Université de Montréal

Structural and functional diversity of bacterial communities in petroleum hydrocarbons contaminated soils subjected to phytoremediation

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Thèse présentée à la Faculté des arts et des sciences
en vue de l'obtention du grade de doctorat
en sciences biologiques

mai 2022

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Université de Montréal
Faculté des Études Supérieures et Postdoctorales

Cette thèse intitulée:
Structural and functional diversity of bacterial communities in petroleum hydrocarbons contaminated soils subjected to phytoremediation

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RÉSUMÉ

L'intensification des activités industrielles et les besoins en énergie font des hydrocarbures pétroliers (HP) un enjeu majeur mondial mais augmentent aussi considérablement les risques environnementaux dans divers écosystèmes. La phytoremédiation est une phytotechnologie qui a fait ses preuves en tant que solution verte pour faire face aux contaminations des sols par des HP. La phytoremédiation des sols contaminés par les HP repose principalement sur l'activité des communautés microbiennes associées aux racines des plantes au niveau de la rhizosphère, qui peuvent non seulement favoriser la croissance des plantes hôtes mais aussi augmenter leur tolérance à divers stress biotiques et abiotiques. Parmi les défis majeurs de la phytoremédiation des sols contaminés par les HP, on compte la forte toxicité de certains composés des HP qui entravent la croissance des plantes et par conséquent l'efficacité de la phytoremédiation. Cependant, la croissance des plantes peut être positivement stimulée par la présence de rhizobactéries favorisant leur croissance (PGPR) qui sont capables d'atténuer le stress des plantes par divers mécanismes.

Dans cette thèse, un total de 438 bactéries PGPR dégradant les hydrocarbures pétroliers, ont été isolées de la rhizosphère et du sol de deux espèces de plantes, *Salix purpurea* et *Eleocharis obusta,* dans un site d'une ancienne raffinerie pétrochimique à Varennes, QC, Canada. Les isolats bactériens ont été classés en 62 genres, appartenant aux phylums Actinobacteria, Bacteroidetes, Firmicutes et aux sous-groupes Alpha-, Beta- et Gamma-Proteobacteria. De plus, cette collection de cultures contient 438 isolats bactériens avec de multiples caractéristiques de dégradation et de stimulation de croissance (PGPR), représentant une diversité fonctionnelle de dégradation des HP et de caractéristiques PGPR qui pourraient être utilisées dans la phytoremédiation assistée par les bactéries, des sols contaminés par les HP.

Parmi ces 438 isolats bactériens, 50 isolats représentant une large diversité taxonomique, ont été sélectionnées pour une caractérisation approfondie supplémentaire concernant leur capacité à favoriser la croissance des plantes en présence de différentes concentrations de n-hexadécane (0%, 1%, 2%, 3%) dans des conditions contrôlées. Les résultats ont indiqué que les isolats bactériens *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27), *Stenotrophomonas pavanii* (EB31), *Bacillus megaterium* (WT10) et *Gordonia amicalis* (WT12) ont significativement

augmenté la croissance des plantes cultivées dans 3% de n-hexadécane par rapport au traitement témoin. De plus, ces isolats possèdent plusieurs traits favorisant la croissance des plantes (PGPR) tels que l'activité 1-aminocyclopropane-1-carboxylate (ACC) désaminase (ACCD), la production d'acide indole-3-acétique (IAA) et la fixation de l'azote. De plus, ces isolats étaient capables d'utiliser le n-hexadécane comme seule source de carbone et possédaient des gènes cataboliques liés à la dégradation des hydrocarbures tels que le gène de l'alcane monooxygénase (*alkB*), le cytochrome P450 hydroxylase (*CYP153*) et le gène de la naphtalène dioxygénase (*nah1*).

Nocardia sp. isolate WB46, a été sélectionné pour le séquençage de son génome afin de déterminer sa diversité génétique et fonctionnelle relatives à la dégradation des HP et les potentiels PGPR. Les résultats ont indiqué que, sur la base des analyses du gène de l'ARNr 16S, l'hybridation ADN-ADN *in silico* (DDH) et l'identité moyenne des nucléotides (ANI), *Nocardia* sp. isolate WB46 représente une nouvelle espèce bactérienne. De plus, l'annotation fonctionnelle de son génome révèle que celui-ci contient de nombreux gènes responsables de la dégradation des hydrocarbures pétroliers tels que l'alcane 1-monooxygénase (*alkB*) et la naphtalène dioxygénase (*ndo*) ainsi que d'autres gènes liés à ses potentiels PGPR. En conclusion, la rhizosphère des espèces S. *purpurea* et *E. obusta* poussant dans un site fortement pollué par les HP représente un biotope diversifié et comprenant des bactéries PGPR avec de multiples potentiels de dégradation des HP. De plus, plusieurs isolats bactériens tels que *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27) et *Stenotrophomonas pavanii* (EB31) démontrent un potentiel d'utilisation comme bioinoculants pour de futures études de phytoremédiation à grande échelle.

Mots-clés: alkanes;1-aminocyclopropane-1-carboxylate (ACC) désaminase (ACCD); bactéries; bioinoculants; *Eleocharis*; hydrocarbures pétroliers (HP); rhizobactéries favorisant leur croissance (PGPR); rhizoremédiation; phytoremédiation; *Salix;* sols contaminés.

ABSTRACT

Petroleum hydrocarbons (PHCs), as a result of intensification of industrial activities, are a global environmental issue especially in soil environments. Phytoremediation represents an ideal solution to tackle this global crisis. Phytoremediation of PHC-contaminated soils proceeds mainly through the activities of microbial communities that colonize the plant rhizosphere which might promote host plants growth and increase its tolerance to various biotic and abiotic stresses. A main challenge in phytoremediation of PHC-contaminated soils is the high toxicity of PHCs which hinder plant growth and reduce the efficiency of phytoremediation. However, plant growth may be positively stimulated by the presence of plant growth-promoting rhizobacteria (PGPR) that are able to alleviate stresses in plants through various mechanisms.

In this thesis, a total of 438 petroleum hydrocarbons degrading-PGPR bacterial isolates were recovered from the rhizosphere and the surrounding bulk soil of *Salix purpurea* and *Eleocharis obusta* plants from the site of a former petrochemical plant in Varennes, QC, Canada. Bacterial isolates were classified into 62 genera, belonging to the phyla Actinobacteria, Bacteroidetes, Firmicutes and the Alpha, Beta and Gamma-subgroups of Proteobacteria. Additionally, this culture collection holds 438 bacterial isolates with multiple degradative and PGP features, representing a rich reservoir of metabolically versatile PGPR-PHC degraders that could be used in holistic, bacterial-aided phytomanagement of PHC-contaminated soils.

Among the above 438 bacterial isolates, 50 bacterial strains representing a wide phylogenetic range were selected for an additional in-depth characterization regarding their ability to promote plant growth under the presence of different concentrations of *n*-hexadecane (0%, 1%, 2%, 3%) under gnotobiotic conditions. Results indicated that bacterial isolates *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27), *Stenotrophomonas pavanii* (EB31), *Bacillus megaterium* (WT10) and *Gordonia amicalis* (WT12) significantly increased the growth of plants grown in 3% *n*-hexadecane compared with the control treatment. Additionally, these isolates possess several plant-growth-promoting (PGP) traits such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD) activity, indole-3-acetic acid (IAA) production and nitrogen fixation. Also, these isolates were able to use *n*-hexadecane as sole source

of carbon and have catabolic genes related to hydrocarbon degradation such alkane monooxygenase (*alkB*) gene, the cytochrome P450 hydroxylase (*CYP153*) and the naphthalene dioxygenase (*nah1*) gene.

The isolate that showed the highest growth stimulation of plants grown in 3% *n*-hexadecane under gnotobiotic conditions, *Nocardia* sp. isolate WB46, was selected for *de novo* genome sequencing to unveil its genetic versatility and the mechanisms of PHCs biodegradation and PGP potentials. Results indicated that based on the 16S rRNA gene analyses, *in silico* DNA-DNA hybridization (DDH) and average nucleotide identity (ANI) *Nocardia* sp. isolate WB46 is a new species. Additionally, the functional annotation of the genome of *Nocardia* sp. isolate WB46 reveals that its genome contains many genes responsible for petroleum hydrocarbon degradation such as alkane 1-monooxygenase (*alkB*) and naphthalene dioxygenase (*ndo*) as well as other genes related to its PGP potentials.

In conclusion, *S. purpurea* and *E. obusta* growing in a site highly polluted with PHCs are rich reservoir of diverse PGPR with multiple PHC-degradation and PGP potentials. In addition, several bacterial isolates such as *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27) and *Stenotrophomonas pavanii* (EB31) demonstrate potential for use as bioinoculants in future large-scale phytoremediation studies.

Keywords: Alkanes; 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD); bacteria; bioinoculants; *Eleocharis*; petroleum hydrocarbons (PHCs); Plant growth-promoting rhizobacteria (PGPR); rhizoremediation; phytoremediation; *Salix*; soil contamination.

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LIST OF ABBREVIATONS

ACC	1-aminocyclopropane-1-carboxylic acid
ANI	Average nucleotide identity
AMF	Arbuscular mycorrhizal fungi
BLAST	Basic local alignment search tool
BSA	Bovine serum albumin
CAS	Chrome azurol S
CFU	Colony forming units
CDS	Coding sequences
DF	Dworkin & Foster
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribonucleic acid
gDNA	Genomic DNA
HDTMA	Hexadecyltrimethyl-ammonium bromide
IAA	Indole-3-acetic acid
LB	Luria-bertani
LSD	Least significant difference
MSM	Mineral salt medium
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
N	Nitrogen
N/D	Not determined
OD	Optical density

P	Phosphorus
PAF	Pseudomonas Agar F
PAHs	Polycyclic aromatic hydrocarbons
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDYA	Potato-dextrose yeast agar
PGP	Plant growth-promoting
PGPR	Plant growth-promoting rhizobacteria
PHCs	Petroleum hydrocarbons
rRNA	Ribosomal DNA
rpm	Rounds per minute
sp.	Species
spp.	Species
TSA	Tryptic soy agar
TSB	Tryptic soy broth
tRNA	Transfer RNA

LIST Of SYMBOLS/UNITS

°C	Degree centigrade
s	Seconds
h	Hour
cm	Centimeter
d	Days
g	Gram
Kg	Kilogram
min	Minute
M	Molar
mg	Milligram
mL	Milliliter
mM	Millimolar
L	Liter
µg	Microgram
µl	Microliter
µmol	Micromole
µM	Micrometer
v/v	Volume per volume
w/v	Weight per volume
-	Negative
%	Percentage
+	Positive

ACKNOWLEDGEMENTS

A journey of this magnitude is never taken alone. The number of people who helped me along the way is far too numerous to list here. First, I give my heartfelt thanks to both of my supervisors, Dr. Marc St-Arnaud and Dr. Mohamed Hijri for offering me the opportunity to pursue my studies at the Université de Montréal and for allowing me to grow up in their lab. Also, I have to give them recognition and appreciation for sticking with me steadily and surely for the long trek that is a doctoral thesis, all the while not forgetting the occasional enthusiastic discussion or question, helping to keep scientific excitement alive. Merci beaucoup. Second, I also greatly appreciate the time and help given by the other members of my advisory committee members, Dr. Michel Labrecque, Dr. Etienne Yergeau, and Dr. Charles Greer. I would also like to acknowledge my examination committee members Dr. Richard Villemur, Dr. Frédéric Pitre and Dr. Rachid Daoud for accepting to examine my dissertation and providing valuable comments and suggestions.

