Varennes (southern Québec, Canada). These plants were tolerant of high levels of PHCs where the concentrations could reach up to 26300 mg/kg [21]. Moreover, not only spontaneously growing indigenous plants could tolerate high concentrations of PHCs, but also introduced pioneering phytoremediator plants such as *Salix* sp. were able to tolerate such a high level of PHCs [10,14,20].

The results of this study support our hypothesis that plants growing in soil chronically contaminated with PHCs would select for rhizospheric bacteria with multiple petroleum hydrocarbon-degrading potential and plant growth-promoting capabilities. In this study, 438 bacterial strains were isolated from bulk soil, and the rhizosphere soil of *S. purpurea* and *E. obtuse* rhizosphere soil using three different isolation strategies to enhance the diversity of bacterial isolates with multiple petroleum-hydrocarbon degradation potentials and plant growth-promoting traits. Our isolation strategies resulted in a culture collection of bacterial strains belonging to Actinobacteria, Alpha- Beta- and Gammaproteobacteria, Bacteroidetes and Firmicutes (Figure 1), encompassing a fairly diverse collection of bacterial genera (62 genera) (Table 1), including *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Chitinimonas*, *Enterobacter*, *Gordonia*, *Klebsiella*, *Microbacterium*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcus*, *Serratia*, *Sphingomonas*, *Stenotrophomonas*, *Streptomyces* and *Variovorax* (Table 1). Several of these genera have previously been shown to hold promising petroleum-hydrocarbons degradation potential and plant growth-promoting activities [30,43–46]. The selected media used in our study did not result in the cultivation of new phyla; however, expandable bacterial culture collections could be established using additional novel cultivation strategies, as previously demonstrated for *Arabidopsis thaliana* At-SPHERE culture collection [47].

Our study revealed that culturable rhizospheric bacteria associated with *S. purpurea* rhizosphere mainly belonged to Actinobacteria and Gammaproteobacteria (Figure 1). In contrast to our results, Bell et al. [10] studied the bacterial community structure and composition in the rhizosphere of several willows cultivar growing in PHCs-contaminated soils using 454-pyrosequencing and found that Betaproteobacteria was the predominant phyla. One possible explanation for this result is that a selective medium was used in this study (Bushnell-Haas medium amended with 1% diesel) to isolate PHC-degrading bacteria, while in Bell et al. [10], all bacteria were potentially amplified and sequenced. In agreement with our explanation, Ferrera-Rodriguez et al. [48] reported that culturable rhizospheric bacteria from five Arctic native plant species growing in PHC-contaminated soils were similarly dominated by Actinobacteria and Gammaproteobacteria when a selective medium was used to isolate PHC-degrading bacteria. The predominant family within the willow rhizosphere was *Enterobacteriaceae* (Figure 2). Recent studies have reported that genera belonging to the family *Enterobacteriaceae* were predominant in the root endosphere of plants growing in Athabasca oil sands reclamation sites [49] and herbaceous plants growing near natural oil seep fields [50]. Endophytic bacteria are thought to be a subset of the larger rhizosphere microbiota [34] and further studies looking at the composition of culturable endophytic bacteria of *Salix* plants growing in PHC-contaminated soils will be required in order to elucidate the role of bacterial endophytes to improve PHC-phytoremediation. Other predominant families included *Micrococaceae*, *Nocardioidae* and *Nocardiaceae* (Figure 2), which have been shown to possess strong petroleum hydrocarbon degradation capabilities [45,46].
To our knowledge, there are no other reports concerning the isolation and identification of rhizospheric bacteria from *E. obusta*. Our study revealed that culturable rhizospheric bacteria associated with the *E. obusta* rhizosphere were mainly affiliated to Gammaproteobacteria, Actinobacteria and Betaproteobacteria phyla (Figure 1). The Dominant families were *Comamonadaceae*, *Xanthomonadaceae* and *Microbacteriaceae* (Figure 2). Comamonad bacteria (phylum Betaproteobacteria), for instance, are known to contain genera such as *Comamonas*, *Delftia* and *Variovorax*, which exhibit an extraordinary capability of degrading wide spectra of PHCs [45,46]. Genera belonging to the phyla Gammaproteobacteria, Actinobacteria are also known to contain bacterial species with efficient petroleum hydrocarbon degradation potentials [45,46] such as *Pseudomonas*, *Streptomyces* and *Rhodococcus* [51,52].

Soil and rhizospheric bacteria can increase the phytoremediation of PHCs by decreasing the level of PHCs in the contaminated soils via their enzymatic machinery mostly under aerobic conditions [53]. The results obtained in our study indicate that many bacterial isolates originating from the contaminated soil and rhizosphere samples have the potential to degrade a wide range of PHC compounds. More than 32% of our bacterial isolates were able to degrade all PHC being tested (Figure 4). Petroleum hydrocarbon-degrading bacteria isolated in this study belonged mainly to Actinobacteria (mostly *Streptomyces*, *Arthrobacter*, *Rhodococcus* and *Nocardia*), Proteobacteria (mostly *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, *Acinetobacter* and *Variovorax*) and Firmicutes (mostly *Bacillus*). Previous reports have shown that many bacterial genera belonging to these phyla were able to degrade a wide range of PHC compounds [36,46,54]. For example, the genus *Rhodococcus* has demonstrated high efficiency in degrading and transforming a wide range of organic substances, including aliphatic and aromatic hydrocarbons, pesticides and petroleum [55,56]. Therefore, there are immense interests in utilizing *Rhodococcus* in bioremediation of polluted soils due to their safe and ease of culturing and maintenance, and high catabolic versatility [52,55,56].

Bacterial isolates with PGP traits provide critical functions for their host plants growing in stressful environments, such as soil contaminated with PHCs. Isolating bacteria from PHC-contaminated environments that have both PGP traits and PHC-degrading activities has been of great interest in a new paradigm of environmental cleanup biotechnology which exploits PGPR. Selecting plants suitable for phytoremediation depends on many criteria, the most important of which is root morphology [15]. PGPR with the capacity to produce the phytohormones IAA, which plays a role in inducing the formation of lateral roots [43], would further stimulate plant growth in PHC-contaminated soils. In this study, 43% of bacterial isolates synthesized IAA (Figure S1), which were mostly affiliated to the genera *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Arthrobacter* and *Microbacterium* (Figure S2). Previous studies confirmed that IAA-producing genera reported in this work were also found to produce IAA by endophytic and rhizospheric bacteria isolated from various plants [24,57,58].

Another mechanism by which PGPR have the potential to improve plant growth under adverse environmental conditions, including PHC contamination, is by producing the enzyme ACC deaminase [30,43]. Stressed plants induce the production of the phytohormone ethylene to bolster their defense. However, ethylene also inhibits plant growth [59]. Certain PGPR can inhibit ethylene biosynthesis via the production of ACC deaminase which cleaves the ethylene precursor ACC into alpha-ketobutyrate and ammonia [30,59]. In this study, 60% of bacterial isolates were found to produce ACC deaminase (Figure S1). Most isolates that could catabolize ACC reported in this work belonged to genera such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Stenotrophomonas* and *Microbacterium* (Figure S2). The high percentage of ACC deaminase-producing bacteria among our isolates corroborate previous studies reporting the widespread nature of this trait in various soil bacteria [60–62].

N fixation, phosphate solubilization, and siderophore production are some of the direct PGP mechanisms making nutrients available to plants. These traits were found among the bacteria isolated of this study (Figure S1). Nitrogen fixation by diazotrophic...
bacteria is an important trait of PGPR that benefits the plant, especially when growing in nutrients-deficient soils [63]. Diazotrophic bacteria isolated in this study belonged mainly to genera such as Pseudomonas, Klebsiella, Bacillus, Enterobacter, Acinetobacter and Variovorax (Figure S2). Low levels of soluble P in soils can restrict the growth and development of plants [43]. Some PGPR solubilize inorganic forms of P and convert it to plant-available forms, thereby facilitating plant growth [64,65]. Our study found that the majority of isolates are able to solubilize inorganic P belonged to the genera Pseudomonas, Acinetobacter, Bacillus and Serratia (Figure S2). Another essential nutrient for plant growth is iron, even if it is present in soils in the highly insoluble form Fe$^{3+}$ [66]. Some PGPR produce low molecular-weight organic compounds, siderophores, that chelate Fe$^{3+}$ ions and render them available for reduction to the soluble Fe$^{2+}$ form preferred by plants [66]. The majority of isolates reported in this study that were able to produce siderophores belonged to the genera Pseudomonas, Acinetobacter, Microbacterium, Rhodococcus and Stenotrophomonas (Figure S2). The widespread ability of our isolates to hold PGP traits related to increasing the concentration and availability of nutrients to plants is of great importance to the plant nutrition balance.

This study highlights the functional potential of this culture collection in which many bacterial isolates, from the genera Acinetobacter, Arthrobacter, Nocardia, Rhodococcus, Streptomyces and Variovorax, possessed petroleum hydrocarbon degradation capabilities. However, only a small proportion of bacterial isolates (5%) had multiple PGP traits. These strains were isolated from the genera Acinetobacter, Enterobacter, Klebsiella, Pseudomonas and Serratia. Interestingly, in our study, only three bacterial isolates were capable of degrading all five PHCs, and had all five PGP traits (Figures S3–S5): Pseudomonas putida strain EB3, Streptomyces sp. strain WT8 and Bacillus sp. strain WT32. These findings corroborate earlier studies which reported that many isolates from these genera can degrade PHCs and promote plant growth [51,67,68]. These bacterial taxa are candidates to look for in follow-up experiments.

4. Materials and Methods

4.1. Site Description, Experimental Design and Sample Collection

Soil samples were collected from Salix purpurea L. cv “Fish Creek” and Eleocharis obtusa (Willd.) Schult. plants growing on a former petrochemical plant located on the south shore of the St-Lawrence River in Varennes, Québec, Canada (45°43′ N, 73°22′ W) (for details on the site, see Bell et al. [10] and Desjardins et al. [21]). The petrochemical plant was fully operated from 1953 until it was closed in 2008 [69]. The soil was contaminated with a mixture of alkanes and PAHs. Previous studies have analyzed contaminated soil samples from the site for F1-F4 hydrocarbons fractions (the sum of aliphatic and aromatic compounds with chain lengths of C6–C50). Analysis showed that the soil contamination was variable but reached concentrations averaging 3590 mg kg$^{-1}$ [10], which exceeds by far the limit for land reuse defined by the government of Québec for industrial areas.

About 10,000 trees of eleven different Salix cultivars were planted in the contaminated soil in a split-plot design in this site in 2011, as part of a large phytoremediation pilot project (see Bell et al. [10] for details), while E. obtusa plants began spontaneously growing in the polluted soil across the site. We took advantage of this larger design to sample five four-year-old S. purpurea trees and five fully-grown E. obtusa plants, which were randomly selected from the site on 13 August 2015. S. purpurea and E. obtusa plants were dug out and shaken vigorously to dislodge the bulk soil attached to the roots; only the soil that remained strongly adhered to the roots (i.e., rhizosphere soil) was collected, from a zone of the root system growing approximately 0 to 15 cm in depth from the surface. Approximately 50 g of rhizospheric soil was collected from each plant, while five bulk soil samples free of any plant materials were randomly collected from the site as bulk samples. Bulk soil samples were taken from the top 15 cm of soil at least 50 cm from the nearest plants. Rhizosphere and bulk soil samples were placed in sterile Whirl-Pack® bags (Sigma-Aldrich, Oakville, ON, Canada) and put on ice until transportation to the laboratory.
A composite sample for chemical analysis was formed from each of the five \( S. \text{ purpurea} \) rhizosphere soil samples, \( E. \text{ obtusa} \) rhizosphere soil samples, and bulk soil samples. Chemical analysis showed that the soil samples had high Total Petroleum Hydrocarbons (TPH) concentrations. The mean TPH concentrations were 10000 mg/kg for the bulk soil samples, 4800 mg/kg for the \( E. \text{ obtusa} \) rhizosphere samples and 1400 mg/kg for the \( S. \text{ purpurea} \) rhizosphere samples. Other soil chemical and physical parameters are listed in Table 3.