Thirdly, I could not have done any of this work without the help and friendship of many people at IRBV. I am grateful for my friends in Drs. St-Arnaud and Hijri labs. A very special thanks to Dr. Saad El-Din Hassan and Dr. Terrence Bell, who provided invaluable assistance in different aspects of this work. I'm also grateful to my office and lab mates Abdelghani, Bachir, TJ, Zakaria, Dimitri, Rim, Charlotte, Chih-Ying Soon-Jae, Jean-Baptiste Floc′H and Stacie.

Fourthly, I am sincerely grateful to my extended family in Saudi Arabia for their continuous prayer, patience, understanding, support and encouragement. I would also like to thank my wife, Bashayer Alshaibani for her patience, moral support and bearing with me during the difficult times during this long journey.

Finally, I am very grateful for the financial support provided by King Saud University Scholarship program managed through the Saudi Cultural Bureau in Ottawa. Funding for this research project provided by the government of Canada through Genome Canada is gratefully acknowledged.

Contribution of authors

This Ph.D. thesis consists of three experimental chapters (Chapters 3, 4, and 5), of which the candidate, Fahad Alotaibi, was fully responsible for the experimental design, development and execution of the laboratory, growth chamber and green house research studies, collection and analysis of the data, discussion of the results, and writing of the thesis. However, this thesis could not have been accomplished without the contribution of the co-supervisors, Dr. Marc St-Arnaud and Dr. Mohamed Hijri, who conceived the initial research idea and the concept of isolating and screening of plant growth-promoting rhizobacteria from plants growing in petroleum hydrocarbon-contaminated soils. Drs. Marc St-Arnaud and Mohamed Hijri provided guidance, advice and funding support during the preparation, laboratory work, interpretation of the findings, and writing of the thesis. Drs. Marc St-Arnaud and Mohamed Hijri also critically revised this thesis.

Chapter One: General Introduction

Environmental contamination resulting from industrial activities, such as mining, extraction of oil and gas and agrochemical-based agriculture, in addition to industrial waste disposal, has become a global challenge. Petroleum hydrocarbons (PHCs) are among the pollutants associated with oil production industry, and they are of great concern and can pose a risk of environmental contamination (Brzeszcz and Kaszycki, 2018; Alotaibi et al., 2021a).

Petroleum hydrocarbons (PHCs) are heterogeneous organic molecules composed mainly of carbon and hydrogen atoms arranged in varying structural configurations with different physical and chemical properties (Gkorezis et al., 2016). The PHCs are formed primarily by hydrocarbons and lesser amounts of other non-hydrocarbon constituents such as sulfur, oxygen and nitrogen (Farrell-Jones, 2003; Wang, 2006). The PHCs are grouped into two major fractions according to their chemical properties, namely aliphatic hydrocarbons and aromatic hydrocarbons. Aliphatic hydrocarbons include both linear or branched chain hydrocarbons, which may be unsaturated (alkenes and alkynes) or saturated (alkanes) (Pandey et al., 2016). Aromatic hydrocarbons include mono (i.e. benzene, toluene, phenol, etc.) and polycyclic aromatic hydrocarbons (PAHs). The ever-increasing dependency of modern civilization on fossil fuel for energy generation in many civil sectors, such as industry, heat, electricity and transportation has resulted in the extensive utilization of PHCs (Gkorezis et al., 2016).

Soil pollution with PHCs is a global concern, and the magnitude of soil contamination is beyond imagination. For instance, in Canada, approximately 22,000 federal-owned sites are estimated to be contaminated by PHCs (Secretariat, 2015), whereas in Australia, around 80,000 sites are identified as being polluted by PHCs (Hoang et al., 2021). These PHCs compounds also pose serious health risks to humans and other organisms as well as their adverse impact on the soil microbiota and fauna, leading to environmental quality degradation. This explains the growing concern with the ever-increasing PHCs pollution and the urgent need to use all possible means to protect the environment and to find a suitable method to remediate contaminated soils (Alotaibi et al., 2021a).

Over the past few decades, several traditional remediation strategies including chemical, physical and thermal technologies have been used to clean-up soils contaminated with PHCs (Salt et al., 1998; Pilon-Smits, 2005). However, these approaches have some limitations such as a prohibitive cost, and some of these methods only working for specific organic compounds, do not often result in a complete degradation of the contaminants, and most importantly, are considered as non-sustainable solutions to cleanup PHCs pollution (Yerushalmi et al., 1998; Inoue and Katayama, 2011; Alotaibi et al., 2021a). On the other hand, phytoremediation is a more recent and promising green-biological technology that is perceived as an environmentally friendly, less expensive, more sustainable and less destructive method to remove pollutants from the environment (Gkorezis et al., 2016; Correa-García et al., 2018).

Phytoremediation is a plant based-biotechnology that relies on the ability of plants and their root-associated rhizospheric and endophytic microbiomes to clean-up PHCs-polluted soils (Pilon-Smits, 2005). Phytoremediation is gaining popularity because it is an eco-friendly, solar-driven, green, and low carbon footprint remediation approach (Pilon-Smits, 2005; Alotaibi et al., 2021a). Over the past decades, phytoremediation has been applied to clean up a wide spectrum of organic and inorganic contaminants in soil and water environments such as chlorinated solvents (Aken and Doty, 2009), explosives (Panz and Miksch, 2012), trace metals (Leguizamo et al., 2017), landfill leachates (Jerez Ch and Romero, 2016), pesticides (Olette et al., 2008), petroleum hydrocarbons (Newman and Reynolds, 2004), radionuclides (Sharma et al., 2015) and salts (Devi et al., 2016).

A subset of phytoremediation is rhizoremediation, which is the breakdown of organic contaminants by root-associated microbial communities (Kuiper et al., 2004). Rhizoremediation has shown a great performance in remediating mildly polluted soils; however, its effectiveness at high levels of contaminants is limited because of the significant reduction in the growth of introduced plants under these conditions. This might be in part related to variations in the associations between plants and their resident microbiomes (Robichaud et al., 2019; Alotaibi et al., 2021b). To promote phytoremediation effectiveness, the use of PHCs-tolerant plant species with vigorous root systems and fast growth patterns, and plant growth-promoting rhizobacteria

(PGPR) has been the focus of research interest in the last decade (Thijs et al., 2016; Correa-García et al., 2018; Alotaibi et al., 2021a; Eze et al., 2022).

In the beginning of phytoremediation trials, many varieties of plants have been evaluated to increase the efficiency of rhizoremediation of PHCs contaminated soils. Plant species that have shown high potential so far encompass a wide range of families such as legumes (alfalfa, clover), herbaceous crops (sunflower, Indian mustard), grasses (tall fescue, annual ryegrass), and woody trees (willows, hybrid poplars) (Frick et al., 1999; Gaskin and Bentham, 2010; Hall et al., 2011). Criteria for selection of suitable plants for phytoremediation purposes include suitability for various soil types, increased tolerance to a broad range of pollutants, root morphology pattern, fast growth, biomass production, the root exudate profile and symbiosis formation with soil microbes (Chaudhry et al., 2005; Wenzel, 2009). In addition, plants ability to stimulate microbial abundance and activity should be taken into consideration (Thijs et al., 2016; Alotaibi et al., 2021a).

Shrubs such as willows (*Salix*) have been candidates for improving rhizoremediation of PHC-polluted soil (Gkorezis et al., 2016) because they are easy to propagate, show increased tolerance toward several stressful environments, exhibit extremely fast growth in marginal soils, produce large root and shoot biomass, and generate widespread deep-rooting systems (Kuzovkina and Volk, 2009). Over the last decade, several reports documented the successful use of willows for the phytoremediation of soils contaminated with different organic and inorganic pollutants, including PHCs (de Cárcer et al., 2007; Bell et al., 2014a; Yergeau et al., 2018). In addition, willows establish two types of mycorrhizal symbiosis with fungi, endomycorrhiza with Glomeromycota and ectomycorrhiza with Basidiomycota and Ascomycota (Dagher et al., 2020).

Recently, screening and identification of native plants growing on PHC-contaminated soils for their potential utilization in promoting rhizoremediation of PHC-polluted soil have been investigated (Desjardins et al., 2014). The use of locally adapted plants in rhizoremediation offers several advantages including a reduced potential risk of introducing alien species that can become invasive and disrupt local fauna and flora (Timmis and Pieper, 1999). In addition, native plants are more genetically diverse and potentially more adapted to a wide range of environmental and climatic conditions compared with foreign plants currently chosen for phytoremediation (Brown, 1976).

For example, (Desjardins et al., 2014) screened native plants growing spontaneously in a highly PHC-contaminated site in Canada and identified three native species (*Alisma triviale*, *Eleocharis obtusa* and *Panicum capillare*) with high tolerance to PAHs and PHCs.

Plants are colonized with taxonomically diverse microbial communities. Recent studies demonstrated the immense role of plant microbiome to plant health and fitness, such as enhanced nutrient acquisition, altered plant hormonal balance, alleviated plant stress and biologically controlled plant diseases (Bulgarelli et al., 2013). Plant-associated bacteria that perform such functions are known as plant growth-promoting rhizobacteria (Lugtenberg and Kamilova, 2009).