Table 3. Chemical and physical characteristics of soils used in this study.

| Soil Type          | pH (1:1) | CEC (meq/100 g) | N (g/Kg) | P (Kg/ha) | K (Kg/ha) | Ca (Kg/ha) | Mg (kg/ha) | Mn (PPM) | O.M (%) | Fe (PPM) |
|--------------------|----------|-----------------|----------|-----------|-----------|------------|------------|----------|---------|----------|
| Salix rhizosphere  | 7.4      | 24.8            | 0.9      | -<10      | 453       | 7323       | 2127       | 34.5     | 3.5     | 291.79   |
| Eleocharis         | 7.3      | 38.7            | 2.2      | -<10      | 566       | 13961      | 1851       | 61.5     | 6.2     | 582.56   |
| Bulk soil          | 7.4      | 37.2            | 2.1      | 13        | 565       | 13121      | 1952       | 99.7     | 7.5     | 580.77   |

4.2. Bacterial Isolation

Three different growing media were used to isolate the bacteria from the soil samples: (1) Bushnell-Haas medium amended with 1% diesel, as the sole carbon source; (2) one-tenth-strength Trypticase Soy Agar (TSA) medium; and (3) Dworkin & Foster (DF) minimal salts medium containing ACC. These media were used for the isolation and cultivation of petroleum hydrocarbon-degrading bacteria, total heterotrophic bacteria and ACC deaminase-producing PGPR, respectively.

Bushnell-Haas agarose plates amended with 1% diesel were prepared as follows (per liter): 0.2 g MgSO\(_4\), 0.020 g CaCl\(_2\), 1 g KH\(_2\)PO\(_4\), 1 g K\(_2\)HPO\(_4\), 1 g NH\(_4\)NO\(_3\), 0.050 g FeCl\(_3\), 17 g agarose; the final pH was adjusted to 7 and the medium was sterilized by autoclaving at 121 °C for 25 min [70]. One percent filter-sterilized (0.2 µm pore size membrane) diesel was added to the Bushnell-Haas medium before pouring the plates. One-tenth-strength TSA plates were prepared by suspending 3 g trypticase soy broth (Difco Laboratories, Detroit, MI, USA) and 15 agar (Difco Laboratories, Detroit, MI, USA) per L of distilled water before sterilizing the medium by autoclaving it at 121 °C for 25 min. The DF minimal salts agar plates were prepared as follows (Dworkin and Foster 1958) (per liter): 4 g KH\(_2\)PO\(_4\), 6 g Na\(_2\)HPO\(_4\), 0.2 g MgSO\(_4\)-7H\(_2\)O, 2.0 g glucose, 2 g gluconic acid, 2 g citric acid, 0.1 mL of trace elements solution (10 mg H\(_3\)BO\(_3\), 11.19 mg MnSO\(_4\)-H\(_2\)O, 124.6 mg ZnSO\(_4\)-7H\(_2\)O, 78.22 mg CuSO\(_4\)-5H\(_2\)O, and 10 mg MoO\(_3\)), 0.1 mL of FeSO\(_4\)-7H\(_2\)O solution and 1.8% Bacto-Agar (Difco Laboratories, Detroit, MI, USA). The pH was adjusted to 7.2 and the medium was sterilized by autoclaving at 121 °C for 25 min. To suppress fungal growth, filter-sterilized cycloheximide (100 mg L\(^{-1}\)) was added to all three media after autoclaving and just before pouring plates.

For the isolation of petroleum hydrocarbon-degrading bacteria and total heterotrophic bacteria, 5 g of rhizosphere or bulk soil samples were suspended in 95 mL of sterile phosphate buffered saline (PBS; Difco Laboratories, Detroit, MI, USA) and shaken on a rotary shaker (150 rpm) for 30 min. Suspensions were serially diluted in 10-fold series in PBS and 100 µL of the appropriate dilutions (10\(^{-4}\) for B-H plates and 10\(^{-5}\) for 1/10 TSA plates) were spread in triplicate onto both the Bushnell-Haas agarose plates amended with 1% diesel and on the 1/10 strength TSA plates. The Bushnell-Haas plates and 1/10 TSA plates were incubated at 28 °C for 14 days or 3 days, respectively [71,72].

For the isolation of ACC deaminase-producing PGPR, an ACC deaminase enrichment culture method was used as described by Penrose and Glick [73]. Briefly, 1 g of rhizosphere or bulk soil samples were added to 50 mL of sterile Pseudomonas Agar F (PAF) medium containing the following (per liter): 10 g proteose peptone, 10 g casein hydrolysate, 1.5 g anhydrous MgSO\(_4\), 1.5 g K\(_2\)HPO\(_4\) and 10 mL glycerol. The culture was incubated in a rotary shaker (200 rpm) at 28 °C for 24 h and a 1-mL aliquot was transferred into a fresh 50-mL sterile PAF medium and incubated under the same environmental conditions. After 24 h, a 1-mL aliquot was transferred into 50-mL sterile DF salts minimal broth medium as described above, except that agar was omitted and 2 g of (NH\(_4\))\(_2\)SO\(_4\) was added as
Plants 2021, 10, 1987

a nitrogen source. The culture was incubated in a rotary shaker (200 rpm) at 28 °C for 24 h and a 1-mL aliquot was transferred into a fresh 50-mL sterile DF salts minimal broth medium containing 3 mM filter-sterilized ACC (instead of (NH_4)_2SO_4) as a nitrogen source, and the culture was incubated under the same environmental conditions. After 24 h, 10-fold serial dilutions in PBS were made and 100 µL of the 10^{-4} dilution spread in triplicate onto solid DF salts minimal agar plates amended with ACC (30 µmol plate^{-1}). The solid DF salts minimal agar plates were incubated for 72 h at 28 °C. Colonies showing growth on the plates indicate ACC deaminase production.

Discrete colonies with a distinctive morphology (shapes, size, colors, etc.) were further sub-cultured in order to obtain pure cultures. Isolates were streaked twice on the original medium and checked for purity. Purified isolates were stored in a 1:1 mixture of half-strength Trypticase Soy Broth (TSB) (Difco Laboratories, Detroit, MI, USA) and 20% glycerol (v/v) and frozen at −80 °C. A total of 438 isolates were collected and further characterized in this study.

Isolated bacterial strains were named based on the medium used for isolation and the rhizosphere zone of origin, and the arbitrary serial number of the strain (i.e., WT15 for the 15th isolate from willow rhizosphere using TSA plates.

4.3. DNA Extraction, PCR Amplification, and Sequencing of Bacterial Isolates

A single colony from each purified isolate was inoculated into 5 mL of 1/10 TSB culture media and grown at 28 °C on a gyratory shaker (150 rpm) for 1–3 days until became turbid. Once the liquid culture was ready, an aliquot of 1.8 mL was used to extract genomic DNA using the DNeasy UltraClean Microbial Kit (Qiagen, Toronto, ON, Canada) following the manufacturer’s protocols.

Isolated genomic DNA was used as a template for the amplification of bacterial 16S rRNA gene by PCR using the primer pair 27F (‘5-AGAGTTTGATCMTGGCTCAG-3’) [74] and 1492R (‘5-GGTTACCTTGTTACGACTT-3’) [75]. PCRs reactions were performed in 50 µL consisting of 1X PCR Buffer (Qiagen, Toronto, ON, Canada), 0.2 µM each primer, 0.5 mM of MgCl_2, 0.2 mM of dNTP mix, 0.2 mg mL^{-1} of BSA (Amersham Biosciences, Mississauga, ON, Canada), 1.25 U of Taq DNA polymerase (Qiagen, Toronto, ON, Canada) and 50 ng of gDNA. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min; 30 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final elongation at 72 °C for 7 min. PCR products were visualized on GelRed-stained 1.5% agarose gels using the Gel-Doc system (Bio-Rad Laboratories, Mississauga, ON, Canada). DNA sequencing was performed on an Applied Biosystems 3730xl DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) at Génome Québec (Montréal, Canada) [76].

4.4. Nucleotide Sequence Analyses and Accession Numbers

Sequences obtained from Sanger sequencing were trimmed by removing ambiguous nucleotide sequences, and a pair of forward and reverse reads of the 16S rRNA target was assembled by Geneious Pro v.6.1.5 (Biomatters Inc., San Diego, CA, USA). Bacteria isolates were identified by comparison with reference 16S rRNA genes from GenBank database using the BLAST algorithm [77].

The partial 16S rRNA gene sequences obtained from the bacterial isolates have been deposited in GenBank under the accession numbers (MZ430069-MZ430506).

4.5. Assessment of Hydrocarbon Degradation Potential of the Bacterial Isolates

Bacterial isolates were assessed for their ability to grow on a Bushnell Haas (BH) mineral salts medium containing various alkanes (n-hexadecane and dodecane) and (PAH) compounds (naphthalene, phenanthrene and pyrene) as the sole carbon source as described by Phillips et al. [78].

For the alkanes screening, the following ingredients were added into separate wells of a 48-well microtitre plate: 720 µL sterile BH medium and 20 µL of each filter-sterilized hydrocarbon (n-hexadecane or dodecane), as the sole carbon source, followed by an addition
of 20 µL bacterial suspension of each isolate. Two negative controls were included in the experimental setup: (1) wells containing alkanes and BH without bacterial inoculum, and (2) wells containing alkanes and BH with an autoclaved bacterial culture. After two weeks, 200 µL of filter-sterilized p-iodonitrotetrazolium violet (INT) (3 g L\(^{-1}\)) (Sigma–Aldrich, Oakville, ON, Canada) was added to each well of the plates, which were then incubated overnight. Wells that were positive for alkane degradation were identified due to the appearance of a red precipitate as a result of the INT reduction to an insoluble formazan that deposits intracellularly [79]. The experiments were repeated twice, each with three replicates for each bacterial isolate.

To screen for PAHs (naphthalene, phenanthrene and pyrene) degradation potential, each PAH compound, of at least 98% purity (Sigma–Aldrich, Oakville, ON, Canada), was first diluted in pentane (5 g L\(^{-1}\)) (Sigma–Aldrich, Oakville, ON, Canada) and 80 µL of naphthalene, phenanthrene, or pyrene, was added to each well of a 48-well plate, as the sole carbon source, and the pentane was allowed to evaporate. Then, 720 µL sterile BH medium was added to each well, followed by the addition of 20 µL suspension of each bacterial isolate. Two negative controls were included in the experimental setup: (1) wells containing PAHS and BH but no bacterial inoculum, and (2) wells containing PAHS and BH with an autoclaved bacterial culture. PAH plates were incubated for two weeks before 200 µL of filter-sterilized INT (3 g L\(^{-1}\)) (Sigma–Aldrich, Oakville, ON, Canada) was added to each well. The plates were incubated for an additional week before wells were scored positive for PAH degradation by the presence of a yellow-brown color due to the partial oxidation of aromatic compounds [79]. The experiments were repeated twice, each with three replicates for each bacterial isolate.

4.6. Assessment of Plant Growth-Promoting (PGP) Traits of the Bacterial Isolates

4.6.1. Phosphate Solubilization

The ability of bacterial isolates to solubilize inorganic phosphate was assessed using a potato-dextrose yeast agar (PDYA, pH 7.0) medium containing freshly precipitated calcium phosphate [80]. The PDYA medium was prepared in three separate solutions including PDYA-calcium phosphate (CaP) as described by de Freitas et al. [80]. Bacterial cultures were grown in half-strength TSB medium at 28 ºC for 48 h with continuous agitation at 150 rpm in a rotary shaker. A loopful of each bacterial isolate growing in liquid culture was streaked in the center of PDYA-calcium phosphate (CaP) plates, and incubated at 28 ºC. The appearances of clear zones around colonies were considered as positive phosphate solublizers and measured after 14 days of incubation. An autoclaved bacterial culture was used as a negative control. The experiment was repeated twice, each with three replicates for each isolate.

4.6.2. Screening for Nitrogen Fixation

The bacterial isolates were evaluated for their ability to grow on an N-deficient combined carbon medium which was prepared in two solutions as described by Rennie [81]. Bacterial cultures were grown in half-strength TSB at 28 ºC for 48 h with continuous agitation at 150 rpm in a rotary shaker, and a loopful of each bacterial isolate growing in liquid culture was streaked into the N-deficient combined carbon medium agar plate and incubated at 28 ºC for up to one week. The formation of colonies on agar plates was considered as positive N-fixers. An autoclaved bacterial culture was used as a negative control. The experiments were repeated twice, each with three replicates for each isolate.

4.6.3. ACC Deaminase Activity

1-Aminocyclopropane-1-carboxylate (ACC)-deaminase activity was assessed by monitoring bacterial isolates' ability to grow on DF minimal salts medium containing ACC as a sole nitrogen source. The DF minimal salts agar plates were prepared as described above and were spread with filter-sterilized ACC (30 µmol plate\(^{-1}\)) [75]. Bacterial cultures were grown in half-strength TSB at 28 ºC for 48 h with continuous agitation at 150 rpm in a
rotary shaker. A loopful of each bacterial isolate growing in liquid culture was streaked into DF minimal salts agar plates containing fresh ACC, which was just spread into the agar plate prior to use. The solid DF salts minimal agar plates were incubated for 72 h at 28 °C. Colonies showing growth on the plates indicate ACC deaminase production. An autoclaved bacterial culture was used as a negative control. The experiments were repeated twice, each with three replicates for each isolate.