PGPR are free-living inhabitants of the rhizospheric zone of plants and can directly or indirectly impact plant growth via various mechanisms. PGPR provide nutrients to host plants, produce different phytohormones that regulate plant growth, and protect their hosts from phytopathogens and abiotic stress. The functions of PGPR include nitrogen fixation, phosphate solubilization, indoleacetic-3-acid production, siderophore biosynthesis, stresses alleviation in plants by production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and induction of systemic resistance (ISR) in plants (Haas and Défago, 2005; Lugtenberg and Kamilova, 2009). Although PGPR inoculants are frequently utilized in agricultural settings, their use in environmental settings, such as in the phytoremediation of PHCs, has only emerged in recent years, thus representing a huge untapped potential. For example Pacwa-Płociniczak et al. (2016) reported that two PHC-degrading *Rhodococcus* sp. with multiple PGP traits are good candidates for rhizoremediation of PHCs-contaminated sites. Similarly, Zuzolo et al. (2021) performed *in vitro* assessment of PGP activities of PHC-degrading bacteria isolated from contaminated soil of petroleum refinery and reported that several bacterial genera such as *Gordonia*, *Pseudomonas*, *Bacillus*, *Comamonas*, *Burkholderia* and *Rhodococcus* possess multiple PGP activities such as IAA production, siderophores synthesis and exopolysaccharides (EPSs) production.

Plants can adapt and confront many unfavorable stressful conditions, such as PHC contamination. However, plant growth has been retarded under highly stressed conditions, e.g., PHC pollutants are expected to lower plant growth than under optimal conditions (Glick and Stearns, 2011). As bacterial communities present in PHCs-polluted sites often possess adaptability and resistance to toxic chronic levels of organic

contaminants, an examination of their plant growth-promoting (PGP) traits will bridge the knowledge gap required to develop effective PGPR inoculants for plants growing in such contaminated soils. Therefore, there is a growing interest in the isolation, identification and characterization of bacterial consortia with the ability to degrade PHCs while enhancing plant growth. The exploitation of such carefully selected PGPR-inoculants would improve the tolerance of plants to PHCs toxicity, stimulate biomass production, and enhance rhizoremediation efficiency of PHCs-contaminated soils (Eze et al., 2022).

We have to keep in mind that for successful PGPR-assisted phytoremediation, it is also preferable to use bacterial strains indigenous to the site. The application of non-native microbial inoculants might become invaders and serious competitors against resident microbes. Furthermore, the use of non-native microbes is usually unacceptable or non-recommended by government regulatory agencies (Xia et al., 2020).

The role of PGPR in association with plants that spontaneously grow in heavily PHC-contaminated areas has not been widely explored (Alotaibi et al., 2021b; Eze et al., 2022). Therefore, the overall goal of this thesis was to generate a structurally and functionally diverse culture collection of PGPR and PHCs-degrading bacteria isolated from the rhizosphere of *Salix* and *Eleocharis* 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.

The main objectives of my PhD project were to:

1- isolate and identify bacterial strains from the rhizosphere of *Salix* and *Eleocharis* plants growing in a PHC-contaminated site,

2- assess the abilities of bacterial isolates to grow in the presence of alkanes and polycyclic aromatic hydrocarbons as the sole carbon source, as well as to characterize their PGP traits;

3- select, characterize and evaluate plant-growth promotion potentials of selected PGPR isolates showing degradative capabilities, and

4- sequence the genome of *Nocardia* sp. strain WB46, a promising novel isolate with multiple PGP and alkanes degradation characteristics.

1.1 Organization of the Thesis

The following research thesis is presented in manuscript format. The thesis incudes an introduction (Chapter 1), literature review (Chapter 2), followed by three research studies (Chapters 3, 4 and 5), an overall general discussion and conclusion (Chapter 6) and future research directions (Chapter 7). The main goal of Chapter 3 was to generate a structurally and functionally diverse bacterial culture collection from the rhizosphere of plants growing in a soil highly contaminated with PHCs. Chapter 4 provides an in-depth selection, characterization and evaluation of plant-growth promotion potentials of selected PGPR with degradative capabilities. Chapter 5 describes the draft genome sequence of *Nocardia* sp. strain WB46, a promising novel isolate with multiple PGP and alkanes degradation characteristics. Finally, Chapters 6 and 7 include a synthesis of major findings of this thesis and suggestions for future research directions.

Chapter Two: Overview of Approaches to Improve Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils

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This chapter was published in:

Fahad Alotaibi, Mohamed Hijri and Marc St-Arnaud. 2021. Overview of Approaches to Improve Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils. **Applied Microbiology**. 2021, 1, 329-351. (https://doi.org/10.3390/applmicrobiol1020023).

Author Contributions: Writing, F.A.; M.H. and M.S.-A. supervised the work, and edited, and revised the manuscript draft. All authors have read and agreed to the published version of the manuscript.

2.1 Abstract: Soil contamination with petroleum hydrocarbons (PHCs) has become a global concern and has resulted from the intensification of industrial activities. This has created a serious environmental issue; therefore, there is a need to find solutions, including application of efficient remediation technologies or improvement of current techniques. Rhizoremediation is a green technology that has received global attention as a cost-effective and possibly efficient remediation technique for PHC-polluted soil. Rhizoremediation refers to the use of plants and their associated microbiota to clean up contaminated soils, where plant roots stimulate soil microbes to mineralize organic contaminants to H_2O and CO_2. However, this multipartite interaction is complicated because many biotic and abiotic factors can influence microbial processes in the soil, making the efficiency of rhizoremediation unpredictable. This review reports the current knowledge of rhizoremediation approaches that can accelerate the remediation of PHC-contaminated soil. Recent approaches discussed in this review include (1) selecting plants with desired characteristics suitable for rhizoremediation; (2) exploiting and manipulating the plant microbiome by using inoculants containing plant growth-promoting rhizobacteria (PGPR) or hydrocarbon-degrading microbes, or a combination of both types of organisms; (3) enhancing the understanding of how the host–plant assembles a beneficial microbiome, and how it functions, under pollutant stress. A better understanding of plant–microbiome interactions could lead to successful use of rhizoremediation for PHC-contaminated soil in the future.

Keywords: phytoremediation; PGPR; hydrocarbon-degrading bacteria; Salix; contaminated soils; alkanes; PAHs.

2.2 Introduction

Industrial activities, including mining and extraction of oil and gas, as well as chemical inputs into agricultural production systems, have led to different degrees of environmental contamination worldwide. Petroleum hydrocarbons (PHCs) are among the major pollutants that can pose a serious environmental threat. PHC products have adversely affected various ecosystems, causing disturbing damage to natural habitats with serious economic consequences (Brzeszcz and Kaszycki, 2018).

PHCs are heterogeneous organic mixtures composed of carbon and hydrogen atoms arranged in varying structural configurations and have different physical and chemical properties (Gkorezis et al., 2016). These compounds consist mainly of hydrocarbons and fewer numbers of other non-hydrocarbon constituents, such as nitrogen, oxygen, and sulfur (Farrell-Jones, 2003; Wang, 2006). They are broadly classified into two major fractions: aliphatic hydrocarbons and aromatic hydrocarbons (Figure 1). Prior to processing, PHCs are composed, on average, of ~57% aliphatic hydrocarbons, ~29% aromatic hydrocarbons, and ~14% asphaltenes and other polar compounds containing nitrogen, oxygen, and sulfur (Tissot and Welte, 2013). Aliphatic hydrocarbons include both linear or branched-chain hydrocarbons, which may be unsaturated (alkenes and alkynes) or saturated (alkanes) (Pandey et al., 2016). Aromatic hydrocarbons include monocyclic (i.e., benzene, toluene, phenol, etc.) and polycyclic aromatic hydrocarbons (PAHs) (Figure 2.1). PHCs are the most common pollutants in soil and ground water worldwide. The ever-increasing dependency of modern society on fuel for energy generation in many vital sectors, such as electricity, heat, industry, and transportation has resulted in the extensive exploitation of PHCs (Gkorezis et al., 2016). Although environmental transition actions have been taken in many countries, dependency on petroleum will last for some decades, contributing to organic pollution risks.

Soil contamination with PHCs is an international issue, and the magnitude of soil pollution is hard to quantify. For example, in Australia, around 80,000 sites are estimated to be contaminated by PHCs (Hoang et al., 2021), whereas in Canada around 22,000 federal-owned sites are identified as being contaminated by PHCs (Secretariat, 2015). In Europe, PHC contamination was observed in at least 342,000 sites

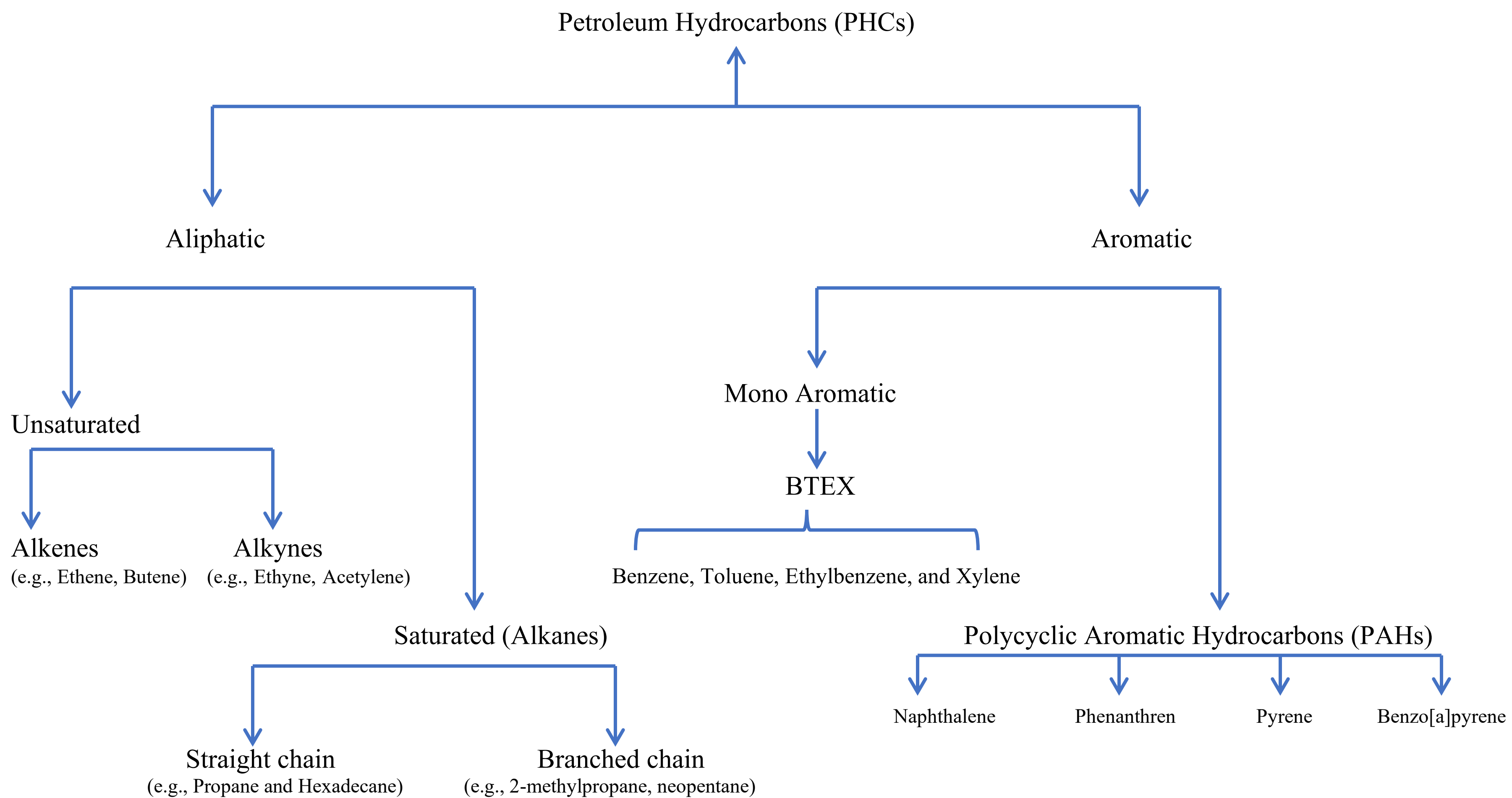

Figure 2.1 Schematic diagram showing the classification of petroleum hydrocarbon (PHCs).