4.6.4. Indole-3- Acetic Acid (IAA) Production

The bacterial isolates were screened for the production of the auxin IAA by using the Salkowski colorimetric assay as originally described by Bric et al. [82] and modified by Ribeiro et al. [83]. Isolates were cultured in 15-mL Falcon tubes containing 3 mL of Luria Bertani (LB) medium supplemented with tryptophan (1 mg mL⁻¹) as an auxin precursor. Bacterial isolates were grown in a shaker (120 rpm) for 1 day at 28 °C. A 1-mL aliquot of bacterial cultures was then centrifuged at 9500× g for 2 min and 100 µL of supernatant were added to 96 microtitre plate wells followed by the addition of 100 µL of Salkowski’s reagent (150 mL of 98% H₂SO₄, 7.5 mL of 0.5 M FeCl₃·6H₂O, and 250 mL distilled water) and the 96 micro-plate was incubated in the dark for 30 min at room temperature. Bacterial isolates producing IAA were characterized by the formation of a distinct red color [82]. An autoclaved bacterial culture was used as a negative control. The experiments were repeated twice, each with three replicates for each isolate.

4.6.5. Siderophore Production

The complex Chrome Azurol S (CAS) solid medium was used to detect siderophore synthesis by the bacterial isolates, as described by Alexander and Zuberer [84]. The assay was performed in 12-well microtitre plates and utilized the ternary complex CAS as an indicator. A change of the color of the indicator from blue to orange designates siderophore production. The CAS-agar medium consists of four solutions as described by Alexander and Zuberer [84]. The CAS-agar medium is poured into 12-microtitre plates by dispensing 5 mL of medium into each well of the plate aseptically. Plates were allowed to solidify before inoculation. Bacterial cultures were grown in half-strength TSB at 28 °C for 48 h with continuous agitation at 150 rpm in a rotary shaker, and 10 µL of liquid bacterial culture was spotted into each well of the micro-titer plate containing the solidified CAS-agar medium. The well plates were incubated at room temperature for 72 h, and the development of an orange-yellow color in the wells indicated siderophore production. An autoclaved bacterial culture was used as a negative control. The experiments were repeated twice, each with three replicates for each isolate.

5. Conclusions

*Salix purpurea* and *Eleocharis obusta* are widespread, native plants in North America, distributed in various habitats and ecosystems, and are able to tolerate chronic levels of PHC pollution. Thus, they are ideal candidates for phytoremediation of PHC-contaminated soils. This culture collection holds 438 bacterial isolates with multiple degradative and PGP features, originating from unique soil environments characterized by high levels of PHC contamination. The functional potential of bacterial isolates reported here represents a rich reservoir of metabolically versatile PGPR-PHC degraders that could be used in holistic, bacterial-aided phytomanagement and remediation of PHC contamination in future research.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10101987/s1, Table S1: Taxonomic affiliations of rhizospheric bacteria isolated from *Salix* rhizosphere on different media based on 16S rRNA gene, Table S2: Taxonomic affiliations of rhizospheric bacteria isolated from *Eleocharis* rhizosphere on different media based on 16S rRNA gene, Table S3: Taxonomic affiliations of bacteria isolated from bulk soil on different media based on 16S rRNA gene, Figure S1: Bars indicate the relative abundance of phyla among isolates that possesses different PGP-associated traits in vitro, Figure S2: Qualitative representation of genera among isolates
presenting different PGP-associated traits in vitro, Figure S3: Venn diagram of comparison between isolates reported with all five PHC degradation and all five PGP traits, Figure S4: Example of production of siderophore by Pseudomonas putida strain ET27 on CAS agar plate, Figure S5: Example of phosphate solubilization by bacterial isolates Pseudomonas monteilii strain SB53, Acinetobacter calcoaceticus strain SB54 and Bacillus indicus strain SB55 as indicated by clear zone on the PDYA-CaP medium.

**Author Contributions:** F.A., M.S.-A. and M.H. conceived and initiated the study; F.A. performed the experiments and analyzed the data; S.-J.L. helped in performing experiments and editing the manuscript; M.S.-A. and M.H. supervised experiments and obtained funds; F.A. writing—original draft preparation; M.S.-A. and M.H. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received funding from Genome Canada, Genome Quebec and Natural Sciences and Engineering Research Council of Canada.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Sequencing Data are deposited in GenBank and their accession number are provided within the article.

**Acknowledgments:** The authors thank Zakaria Lahrach and Saad El Din Hassan for their technical assistance and Andrew Blakney for English editing of the manuscript. FA was also supported by a grant from King Saud University.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Alotaibi, F.; Hijri, M.; St-Arnaud, M. Overview of Approaches to Improve Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils. *Appl. Microbiol.* 2021, 1, 329–351. [CrossRef]
2. Gkorezis, P.; Daghio, M.; Franzetti, A.; Van Hamme, J.; Sillen, W.; Vangronsveld, J. The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. *Front. Microbiol.* 2016, 7, 1836. [CrossRef]
3. Pandey, P.; Pathak, H.; Dave, S. Microbial Ecology of Hydrocarbon Degradation in the Soil: A Review. *Res. J. Environ. Toxicol.* 2016, 10, 1–15. [CrossRef]
4. Pilon-Smits, E.A.; Freeman, J.L. Environmental cleanup using plants: Biotechnological advances and ecological considerations. *Front. Ecol. Environ.* 2006, 4, 203–210. [CrossRef]
5. Brzeszcz, J.; Kaszycki, P. Aerobic bacteria degrading both n-alkanes and aromatic hydrocarbons: An undervalued strategy for metabolic diversity and flexibility. *Biodegradation* 2018, 29, 359–407. [CrossRef]
6. Khan, F.I.; Husain, T.; Hejazi, R. An overview and analysis of site remediation technologies. *J. Environ. Manag.* 2004, 71, 95–122. [CrossRef]
7. Pilon-Smits, E. Phytoremediation. *Ann. Rev. Plant Biol.* 2005, 56, 15–39. [CrossRef] [PubMed]
8. Thijs, S.; Sillen, W.; Rineau, F.; Weyens, N.; Vangronsveld, J. Towards an Enhanced Understanding of Plant-Microbiome Interactions to Improve Phytoremediation: Engineering the Metaorganism. *Front Microbiol.* 2016, 7, 341. [CrossRef] [PubMed]
9. Pulford, I.; Watson, C. Phytoremediation of heavy metal-contaminated land by trees—a review. *Environ. Int.* 2003, 29, 529–540. [CrossRef]
10. Bell, T.; Hassan, S.E.-D.; Lauron-Moreau, A.; Al-Otaibi, F.; Hijri, M.; Yergeau, E.; St-Arnaud, M. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *ISME J.* 2013, 8, 331–343. [CrossRef] [PubMed]
11. Dagher, D.J.; De La Providencia, I.E.; Pitre, F.E.; St-Arnaud, M.; Hijri, M. Plant Identity Shaped Rhizospheric Microbial Communities More Strongly Than Bacterial Bioaugmentation in Petroleum Hydrocarbon-Polluted Sediments. *Front. Microbiol.* 2019, 10, 2144. [CrossRef] [PubMed]
12. Marchand, C.; Mench, M.; Jani, Y.; Kaczala, F.; Notini, P.; Hijri, M.; Hogland, W. Pilot scale aided-phytoremediation of a co-contaminated soil. *Sci. Total Environ.* 2018, 618, 753–764. [CrossRef]
13. Robichaud, K.; Stewart, K.; Labrecque, M.; Hijri, M.; Cherevyk, J.; Amyot, M. An ecological microsystem to treat waste oil contaminated soil: Using phytoremediation assisted by fungi and local compost, on a mixed-contaminant site, in a cold climate. *Sci. Total Environ.* 2019, 672, 732–742. [CrossRef]
14. Yergeau, E.; Sanschagrin, S.; Maynard, C.; St-Arnaud, M.; Greer, C.W. Microbial expression profiles in the rhizosphere of willows depend on soil contamination. *ISME J.* 2013, 8, 344–358. [CrossRef]
15. Correa-Garcia, S.; Pande, P.; Seguin, A.; St-Arnaud, M.; Yergeau, E. Rhizoremediation of petroleum hydrocarbons: A model system for plant microbiome manipulation. *Microb. Biotechnol.* 2018, 11, 819–832. [CrossRef]
16. Kuzovkina, Y.A.; Volk, T.A. The characterization of willow (Salix L.) varieties for use in ecological engineering applications: Co-ordination of structure, function and autecology. Ecol. Eng. 2009, 35, 1178–1189. [CrossRef] [PubMed]
17. Newman, L.A.; Reynolds, C.M. Phytodegradation of organic compounds. Curr. Opin. Biotechnol. 2004, 15, 225–230. [CrossRef]
18. González, E.; Pitre, F.E.; Pagé, A.P.; Marleau, J.; Nissim, W.G.; St-Arnaud, M.; Labrecque, M.; Joly, S.; Yergeau, E.; Breton, N.J.B. Trees, fungi and bacteria: Tripartite metatranscriptomics of a root microbiome responding to soil contamination. Microbiome 2018, 6, 53. [CrossRef]
19. Yergeau, E.; Bell, T.; Champagne, J.; Maynard, C.; Tardif, S.; Tremblay, J.; Greer, C.W. Transplanting Soil Microbiomes Leads to Lasting Effects on Willow Growth, but not on the Rhizosphere Microbiome. Front. Microbiol. 2015, 6, 1436. [CrossRef] [PubMed]
20. Hassan, S.E.-D.; Bell, T.H.; Stefani, F.O.P.; Denis, D.; Hijri, M.; St-Arnaud, M. Contrasting the Community Structure of Arbuscular Mycorrhizal Fungi from Hydrocarbon-Contaminated and Uncontaminated Soils following Willow (Salix spp. L.) Planting. PLoS ONE 2019, 9, e102838. [CrossRef]
21. Desjardins, D.; Nissim, W.G.; Pitre, F.E.; Naud, A.; Labrecque, M. Distribution patterns of spontaneous vegetation and pollution at a former decantation basin in southern Quebec, Canada. Ecol. Eng. 2014, 64, 385–390. [CrossRef]
22. Lumactud, R.; Shen, S.Y.; Lau, M.; Fulthorpe, R. Bacterial endophytes isolated from plants in natural oil seep soils with chronic hydrocarbon contamination. Front. Microbiol. 2016, 7, 755. [CrossRef] [PubMed]
23. Iqbal, A.; Arshad, M.; Karthikeyan, R.; Gentry, T.J.; Rashid, J.; Ahmed, I.; Schwab, A.P. Diesel degrading bacterial endophytes with plant growth promoting potential isolated from a petroleum storage facility. 3 Biotech 2019, 9, 35. [CrossRef]
24. Pawlik, M.; Cania, B.; Thijs, S.; Vangronsveld, J.; Piotrowska-Seteg, Z. Hydrocarbon degradation potential and plant growth-promoting activity of culturable endophytic bacteria of Lotus corniculatus and Oenothera biennis from a long-term polluted site. Environ. Sci. Pollut. Res. 2017, 24, 19640–19652. [CrossRef] [PubMed]
25. Pérez-Jaramillo, J.E.; Mendes, R.; Raaijmakers, J.M. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 2015, 90, 635–644. [CrossRef]
26. Escaray, F.J.; Menendez, A.B.; Gárriz, A.; Pieknenstain, F.L.; Estrella, M.J.; Castagno, L.N.; Carrasco, P.; Sanjuan, J.; Ruiz, O.A. Ecological and agronomic importance of the plant genus Lotus. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. Plant Sci. 2012, 182, 121–133. [CrossRef] [PubMed]
27. Johnson, N.C. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol. 2009, 185, 631–647. [CrossRef]
28. Backer, R.; Rokem, J.S.; Langumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Front. Plant Sci. 2018, 9, 1473. [CrossRef]
29. Kotoky, R.; Rajkumari, J.; Pandey, P. The rhizosphere microbiome: Significance in rhizoremediation of polyaromatic hydrocarbon contaminated soil. J. Environ. Manag. 2018, 217, 858–870. [CrossRef]
30. Glick, B.R.; Stearns, J.C. Making Phytoremediation Work Better: Maximizing a Plant’s Growth Potential in the Midst of Adversity. Int. J. Phytoremediat. 2011, 13, 4–16. [CrossRef]
31. Khan, S.; Afzal, M.; Iqbal, S.; Khan, Q.M. Plant–bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere 2013, 90, 1317–1332. [CrossRef]
32. Singh, L.P.; Sinha, N.; Pandey, P. Rhizoremediation prospects of Polyaromatic hydrocarbon degrading rhizobacteria, that facilitate glutathione and glutathione-S-transferase mediated stress response, and enhance growth of rice plants in pyrene contaminated soil. Ecotoxicol. Environ. Saf. 2018, 164, 579–588. [CrossRef]
33. Jambon, I.; Thijs, S.; Weymens, N.; Vangronsveld, J. Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants. J. Plant Interact. 2018, 13, 119–130. [CrossRef]
34. Hardoim, P.R.; van Overbeek, L.S.; Elsas, J.D. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 2008, 16, 463–471. [CrossRef]
35. Iffis, B.; St-Arnaud, M.; Hijri, M. Petroleum hydrocarbon contamination, plant identity and arbuscular mycorrhizal fungal (AMF) community determine assemblages of the AMF spore-associated microbes. Environ. Microbiol. 2016, 18, 2689–2704. [CrossRef]
36. Marchand, C.; St-Arnaud, M.; Hogland, W.; Bell, T.; Hijri, M. Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. Int. Biodeterior. Biodegrad. 2017, 116, 48–57. [CrossRef]
37. Iffis, B.; St-Arnaud, M.; Hijri, M. Bacteria associated with arbuscular mycorrhizal fungi within roots of plants growing in a soil highly contaminated with aliphatic and aromatic petroleum hydrocarbons. FEMS Microbiol. Lett. 2014, 358, 44–54. [CrossRef] [PubMed]
38. Lee, S.-J.; Kong, M.; St-Arnaud, M.; Hijri, M. Arbuscular Mycorrhizal Fungal Communities of Native Plant Species under High Petroleum Hydrocarbon Contamination Highlights Rhizophagus as a Key Tolerant Genus. Microorganisms 2020, 8, 872. [CrossRef] [PubMed]
39. Yergeau, E.; Tremblay, J.; Joly, S.; Labrecque, M.; Maynard, C.; Pitre, F.E.; St-Arnaud, M.; Greer, C.W. Soil contamination alters the willow root and rhizosphere metatranscriptome and the root–rhizosphere interactome. ISME J. 2018, 12, 869–884. [CrossRef] [PubMed]
40. Baek, K.-H.; Kim, H.-S.; Oh, H.-M.; Yoon, B.-D.; Kim, J.; Lee, I.-S. Effects of crude oil, oil components, and bioremediation on plant growth. J. Environ. Sci. Health Part A 2004, 39, 2465–2472. [CrossRef] [PubMed]
41. Chaineau, C.; Morel, J.-L.; Oudot, J. Phytotoxicity and Plant Uptake of Fuel Oil Hydrocarbons; Wiley Online Library: Hoboken, NJ, USA, 1997.