(Panagos et al., 2013). These organic contaminants also pose serious health risks to humans and other organisms in addition to their adverse impact on the soil microflora, leading to environmental quality degradation. For instance, some aromatic substances, such as BTEX and PAHs, are notorious mutagens and carcinogens that can enter our food chain together with lipophilic compounds (Henner et al., 1997), and they have been linked with probable causes of bladder, kidney, liver, lung, and skin cancers. This explains the growing concern with these contaminants and the urgent need to use all possible means to protect the environment and to find the appropriate technique to remediate polluted soils.

Various chemical, physical, and thermal conventional techniques have been used to remediate soils contaminated with PHCs. These conventional methods, which can contain, destroy, or separate the pollutants, include a wide range of both *in situ* and *ex situ* cleanup technologies, such as asphalt batching, biopiles, chemical oxidation, excavation, hydrolysis, incineration, photolysis, pump and treat, multi-phased slurry reactors, soil vapor extraction, soil washing, and thermal desorption. However, these methods have particular limitations. First, their cost is often prohibitive; for example, it can cost between USD 480 and 813 per m^3 for extraction (Inoue and Katayama, 2011). Second, chemical procedures only work for specific organic compounds, and they most often destroy soil microbial communities. Third, these methods do not often result in a complete degradation of the pollutants (Yerushalmi et al., 1998; Gkorezis et al., 2016). Finally, PHC-contaminated soil contains numerous classes and types of toxic organic compounds, which make the choice of the proper method a challenging task. Hence, phytoremediation is a more recent and promising green-biotechnology that is perceived as an environmentally friendly, more cost-effective, and less destructive approach to cleanup contaminants in the environment.

2.3 Phytoremediation

Phytoremediation is a remediation technique that relies on the ability of plants and their associated microbiomes to accumulate, degrade, sequester, or stabilize harmful environmental contaminants (Salt et al., 1998; Pilon-Smits, 2005). Over the past two decades, the deployment of plants (and their associated microbiomes) to remediate a wide spectrum of inorganic and organic pollutants in soil and water environments has been carried out. This technique has been applied to remediate

various types of pollutants such as chlorinated solvents (Aken and Doty, 2009), explosives (Panz and Miksch, 2012), heavy metals (Leguizamo et al., 2017), landfill leachates (Jerez Ch and Romero, 2016), pesticides (Olette et al., 2008), PHC (Newman and Reynolds, 2004), radionuclides (Sharma et al., 2015), and salts (Devi et al., 2016). Although phytoremediation is still very much in its infancy, its application has been adopted by a growing number of companies. For example, the phytoremediation market has grown continuously at a rapid rate, with an estimated value of USD 32.2 billion in 2016 and is expected to reach USD 65.7 billion by 2025 (Transparency Market Research, 2020).

Phytoremediation is an innovative technique that has gained broad public acceptance, not only because it is an environmentally friendly approach but also as it requires less maintenance efforts, minimize site disturbance, and cost-effective process, which is powered by solar energy. However, phytoremediation still remains a marginal option for *in situ* soil remediation (Mench et al., 2010). As any other technique, phytoremediation has some limitations that affect its efficiency, performance, and time consuming. For example, phytoremediation efficiency varies with environmental conditions, such as soil physiochemical properties, contaminant level, and seasonal temperature fluctuations (Arthur et al., 2005; Pilon-Smits, 2005; Vangronsveld et al., 2009).

Phytoremediation efficiency is dependent on many factors, including plant selection (Wenzel, 2009), environmental parameters such as nutrient status, contaminant concentration, and bioavailability, soil pH, etc. (Vangronsveld et al., 2009), in addition to the composition and activity of plant associated microbiomes. Plants and their associated microbiomes facilitate pollutant uptake from the environment via different processes, including degradation, extraction, stabilization, transformation, and volatilization (Salt et al., 1998; Pilon-Smits, 2005). The type of plant and pollutants plus the environmental conditions are key factors for determining the way in which phytoremediation techniques can be applied. Generally, phytoremediation technologies are divided into five different categories (Table 2.1). The phytoremediation method suitable for petroleum hydrocarbon-contaminated soil is called rhizoremediation (Kuiper et al., 2004), which is defined as the breakdown of organic pollutants by using plants and their root-associated microbiomes.

Table 2.1 Phytoremediation mechanisms whereby plants remediate polluted soils.

Category	Mechanisms	Target Pollutants	Region of Activity	Reference
Phytoextraction	Uptake and concentrate contaminants	Metals (e.g., Cd,Ni), radionuclides (e.g., Pu)	Shoot tissue	(Ali et al., 2013; Sharma et al., 2015)
Phytostabilization	Immobilization and sequestration of contaminants	Primarily metals (e.g., Cu, Zn, Pb)	Root tissue	(Behera, 2014)
Phytotransformation	Enzymatic actions	Chlorinated solvents, ammonium waste, herbicides, monoaromatic hydrocarbons	Plant tissue	(Aken and Doty, 2009; Behera, 2014)
Phytovolatilization	Uptake and evatranspiration	Volatile organics (e.g., TCE, toluene, MTBE)	Shoot tissue	(Salt et al., 1998)
Rhizoremediation	Breakdown of organic pollutants by using plants and root-associated microbiomes	PHC (e.g., diesel), pesticides (e.g., dimethomorph)	Root	(Kuiper et al., 2004; Newman and Reynolds, 2004)

Rhizoremediation of PHCs is facilitated through a process known as the ‘rhizosphere effect’ (Anderson et al., 1993), in which plants exude a variety of organic compounds into their root-surrounding zone (the rhizosphere), resulting in an increase abundance and activity of certain rhizospheric microbes, which in turn can degrade or metabolize hydrocarbon contaminants (Martin et al., 2014). Understanding the plant–microbiome partnerships, and the underlying processes that govern and control PHC degradation, is a priority challenge in rhizoremediation research nowadays (Gkorezis et al., 2016; Thijs et al., 2016; Correa-García et al., 2018).

2.4 The Rhizosphere Microbiome

By definition, the rhizosphere refers to the narrow zone of nutrient-rich soil in close proximity to plant roots and influenced greatly by plant exudates (Philippot et al., 2013). The rhizosphere is a hot spot for a myriad of organisms, including algae, archaea, arthropods, bacteria, fungi, nematodes, protozoa, and viruses (Raaijmakers et al., 2009), and it has been estimated that one gram of fresh roots contains up to 10^{11} microbial cells representing more than 30,000 prokaryotic species (Berendsen et al., 2012). The structure of the rhizosphere microbiome depends on many factors such as soil type, environmental factors, the period of the year, plant development stage, and plant species and genotypes (Berg and Smalla, 2009). The rhizosphere microbiome is part of the larger root microbiome that also includes the rhizoplane microbiome and root interior microbiome (endophytes) (Bakker et al., 2013) (Figure 2.2). The rhizosphere microbiome is one of the most complex habitats on our planet, and microbial functions occurring within the rhizosphere have critical influences on plant growth and productivity, soil fertility, carbon sequestration, and degradation of environmental contaminants (Berendsen et al., 2012).

Plant roots exude a myriad of organic substances into the surrounding soil, comprising both low molecular weight organic compounds (amino acids, organic acids, sugars, phenolics, secondary metabolites, etc.) and high molecular weight organic compounds (polysaccharides, proteins, etc.). It has been estimated that 6–21% of photosynthetically fixed carbon in plants is released through root systems (Hoang et al., 2021). Therefore, root exudates are the major driver in shaping the rhizosphere microbiome. This countless and steady release of fixed carbon compounds into the

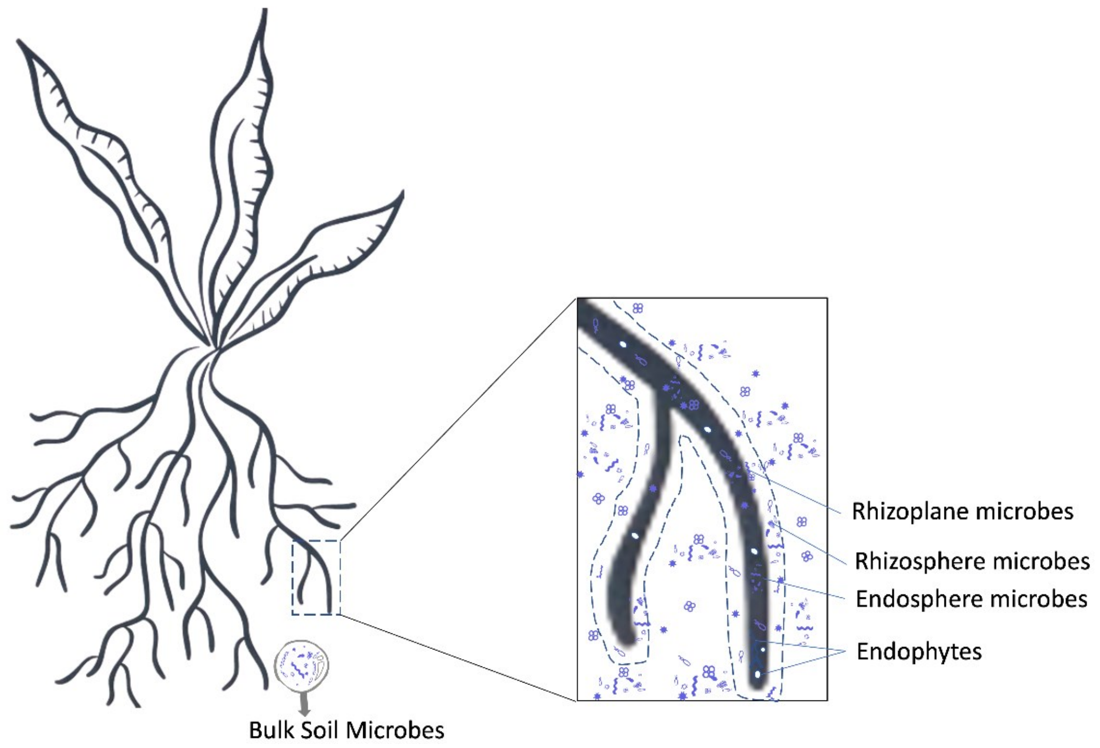

Figure 2.2 Model of the root microbiome.

rhizosphere, a process referred to as the rhizosphere effect, increases the activity and abundance of the rhizosphere microbial community compared to nearby bulk soil (Smalla et al., 2001; Kuzyakov and Blagodatskaya, 2015). The magnitude of bacterial density in the rhizosphere is 10 to 1000 times higher than that in adjacent bulk soil; however, microbial community diversity in the rhizosphere is generally lower than that of bulk soils since rhizodeposition selectively enhances specific microbial taxa (Berendsen et al., 2012; Loeppmann et al., 2016). Although recruitment of the rhizosphere microbiome by plants is strongly dependent on the structure and composition of the bulk soil microbiota (Bulgarelli et al., 2012), different plant genotypes were found to select for different rhizosphere microbiomes (Lundberg et al., 2012), inferring that differential recruitment of beneficial microbiomes is also dependent on the genetic variation across plant species (Bulgarelli et al., 2012; Lundberg et al., 2012).

In addition to shaping the microbial communities in the rhizosphere, root exudates have other functions that benefit the plant itself. Through root exudation, plants can change the soil physicochemical properties, contributing to nutrient assimilation, reducing the growth of competitor plant species, increasing the abundance of certain beneficial microbes, and regulating the microbiome composition in the rhizosphere (Vieira et al., 2020; Vives-Peris et al., 2020).

The important role of root exudation, secreted by plants growing in PHC-contaminated soils, as facilitators of hydrocarbon rhizoremediation has been recognized recently (Rohrbacher and St-Arnaud, 2016; Correa-García et al., 2018). This microbial process can function through different mechanisms. First, root exudates include degradable low molecular weight organic compounds such as carbohydrates, amino acids, and organic acids, all of which are readily available energy and nutrient sources for microbial utilization, stimulating the proliferation of microbial biomass and activities (Kuiper et al., 2004). For instance, the addition of sugar and amino acids into soils causes an instant response (within 1 h) in microbial respiration (Jones and Murphy, 2007). Additionally, compounds essential in plant nutrient acquisition secreted by roots, such as enzymes (e.g., acid phosphatases) and chelating agents (phytosiderophores), provide microbial communities in the rhizosphere with a source of nutrients (Rohrbacher and St-Arnaud, 2016). Second, plant root exudation can enhance PHC degradation by emitting a wide range of enzymes, such as cytochrome P450

monooxygenases, dehalogenases, dioxygenase, laccases, and peroxidases (Gao et al., 2011; Martin et al., 2014; Hoang et al., 2021). Plant-secreted enzymes play a key role in the oxidation of PHCs (Muratova et al., 2015), and the initial attack on the pollutant itself is primarily performed by soil microbial enzymes (Muratova et al., 2015). Third, secondary metabolites released by the plant roots, such as flavonoids and phenols, are analogous to many organic pollutants, thus increasing the abundance and activity of microbial communities equipped with genes relevant to degradation of organic pollutants within the rhizosphere, even in unpolluted soils (Yergeau et al., 2014). Fourth, root-released exudates have been shown to increase the availability of organic pollutants for microbial metabolisms (Martin et al., 2014). For example, (Gao et al., 2010) reported that the availability of phenanthrene and pyrene increased in the soil after the addition of citric acid and oxalic acid.

Considering the above-mentioned role of root exudates, the rhizosphere is hypothesized to be a suitable niche for rhizoremediation of PHC-contaminated soil (Correa-García et al., 2018). Additionally, the rhizosphere is one of the environmental niches that is conducive to horizontal gene transfer (HGT) (van Elsas and Bailey, 2002). HGT is a mechanism used by bacterial communities to adapt to the presence of organic contaminants in their environments (Top and Springael, 2003). Bacteria may acquire genetic information from either closely related or phylogenetically distinct taxa in the community by HGT via different routes, such as plasmids and transposons (Top and Springael, 2003). Several studies have reported that plasmids were shown to help bacterial communities adapt to environmental pollution stress (Top and Springael, 2003; Sentchilo et al., 2013).

To overcome the limitations and improve the efficiency of rhizoremediation, current research trends focus on several auxiliary strategies, such as (1) selecting plants with desired characteristics suitable for rhizoremediation (such as increased contaminant tolerance or production of vigorous root system and shoot biomass (Wenzel, 2009), but also abilities to form symbiotic interactions with microorganisms); (2) exploiting and manipulating the plant microbiome by using inoculants containing plant growth-promoting rhizobacteria (PGPR) or hydrocarbon-degrading microbes or their combination (Correa-García et al., 2018); and (3) enhancing the understanding of the mechanisms through which host plants assemble a beneficial microbiome, and how it functions, under pollutant stress (Thijs et al., 2016).

2.5 Plant Selection

Since the beginning of phytoremediation research, many plant species have been tested for their potential to enhance rhizoremediation of PHCs (Khan et al., 2013). Plants enhance the degradation of PHCs principally by the unique properties of the plant itself and by providing optimal conditions for microbial proliferation in the rhizosphere (Chaudhry et al., 2005). In general, selection of plants suitable for rhizoremediation of PHCs should be based on the following criteria: tolerance to a broad range of PHCs, speed of growth, root morphology, ability to grow in many soil types, and the root exudate profile (Aprill and Sims, 1990; Chaudhry et al., 2005; Gaskin and Bentham, 2010). Additionally, plants should not be selected based solely on the contaminant uptake efficiency; their ability to stimulate microbial activity and abundance also should be considered (Reynolds et al., 1999; Hall et al., 2011).

Plants that have been used thus far in rhizoremediation span a wide range of families. Grasses (annual ryegrass, tall fescue) and other herbaceous crops (Indian mustard, sunflower), legumes (alfalfa, clover), and woody trees (hybrid poplars, willows), among others, have shown a high potential in the rhizoremediation of soil contaminated with PHCs (Frick et al., 1999; Hall et al., 2011).

Grasses have been studied extensively regarding their potential to facilitate the rhizoremediation of PHC-impacted soil (Gaskin and Bentham, 2010). Grasses are often chosen for rhizoremediation applications because of their fast growth, high tolerance to PHCs, extensive fibrous root systems, large root surface area, and deeper root penetration into the soil matrix to depths of up to 3 m (Aprill and Sims, 1990; Frick et al., 1999). These unique characteristics of grass root systems allow microbial colonization and establishment of abundant microbial populations. For example, bacterial populations found in the rhizosphere of goosegrass (*Eleusine indica*) cultivated in PHC contaminated soil were 72 times more abundant than bacterial populations observed in the nearby uncultivated soil (Lu et al., 2010).

Legumes have also been tested for their potential to enhance rhizoremediation of PHC contaminated soil (Hall et al., 2011). The remarkable ability of legumes to form symbiotic relationships with the N-fixing rhizobia is of great importance in PHC-contaminated soil, which is characterized by low nutrient availability and high C/N ratio (Frick et al., 1999). In addition, some legumes species such as alfalfa have a deep-

rooting system that can penetrate highly compacted soil layers and create soil macropore spaces, thus increasing oxygenation of the soil matrix and, consequently, promoting microbial degradation (Hall et al., 2011).

Trees, such as willows (*Salix*), have also gained attention regarding their potential to improve rhizoremediation of PHC-polluted soil (Gkorezis et al., 2016). Willows are attractive for rhizoremediation of PHCs because they are easy to propagate, exhibit extremely fast growth in low-fertility soils, have high tolerance to several stressful environments, produce large biomass, and generate widespread deep-rooting systems (Kuzovkina and Volk, 2009). Additionally, the large diversity of willows (~350–500 genetically distinct species), with a wide range of tolerance to various environmental conditions, facilitates selection of the most appropriate species suitable for a particular environment (Kuzovkina and Volk, 2009). Compelling evidence has been reported about the use of willows for the rhizoremediation of soils polluted with organic contaminants, including PHCs (de Cárcer et al., 2007; Bell et al., 2014a; Yergeau et al., 2018).

More recently, promising approaches including the screening and identification of native plants grown on PHC-contaminated soil have been used. For example, Pérez-(Pérez-Jaramillo et al., 2016) proposed a "back to the roots" framework that involves surveying indigenous plants and associated microbiomes, and their native habitats, to identify plants and microbial traits with the goal to restore associations that may have been diluted during plant domestication (Pérez-Jaramillo et al., 2016). In fact, using native plant species in rhizoremediation offers many advantages over non-native species, including minimizing the potential of introducing alien species that can became invasive and disturb local flora and fauna (Timmis and Pieper, 1999), in addition to the fact that indigenous plants are more genetically diverse and more adapted to a wide range of climatic conditions compared to other plants currently chosen for rhizoremediation (Brown, 1976). Following this approach, (Desjardins et al., 2014) described plants growing spontaneously in highly petroleum-contaminated decantation basins of a former petrochemical plant in Varennes (southern Québec, Canada) and identified three plants species (*Alisma triviale*, *Eleocharis obtusa*, and *Panicum capillare*) that were tolerant to PAHs and PHCs. Additionally, (Lee et al., 2020) studied the diversity of arbuscular mycorrhizal fungal communities of native plant species grown in highly PHC-contaminated soil and identified *Rhizophagus* as a key PHC-

tolerant genus. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts with approximately 80% of plant species on earth (Brundrett, 1991). In agricultural settings, AMF are known for their plant growth-promoting effects including improved plant uptake of mineral nutrients, in particular phosphorus (Roy-Bolduc and Hijri, 2011). Additionally, AMF were shown to enhance plant tolerance toward several biotic and abiotic stresses such as nutrient deficiencies, plant pathogens, drought, salinity, and contaminants (St-Arnaud, 2007; Smith and Read, 2008; Miransari, 2011; Roy-Bolduc and Hijri, 2011; Porcel et al., 2012). Therefore, AMF have more recently gained attention regarding their use in phytoremediation of soils contaminated with different pollutants, such as heavy metals and PHCs (Hassan et al., 2013; Hassan Sel et al., 2014; Lee et al., 2020).