42. Liu, R.; Jadeja, R.N.; Zhou, Q.; Liu, Z. Treatment and remediation of petroleum-contaminated soils using selective ornamental plants. Environ. Eng. Sci. 2012, 29, 494–501. [CrossRef] [PubMed]

43. Lugtenberg, B.; Kamiliova, F. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 2009, 63, 541–556. [CrossRef] [PubMed]

44. Schlaeppi, K.; Bulgarelli, D. The plant microbiome at work. Mol. Plant Microbe. Interact 2015, 28, 212–217. [CrossRef] [PubMed]

45. Ghosal, D.; Ghosh, S.; Dutta, T.K.; Ahn, Y. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Front. Microbiol. 2017, 8, 1369. [CrossRef] [PubMed]

46. Xu, X.; Liu, W.; Tian, S.; Wang, W.; Qi, Q.; Jiang, P.; Gao, X.; Li, F.; Li, H.; Yu, H. Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. Front. Microbiol. 2018, 9, 2885. [CrossRef] [PubMed]

47. Bai, Y.; Müller, D.B.; Srinivas, G.; Garrido-Oter, R.; Potthoff, E.; Rott, M.; Dombrowski, N.; Münch, P.C.; Spaepen, S.; Remus-Emsermann, M.; et al. Functional overlap of the Arabidopsis leaf and root microbiota. Nature 2015, 528, 364–369. [CrossRef]

48. Ferrera-Rodríguez, O.; Greer, C.W.; Juck, D.; Consaul, L.; Martínez-Romero, E.; Whyte, L. Hydrocarbon-degrading potential of microbial communities from Arctic plants. J. Appl. Microbiol. 2013, 114, 71–83. [CrossRef] [PubMed]

49. Mitter, E.K.; De Freitas, J.R.; Germida, J.J. Bacterial Root Microbiome of Plants Growing in Oil Sands Reclamation Covers. Front. Microbiol. 2021, 10, 520. [CrossRef] [PubMed]

50. Lumactus, R.; Fulthorpe, R.R. Endophytic Bacterial Community Structure and Function of Herbaceous Plants from Petroleum Hydrocarbon Contaminated and Non-contaminated Sites. Front. Microbiol. 2018, 9, 1926. [CrossRef] [PubMed]

51. Dwivedi, A.; Chitranshi, S.; Gupta, A.; Kumar, A.; Bhat, J.L. Assessment of the Petroleum Oil Degradation Capacity of Indigenous Bacterial Species Isolated from Petroleum Oil-Contaminated Soil. Int. J. Environ. Res. 2019, 13, 735–746. [CrossRef]

52. Vieser, J.A.; Guériós, M.; Malucelli, I.C.; Pincercati, M.R.; Karpe, S.G.; Marano, L.T. Petroleum-Tolerant Rhizospheric Bacteria: Isolation, Characterization and Bioremediation Potential. Sci. Rep. 2020, 10, 1–11. [CrossRef] [PubMed]

53. Das, N.; Chandran, P. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnol. Res. Int. 2011, 2011, 1–13. [CrossRef] [PubMed]