Sequestration and transportation of contaminants inside plant tissues enable plants to remediate PHC-polluted soil (Sandermann, 1992; Reichenauer and Germida, 2008). Plants can also degrade or transform organic pollutants into less toxic forms via their enzymatic machinery, or synthesizing a variety of defensive proteins and metabolites (Pilon-Smits, 2005; Singer, 2006). Therefore, plants can adapt and confront many unfavorable stressful conditions, such as PHC contamination. However, plant growth has been retarded under highly stressed conditions, e.g., PHC pollutants are expected to be lower than those under optimal conditions (Glick and Stearns, 2011). Therefore, plant growth may be positively enhanced by the presence of plant growth-promoting rhizobacteria (PGPR) that are able to alleviate stresses in plants via many mechanisms, such as reducing soil nutrient deficiencies (fixing nitrogen, solubilizing phosphorus, and enhancing iron uptake), synthesizing plant hormones, suppressing ethylene production via 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Hardoim et al., 2008; Glick and Stearns, 2011), and degrading a broad range of PHCs (Gkorezis et al., 2016).

2.6 Exploiting and Manipulating the Plant Microbiome through Inoculation

2.6.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Bacteria are the predominant group within the soil microbiome community. It has been estimated that one gram of soil contains around 10^8–10^9 bacterial cells (Rughöft et al., 2016) representing tens of thousands of different species (Berendsen et al., 2012). The capacity of bacteria to utilize a wide range of many compounds as nutrient and energy sources, and their diverse metabolism, make them ideal associates

in plant–microbiome partnerships (Berendsen et al., 2012). Among bacterial communities dwelling in the rhizosphere are PGPR. PGPR are free-living and beneficial soil-borne bacteria associated with the root microbiome, enhancing plant growth and development by direct and indirect means (Vessey, 2003; Richardson et al., 2009; Schlaeppi and Bulgarelli, 2015). The direct means by which PGPR may promote plant growth occur through fixing atmospheric N, increasing nutrient acquisition such as phosphorous, stimulating plant growth by producing different phytohormones, sequestration of iron by synthesis of siderophores, and alleviating stresses in plants by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Vessey, 2003; Schlaeppi and Bulgarelli, 2015). Indirect means of growth stimulation occur through biocontrol activities of PGPR against many plant phytopathogens via different mechanisms, including production of antimicrobial metabolites such as siderophores, antibiotics, and bacteriocins as well as induced systemic resistance (ISR) in plants (Bloemberg and Lugtenberg, 2001; Haas and Défago, 2005)[90,91].

A plethora of bacterial genera, such as *Acetobacter*, *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Flavobacterium*, *Enterobacter*, *Erwinia*, *Herbaspirillum*, *Klebsiella*, *Micrococcus*, *Paenibacillus*, *Rhizobium*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, *Variovorax*, and *Xanthomonas* have been shown to stimulate plant growth and development (Vessey, 2003; Schlaeppi and Bulgarelli, 2015). These phylogenetically diverse bacterial group have wide spectrum plant growth-promoting capabilities, and they can be categorized as biocontrol, biofertilizer, and phytostimulation agents (Vessey, 2003; Haas and Défago, 2005; Lugtenberg and Kamilova, 2009).

2.6.2 Enhanced Nutrient Acquisition (Biofertilizer)

A major mechanism used by PGPR to stimulate crop growth and development is biofertilization. Several mineral nutrients, such as nitrogen, phosphorus, and iron can be limited in the soil, thus limiting plant growth and development (Lugtenberg and Kamilova, 2009). Nitrogen is the most liming factor for crop growth, although the geosphere contains 1.6×10^{17} t, most of which is found in the atmosphere with an estimated 3.86×10^{15} t (Stevens, 2019). Nitrogen (N_2) represents around 78% of the atmosphere, and it is inaccessible to all plants and other eukaryotic life. Biological nitrogen fixation (BNF) is a process carried out by a few adapted prokaryotic

diazotroph, that possess the enzyme nitrogenase, which catalyzes the reduction of N_2 to ammonia, a form of N utilized by plants (Boddey et al., 1995). Diazotrophic bacteria can be classified according to the degree of intimacy with plants: symbiotic N-fixing bacteria, such as rhizobia, associative N-fixing bacteria, such as *Azospirillum* spp., and free-living N-fixing bacteria, such as *Azotobacter* spp. (Vessey, 2003). The efficiency and significant contribution of BNF from PGPR is well documented for several crops such as legumes, sugarcane, and grasses (Boddey et al., 1995; Sessitsch et al., 2002; Dobbelaere et al., 2003).

The other major nutrient limiting plant growth is phosphorus (P). Although soil often has abundant quantities of P (~0.05% w/w), only a small fraction of this P (~0.1%) is readily available for plant uptake (Stevenson and Cole, 1999; Alori et al., 2017). Low availability of P in soils is due to the fact that the majority of soil-bounded P is present in insoluble form (Stevenson and Cole, 1999). Plants can take-up P in two soluble forms, either as monobasic (H2PO4 −) or dibasic (HPO4 2−) ions (Glass, 1989). A subset of bacteria, known as phosphate solubilizing bacteria (PSB), can influence the availability of P (Kim et al., 1997; Rodríguez and Fraga, 1999). PSB are commonly found in the rhizosphere of plants and encompass genera such as *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Paenibacillus*, *Pseudomonas*, and *Serratia* (Richardson et al., 2009; Alori et al., 2017). These PSB can solubilize insoluble forms of P to plant-available forms through different mechanisms, such as the secretion of organic acids, siderophores, protons, hydroxyl ions, and CO2, as in the case of inorganic P solubilization (Rodríguez and Fraga, 1999; Sharma et al., 2013). These bacteria also produce many extracellular enzymes, such as acid and alkaline phosphatases and phytases that stimulate organic P mineralization (Kim et al., 1997; Rodríguez and Fraga, 1999; Jorquera et al., 2011).

Iron is another essential plant nutrient that plays a key role in plant growth and development. Despite its abundancy in the soil, most of iron is present in insoluble forms, mainly as ferric hydroxide (Zhang et al., 2019). Plant roots prefer to take-up the reduced form of iron, the ferrous (Fe^{+2}) ion compared to the ferric (Fe^{+3}) ion (Vessey, 2003). Siderophores are low-molecular-weight iron-chelating agents that are produced by many soil bacteria and fungi under stressed low iron conditions (Crowley and Kraemer, 2007). Bacterial produced-siderophores can enhance plant growth by enhancing plant iron nutrition through binding Fe+3 and render it available for

reduction to Fe^{+2} (Vessey, 2003; Lugtenberg and Kamilova, 2009). Apart from improving plant iron nutrition, siderophores also stimulate plant growth indirectly via suppressing plant pathogen activities in the rhizosphere by depriving pathogens of Fe^{+2} required for their cellular growth and development, thus lowering the probability of plant disease (Haas and Défago, 2005). Additionally, siderophore-producing bacteria were shown to play an important role in enhancing plant growth in heavy metal-contaminated soils by alleviating heavy metal toxicity (Rajkumar et al., 2010; Sessitsch et al., 2013). Several reports indicated that microbial siderophores bind and form stable compounds with other heavy metals such as Al, Cd, Cu, Pb, and Zn (Rajkumar et al., 2010; Gururani et al., 2013; Sessitsch et al., 2013).

In addition to pseudomonads, which synthesize high-affinity Fe^{3+}-binding siderophores (Sharma et al., 2003), several other PGPR are capable of producing siderophores including *Azospirillum* spp., *Azotobacter* spp., *Bacillus* spp., *Klebsiella* spp., *Nocardia* spp., *Paenibacillus* spp., *Pantoea* spp., *Serratia* spp., and *Streptomyces* spp. (Vessey, 2003; Crowley and Kraemer, 2007).

The role of PGPR in solubilizing and oxidizing other essential plant nutrients, such as potassium, sulfur, and micronutrients, and their effects on plant growth-promotion are far less studied compared to N, P, and Fe. For more information regarding the role of PGPR in providing these elements to plants, the reader can consult recent publications on this topic (dos Santos et al., 2020; Mitter et al., 2021).

2.6.3 Plant Growth Regulation (Phytostimulation)

Other direct modes of action employed by PGPR to stimulate plant growth and development is through production of phytohormones (Lugtenberg and Kamilova, 2009). Various PGPR are known to synthesize different classes of phytohormones, including abscisic acid (ABA), auxins, ethylene, gibberellins, and cytokinins (Vessey, 2003; Lugtenberg and Kamilova, 2009). These phytohormones are responsible for many processes in plants during their different development stages. Auxins, for example, are responsible for cell enlargement and cell division, root initiation, increased fruit development, and leaf senescence (McSteen, 2010). Cytokinins on the other hand play a major role in the promotion of cell division and root hair formation, initiation, and expansion of shoots and other plant parts, and decreased root growth (Salisbury, 1994). Phytohormone gibberellins take part in regulating seed dormancy and germination, speeding up fruit and flowering processes, and modifying plant

morphology, particularly stem elongation (Martínez et al., 2018; Shu et al., 2018). When produced at low concentration, the plant growth regulator ethylene is involved in many plant growth stages including stimulation of seed germination, formation and elongation of roots, and fruit and leaf maturation (Abts et al., 2017). Finally, the phytohormone ABA plays main roles in seed development and maturation and mediating stomatal opening (Shu et al., 2018). The most studied phytohormone, to date, produced by PGPR is auxin indole-3-acetic acid (IAA), which is involved in enhancing root growth and root length as well as formation and proliferation of lateral root hairs (McSteen, 2010). IAA-synthesizing PGPR include bacterial genera such as *Aeromonas*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bradyrhizobium*, *Comamonas*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Rhizobium*, and *Pseudomonas* (Patten and Glick, 1996; Vessey, 2003; Weyens et al., 2009).

2.6.4 Reduction of Plant Ethylene (Stress Alleviating)

Ethylene production by plants at low concentrations can be beneficial, as mentioned above. However, when produced at high concentrations, it can stunt plant growth and development by inhibiting root growth (Vacheron et al., 2013). In response to various biotic and abiotic stressor conditions, plants synthesize different enzymes, metabolites, and stress proteins to alleviate the adverse effects of stress (Li et al., 2012); of particular interest is ethylene. Once plants encounter stress, such as flooding, drought, or presence of toxic compounds, plant growth is inhibited because the ethylene precursor, 1-aminocyclopropane-1-carboxylate, is induced (Li et al., 2005). However, certain PGPR can hinder ethylene biosynthesis via production of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) that cleaves the ethylene precursor ACC into alpha-ketobutyrate and ammonia (Glick, 2005), thus balancing ethylene levels and reducing its adverse impact on plant growth (Glick, 2005).

The beneficial roles of PGPR-containing ACCD have been studied in plants grown under different stress conditions, such as drought (Sandhya et al., 2010), waterlogging (Ali and Kim, 2018), high salinity (Mayak et al., 2004), and heavy metal contamination (Belimov et al., 2001). Several PGPR are known for their production of ACCD such as *Achromobacter* spp., *Azotobacter* spp., *Bacillus* spp., *Enterobacter* spp. *Herbaspirillum* spp., *Ochrobactrum* spp., *Pseudomonas* spp., and *Serratia* spp. (Belimov et al., 2001; Mayak et al., 2004; Glick, 2005; Sandhya et al., 2010; Ali and Kim, 2018).

2.6.5 Plant Growth-Promoting Rhizobacteria-Assisted Phytoremediation

Over the last few decades, the immense interest in exploiting PGPR as a biofertilizer or biocontrol agent in agriculture has resulted in the development of successful commercial inoculants in many parts of the world, including Canada, Europe, and the United States (Bashan et al., 2014; Owen et al., 2015; Backer et al., 2018). Considerable research investigations have been conducted over the last decade to utilize PGPR in bioremediation of heavy metal-contaminated soils by promoting plant growth and also ameliorating the phytostabilization or phytoextraction efficiency (Khan et al., 2009; Ma et al., 2011; Oleńska et al., 2020). However, the utilization of PGPR in rhizoremediation of PHC contaminated soil is new and represents a large, untapped potential (Gkorezis et al., 2016; Correa-García et al., 2018). Recent reports of PGPR-assisted phytoremediation of PHC-contaminated soil and its host plants are summarized in Table 2.2.

Table 2.2 Example of the use of PGPR in rhizoremediation of PHC-contaminated soil.

Plant	Contaminants	Conditions	Bacteria	Role of PGPR	Reference
Lolium perenne	Diesel	Greenhouse	*Pantoea* sp. BTRH79	ACCD	(Arslan et al., 2014)
Cytisus striatus	Diesel	Greenhouse	*Bradyrhizobium* sp. ER33	IAA, organic acids	(Balseiro-Romero et al., 2017b)
Lupinus luteus	Diesel	Greenhouse	*Streptomyces* sp. RP92	IAA, siderophore, organic acid	(Balseiro-Romero et al., 2017b)
Trifolium repens	Oil refinery sludge	Field trial	*Psudomonas* putida BIRD-1	P-solub, IAA, siderophore	(Pizarro-Tobías et al., 2015)
Festuca arundinacea	Aliphatic hydrocarbons	Field trial	PGPR consortia	N/A	(Hou et al., 2015)
Lolium perenne	Aged PHCs	Greenhouse	*Rhodococcus erythropolis* CDEL254	Several PGP traits	(Natalia Ptaszek, 2020)
Lolium perenne	Aged PHCs	Greenhouse	*Rhodococcus erythropolis* CD 106	Several PGP traits	(Tomasz Płociniczak, 2017)

2.7 Hydrocarbon-Degrading Bacteria

2.7.1 Ecology and Diversity of PHC-Degrading Bacteria

The fate of most PHCs encountered in the terrestrial environment is degradation and/or biotransformation by soil bacteria. These bacteria are heterotroph thus, they utilize PHC compounds as nutrient and energy sources for their cellular growth and development. PHC-degrading bacteria are widespread in nature and have been found in Arctic and Antarctic soils (Whyte et al., 2002a), aquatic environments (Yakimov et al., 2007), and pristine environments (Afzal et al., 2013). The abundance of PHCs in the environment maintains the degradation potential within most bacterial communities (Johnsen and Karlson, 2005). Interestingly, certain obligate hydrocarbonoclastic bacteria (OHCB) such as *Alcanivorax* spp., *Cycloclasticus* spp., *Marinobacter* spp., *Oleispira* spp. *Planomicrobium* spp., and *Thalassolituus* spp. are found undetectable or in low abundance in unpolluted environments; however, they prevail after PHC pollution occurs (Yakimov et al., 2007; Tremblay et al., 2017; Xu et al., 2018).

Over the last few decades, many bacterial species have been isolated and identified from various terrestrial and aquatic environments (Yakimov et al., 2007; Tremblay et al., 2017; Varjani, 2017). Some of these bacteria can utilize a wide spectrum of PHC compounds; for example, the bacterial strain *Dietzia* sp. DQ12-45-1b could grow on many n-alkanes (C6–C40) and other monoaromatic and polyaromatic hydrocarbons as the sole carbon source and energy (Wang et al., 2011). To date, more than 79 bacterial genera that can degrade PHCs have been isolated and identified (Tremblay et al., 2017; Xu et al., 2018), such as *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Dietzia*, *Enterobacter*, *Kocuria*, *Marinobacter*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Streptomyces*, and *Variovorax* (Sarkar et al., 2017; Varjani, 2017; Xu et al., 2018). As different bacteria vary in their catalytic enzyme activity, no single bacterial species can break down the entire PHC fraction completely (Varjani, 2017; Xu et al., 2018); therefore, their effectiveness in remediating PHC-polluted sites also varies widely (Xu et al., 2018).

2.7.2 Alkane-Degrading Bacteria

Alkanes are saturated hydrocarbons that can be further classified as branched (isoalkanes), cyclic (cyclo-alkanes), or linear (n-alkanes) (Rojo, 2009). Although many living organisms, such as bacteria, plants, and green algae produce alkanes (Post-Beittenmiller, 1996; Schirmer et al., 2010), the main source of alkanes in terrestrial

environments comes from PHC contamination, as alkanes are the main constituent of crude oil and natural gas (Rojo, 2009; Ji et al., 2013a). Bacterial alkane degradation is of great significance for the bioremediation of PHC-contaminated soil as well as for microbial enhanced oil recovery (Nie et al., 2014b). Bacteria metabolize alkanes under both aerobic and anaerobic conditions (Rojo, 2009). Most bacteria degrade alkanes aerobically; therefore, aerobic degradation will be discussed hereafter.

Aerobic degradation of alkanes starts with terminal or sub-terminal incorporation of oxygen atoms (O2) into the hydrocarbon substrate by an alkane hydroxylase enzyme (Ji et al., 2013a). Alkane hydroxylases (AHs) are a class of several specific enzymes that insert O2 into the hydrocarbons to initiate degradation (van Beilen and Funhoff, 2007). Depending on the chain length of the alkane substrate, there are different enzyme classes that carry out the oxygenation of hydrocarbons (van Beilen and Funhoff, 2007) (Table 3). For example, bacteria degrading short-chain alkanes (C2–C4) have enzymes related to methane monooxygenases, while bacterial strains degrading medium-chain alkanes (C5–C20) usually contain alkane 1-monooxygenase and soluble cytochrome P450 enzymes, and bacterial strains degrading long-chain alkanes (>C20) contain several recently discovered types of AHs, such as flavin-binding monooxygenase and thermophilic flavin-dependent monooxygenase (van Beilen and Funhoff, 2007; Rojo, 2009; Ji et al., 2013a; Wang and Shao, 2013) (Table 2.3).

Among the above-mentioned (AHs) enzyme systems, alkane 1-monooxygenase (encoded by alkB) is the most common found in alkanes degrading α-, β-, and γ-Proteobacteria and high G+C content Gram-positive bacteria (van Beilen and Funhoff, 2007; Wang and Shao, 2013). The substrates for AlkB-harboring bacteria comprise alkanes ranging from C10 to C16 (van Beilen and Funhoff, 2007); however, some AlkB-harboring Actinobacteria, such as *Dietzia* sp. and *Gordonia* sp., can degrade alkanes with chain lengths up to C32 (Bihari et al., 2011; Lo Piccolo et al., 2011). Another bacterial AH enzyme system for degradation of short- and medium-chain substrates is cytochrome P450 hydroxylase of the CYP153 family, which is frequently found in alkane-degrading bacteria lacking the AlkB enzyme (van Beilen et al., 2006; Nie et al., 2014c). It is common that bacterial strains contain more than one alkB homologous gene, as in the case of *Rhodococcus* strain Q15, which contains at least

Table 2.3 Examples of alkane-degrading genes, enzymes, and their bacterial sources.

Enzyme class	Substrate Range	Gene	Bacterial species
Soluble methane Monooxygenase	C1-C8	*mmoX*	*Gordonia, Methylococcus, Methylosinus, Methylocystis, Methylomonas, Methylocella*
Particulate methane Monooxygenase	C1-C5	*pmoC*	*Methylococcus, Methylosinus, Methylocystis, Methylobacter, Methylomonas, Methylomicrobium, Nocardioides*
Alkane 1-monooxygenase	C10-C20	*alkB*	*Acinetobacter, Alcanivorax, Burkholderia, Mycobacterium, Pseudomonas, Rhodococcus*
Soluble cytochrome P450	C5-C16	*CYP153*	*Acinetobacter, Alcanivorax, Caulobacter, Mycobacterium, Rhodococcus, Sphingomonas*
Flavin-binding monooxygenase	C20-C36	*Alma*	*Alcanivorax, Marinobacter, Acinetobacter*
Thermophilic flavin-dependent monooxygenase	C10-C36	*LadA*	*Geobacillus thermodenitrificans NG80-2*

Adapted and modified from (van Beilen and Funhoff, 2007; Rojo, 2009; Ji et al., 2013a; Wang and Shao, 2013).

four alkane 1-monooxygenases (Whyte et al., 2002b). Additionally, several bacterial strains have more than one AH system, as has been shown in *Dietzia* sp. strain DQ12-45-1b, which has AlkB and CYP153 systems co-existing together (Nie et al., 2014c). The co-existence of more than one AH system in bacteria can expand its ability to degrade a wider alkane range (van Beilen et al., 2006; Nie et al., 2014c). AlkB and CYP153 genes are commonly assessed to determine the degradation potential of bacterial communities in PHC-impacted soil and water environments (Wang et al., 2010a; Long et al., 2017).

2.7.3 Polycyclic Aromatic Hydrocarbon-Degrading Bacteria

The other major fraction of PHCs is polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous in nature. They have two or more aromatic benzene rings in their structure (Varjani et al., 2017). PAHs are found in nature as a byproduct of many biogeochemical and biological processes as well as incomplete combustion of woods, coal, and gasoline (Pe´rez-Pantoja, 2010). However, the main entry source of PAHs in the environment is industrial activities related to the petroleum and gas industry (Pe´rez-Pantoja, 2010; Varjani et al., 2017). Due to their electrochemical stability, high persistence in terrestrial environments, bio-accumulative behaviors, and their "multi-faceted disease-causing" effects (carcinogenic, mutagenic, teratogenic), the United States Environmental Protection Agency (U.S. EPA), as well as agencies in many other countries, has listed 16 PAH compounds as priority pollutants (Varjani et al., 2017; Kotoky et al., 2018).