54. Wolińska, A.; Gałążka, A.; Kuźniar, A.; Goraj, W.; Jastrzębska, N.; Grzadziel, J.; Stepniewska, Z. Catabolic Fingerprinting and Biodegradation Capacities of the Microbial Community from a Military Soil. FEMS Microbiol. Lett. 2017, 356–362. [CrossRef] [PubMed]

55. Martínez-Romero, E.; Rodríguez-Contreras, H.; Fraga, R. Phosphate-solubilizing bacteria and their role in plant growth promotion. Curr. Opin. Biotechnol. 2005, 16, 282–290. [CrossRef] [PubMed]

56. Martinková, L.; Uhnaková, B.; Pátek, M.; Nesvera, J.; Kren, V. Biodegradation potential of the genus Rhodococcus. Environ. Int. 2009, 35, 162–177. [CrossRef] [PubMed]

57. Hynes, R.K.; Leung, G.C.; Hirkala, D.L.; Nelson, L.M. Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. Can. J. Microbiol. 2008, 54, 248–258. [CrossRef] [PubMed]

58. Dutta, J.; Handique, P.J.; Thakur, D. Assessment of culturable tea rhizobacteria isolated from tea estates of Assam, India for growth promotion in commercial tea cultivars. Front. Microbiol. 2015, 6, 1252. [CrossRef] [PubMed]

59. Glick, B.R. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol. Lett. 2005, 251, 1–7. [CrossRef]

60. Grichko, V.P.; Glick, B.R. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol. Biochem. 2001, 39, 11–17. [CrossRef]

61. Arshad, M.; Saleem, M.; Hussain, S. Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol. 2007, 25, 356–362. [CrossRef]

62. Thijs, S.; Weyens, N.; Sillen, W.; Gkorezis, P.; Carleer, R.; Vangronsveld, J. Potential for plant growth promotion by a consortium of stress-tolerant 2, 4-dinitrotoluene-degrading bacteria: Isolation and characterization of a military soil. Microb. Biotechnol. 2015, 8, 294–306. [CrossRef] [PubMed]

63. Vosjes, J.K. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 2003, 255, 571–586. [CrossRef]

64. Kim, K.Y.; Jordan, D.; McDonald, G.A. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fertil. Soils 1997, 26, 79–87. [CrossRef]

65. Rodriguez, H.; Fraga, R. Phosphate-solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 1999, 17, 319–339. [CrossRef]

66. Crowley, D.E.; Kraemer, S.M. Function of siderophores in the plant rhizosphere. In The Rhizosphere, Biochemistry and Organic Substances at the Soil–Plant Interface; CRC Press: Boca Raton, FL, USA, 2007; pp. 73–109.

67. Nogales, J.; García, J.L.; Díaz, E. Degradation of aromatic compounds in pseudomonas: A systems biology view. In Aerobic Utilization of Hydrocarbons, Oils and Lipids; Springer: Berlin/Heidelberg, Germany, 2017; pp. 1–49. [CrossRef]

68. Ansari, F.A.; Ahmad, I. Fluorescent Pseudomonas -FAP2 and Bacillus licheniformis interact positively in biofilm mode enhancing plant growth and photosynthetic attributes. Sci. Rep. 2019, 9, 4547. [CrossRef]

69. Faubert, M.F.; Hijri, M.; Labrecque, M. Short rotation intensive culture of willow, spent mushroom substrate and ramial chipped wood for bioremediation of a contaminated site used for land farming activities of a former petrochemical plant. Plants 2021, 10, 520. [CrossRef] [PubMed]

70. Bushnell, L.D.; Haas, H.F. The Utilization of certain hydrocarbons by microorganisms. J. Bacteriol. 1941, 41, 653–673. [CrossRef]
71. Daane, L.L.; Harjono, I.; Zylstra, G.J.; Häggblom, M.M. Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. *Appl. Environ. Microbiol.* 2001, 67, 2683–2691. [CrossRef]

72. Yousaf, S.; Andria, V.; Reichenauer, T.G.; Smalla, K.; Sessitsch, A. Phylogenetic and functional diversity of alkane degrading bacteria associated with Italian ryegrass (Lolium multiflorum) and Birdsfoot trefoil (Lotus corniculatus) in a petroleum oil-contaminated environment. *J. Hazard. Mater.* 2010, 184, 523–532. [CrossRef] [PubMed]

73. Penrose, D.M.; Glick, B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* 2003, 118, 10–15. [CrossRef]

74. Lane, D. 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*; John, Wiley and Sons: Hoboken, NJ, USA, 1991; pp. 115–175.

75. Turner, S.; Pryer, K.M.; Miao, V.P.W.; Palmer, J.D. Investigating Deep Phylogenetic Relationships among Cyanobacteria and Plastids by Small Subunit rRNA Sequence Analysis. *J. Eukaryot. Microbiol.* 1999, 46, 327–338. [CrossRef] [PubMed]

76. Stefani, F.O.P.; Bell, T.; Marchand, C.; De La Providencia, I.E.; El Yassimi, A.; St-Arnaud, M.; Hijri, M. Culture-Dependent and -Independent Methods Capture Different Microbial Community Fractions in Hydrocarbon-Contaminated Soils. *PLoS ONE* 2015, 10, e0128272. [CrossRef]

77. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 1997, 25, 3389–3402. [CrossRef] [PubMed]

78. Phillips, L.; Greer, C.; Germida, J. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon-contaminated flare-pit soil. *Soil Biol. Biochem.* 2006, 38, 2823–2833. [CrossRef]

79. Wrenn, B.A.; Venosa, A.D. Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. *Can. J. Microbiol.* 1996, 42, 252–258. [CrossRef] [PubMed]

80. De Freitas, J.R.; Banerjee, M.R.; Germida, J. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). *Biol. Fertil. Soils* 1997, 24, 358–364. [CrossRef]

81. Rennie, R.J. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. *Can. J. Microbiol.* 1981, 27, 8–14. [CrossRef]

82. Bric, J.M.; Bostock, R.M.; Silverstone, S.E. Rapid In Situ Assay for Indoleacetic Acid Production by Bacteria Immobilized on a Nitrocellulose Membrane. *Appl. Environ. Microbiol.* 1991, 57, 535–538. [CrossRef]

83. Ribeiro, C.M.; Cardoso, E.J.B.N. Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (Araucaria angustifolia). *Microbiol. Res.* 2012, 167, 69–78. [CrossRef]

84. Alexander, D.B.; Zuberer, D.A. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils* 1991, 12, 39–45. [CrossRef]