The main principle of PAH biodegradation, mediated by aerobic bacteria, involves activation and subsequent cleavage of the thermodynamically stable benzene ring in PAH substrates (Pe´rez-Pantoja, 2010). Under aerobic conditions, the initial step is the hydroxylation of the benzene ring by dioxygenase enzymes, resulting in the formation of cis-dihydrodiols. After this step, cis-dihydrodiols are further dehydrogenated, via the action of dehydrogenase enzymes, to form several dihydroxylated intermediates. Subsequently, these diol intermediates are cleaved by intradiol or extradiol ring-cleaving dioxygenase enzymes, leading to the formation of central intermediates, such as protocatechuates and catechols that can be further metabolized to acetone, succinate, or pyruvate, which then enter the tricarboxylic acid cycle (TCA) (Cerniglia, 1992; Peng et al., 2008b; Mallick et al., 2011; Ghosal et al., 2016).

PAHs are broadly classified into low-molecular-weight (LMW PAHs) compounds with fewer than three rings (3 rings) (Pe´rez-Pantoja, 2010). LMW PAHs such as naphthalene, phenanthrene, anthracene, fluorene, acenaphthene, and acenaphthylene are found in high quantities in PHC-contaminated sites, and diverse bacterial communities have the ability to utilize these LMW PAHs as the sole carbon and energy source (Mallick et al., 2011; Ghosal et al., 2016). LMW PAH-degrading bacteria are ubiquitous in nature, and the isolation, degradation potential, and

elucidation of catabolic pathways, enzymatic machineries, and genetic regulations within these bacteria are well documented (Peng et al., 2008b; Mallick et al., 2011; Ghosal et al., 2016). For example, several bacterial genera are well known for their high efficiency to degrade LMW PAHs, such as *Acinetobacter*, *Comamonas*, *Novosphingobium*, *Ochrobactrum*, *Ralstonia*, *Rhodococcus*, *Pseudomonas*, *Sphingomonas*, *Sphingobium*, and *Staphylococcus* (Peng et al., 2008b; Mallick et al., 2011; Ghosal et al., 2016).

Unlike LMW PAHs, HMW PAHs, due to their stable physicochemical structures, do not biodegrade easily; however, research on bacterial degradation of HMW PAHs has advanced significantly over the last two decades (Ghosal et al., 2016; Kweon, 2018). Several HMW PAH bioavailability-enhancing strategies and adaptation mechanisms have been identified and include biofilm formation, cell surface hydrophobicity, low requirements for energy and O2 for cell growth and maintenance, high substrate uptake affinity, production of biosurfactants, and wide substrate utilization patterns (Wick et al., 2002; Peng et al., 2008b; Kweon, 2018). More importantly, the functions of diverse, versatile catabolic genes involved in HMW PAH degradation and enzymatic activities, as well as their regulation mechanisms, have been discovered in various HMW PAH-degrading bacteria (Kweon, 2018). Table 2.4 lists genes and enzymes involved in both LMW and HMW PAH biodegradation.

Although most of the HMW PAH-degrading bacteria described so far belong to the Actinobacteria phylum, representing genera such as Arthrobacter, Corynebacterium, *Dietzia*, *Gordonia*, *Microbacterium*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Streptomyces* (Ghosal et al., 2016; Brzeszcz and Kaszycki, 2018), a variety of non-actinomycete bacterial genera such as *Achromobacter*, *Burkholderia*, *Pseudomonas*, *Sphingomonas*, *Sphingobium*, and *Stenotrophomonas* have been reported as well (Peng et al., 2008b; Ghosal et al., 2016).

Table 2.4 examples of PAH-degrading genes, enzymes, and their bacterial sources.

Enzyme	Gene	Bacterial source	References
Naphthalene dioxygenase	*Nah*	*Pseudomonas putida* strain G7	(Simon et al., 1993)
Phenanthrene dioxygenase	*phnAc*	*Burkholderia* sp. strain RP007	(Laurie and Lloyd-Jones, 1999)
Pyrene dioxygenase	*nidA*	*Mycobacterium* sp. strain PYR-1	(Khan et al., 2001)
Extradiol dioxygenase	*phdF*	*Mycobacterium* sp. strain SNP11	(Pagnout et al., 2007)
Catechol 1,2-dioxygenase	*C12O*	*Pseudomonas* sp. strain EST1001	(Kivisaar et al., 1991)

2.8 Enhancing the Understanding of Mechanisms through Which Host Plants Assemble a Beneficial Microbiome, and How It Functions, under Pollutant Stress

A challenging problem facing studies of the microbiome in many disciplines is the fact that the majority of microbial taxa are resistant to cultivation using current culture-dependent techniques. However, over the past decade or so, the advancement of next-generation sequencing and bioinformatics has paved the way to enhance our understanding of the structure, function, and composition of microbial communities in different habitats and environmental conditions, including PHC-contaminated soil (Hiraoka et al., 2016).

2.8.1 High-Throughput Amplicon Sequencing

Studies of the rhizosphere microbiome in natural and agricultural settings have generated most of our knowledge about host plant selection processes and plant–microbiome interactions taking place in the rhizosphere and how plants recruit different microbiota from surrounding environments (Quiza et al., 2015; Thijs et al., 2016). For example, previous studies using 16S rRNA amplicon sequencing revealed that microbial communities in the rhizosphere and adjunct bulk soils are different; the recruitment of rhizosphere microbiota by plants is strongly dependent on the structure and composition of the bulk soil microbiome (Bulgarelli et al., 2012), and different plant genotypes were found to select for different rhizosphere microbiomes (Lundberg et al., 2012).

Over the past few years, several experiments have been conducted to optimize phytoremediation systems and improve their efficacy using high-throughput sequencing approaches. For example, Bell et al. (2014a) used high-throughput 454-pyrosequencing of bacterial 16S rRNA genes and the fungal internal transcribed spacer (ITS) region to compare the community structure and composition of the rhizosphere microbiome of native and non-native *Salix* cultivars across uncontaminated and PHC-contaminated soil. Their results indicated higher fungal sensitivity to PHC contamination than that found for bacterial communities. Additionally, certain fungal class (*Pezizomycetes*) reacted differently following plant introduction to soils (Bell et al., 2014a), implying the importance of plant species selection in phytoremediation with regard to their impact on plant-associated microbiomes (Thijs et al., 2016). Similarly, (Hassan Sel et al., 2014) used 454-pyrosequencing of the AMF 18S rDNA gene to

examine how rhizospheric AMF communities are shaped within the rhizosphere of 11 *Salix* cultivars introduced across non-contaminated and PHC-contaminated soil. While PHC contamination levels had a strong impact on AMF community structure, *Salix* planting increased the abundance of several AMF families (Hassan Sel et al., 2014), inferring that AMF, possibly due to opportunistic associations with the plant, are involved in plant adaptation to PHC contamination (Thijs et al., 2016).

Tardif et al. (2016) amplified the bacterial 16S rRNA gene and fungal ITS regions using Ion Torrent sequencing in order to characterize the variations between plant compartments (bulk soil, rhizosphere soil, roots, and stems) in the microbiome of two *Salix* cultivars growing under three PHC contamination levels at a former petrochemical site. PHC contamination was found to be the main factor, shaping not only the rhizosphere but also the root and stem microbiome structure (Tardif et al., 2016). Additionally, the presence of the plant offered a protective buffer zone against PHC pollution in the rhizosphere and other plant tissues, subsequently minimizing the severe effects of PHC contamination on the microbiome composition, as compared with adjunct bulk soil (Tardif et al., 2016). Finally, increasing PHC contamination caused a shift in the microbial community composition, favoring beneficial microbiome communities such as putative PHC-degraders and PGPR (Tardif et al., 2016).

In a recent study, Mitter et al. (2017) used high-throughput Illumina MiSeq amplicon sequencing of the 16S rRNA gene to characterize the bacterial root microbiome associated with annual barley and sweet clover growing in an oil sands reclamation site. Results confirmed that, consistent with previous reports, the rhizosphere compartment produced the strongest differentiation of the root microbiome community structure (Bulgarelli et al., 2012; Ofek-Lalzar et al., 2014; Mitter et al., 2017); for example, Proteobacteria was the predominant phyla in the endosphere microbiome, whereas phyla such as Acidobacteria and Gemmatimonadetes were restricted only to the rhizosphere microbiome (Mitter et al., 2017). Additionally, host plants play a major role in shaping the root microbiome community structure (Mitter et al., 2017), implying plants have the ability to select for specific soil microbiota (Mitter et al., 2017).

2.8.2 Metatranscriptomics

Metatranscriptomics refers to the study of mRNA expression profiles of complex microbial communities within natural environments (Bell et al., 2014b). Unlike metagenomics, metatranscriptomics can enhance our understanding about how microbiome functions can be altered due to PHC contamination (Bell et al., 2014b). In the context of phytoremediation, metatranscriptomics has been used to study plant-associated microbial activities in PHC-contaminated soil. For example, in a greenhouse experiment, Yergeau et al. (2014) compared the expression of functional genes in the rhizosphere and bulk soil of willow plants growing in contaminated and uncontaminated soil using a metatranscriptomics approach. Combined selective pressure of the pollutants and rhizosphere resulted in an increased expression of genes related to competition, such as antibiotic resistance and biofilm formation, in the contaminated rhizosphere (Yergeau et al., 2014). Additionally, genes related to PHC degradation were more expressed in polluted soils (Yergeau et al., 2014).

More recently, Yergeau et al. (2018) sequenced the rhizosphere metatranscriptome of four willow species and the plant root metatranscriptome for two willow species growing in PHC-contaminated and non-contaminated soil at a former petroleum refinery site. The abundance of transcripts for many microbial taxa and functions were significantly higher in contaminated rhizosphere soil for *Salix eriocephala*, *S. miyabeana*, and *S. purpurea*, compared to the rhizosphere of *S. caprea* (Yergeau et al., 2018). The root metatranscriptomes of two willow cultivars were compared, showing that plant transcripts were mostly influenced by willow species, while microbial transcripts primarily responded to contamination level (Yergeau et al., 2018).

Pagé et al. (2015) used a transcriptomics-based approach to identify microbes involved in willow–microbes PHC degradation systems. Enhanced expression of the four genes related to PHC degradation was observed within the bacterial orders Actinomycetales, Rhodospirillales, Burkholderiales, Alteromonadales, Solirubrobacterales, Caulobacterales, and Rhizobiales, implying that members of these microbial taxa are active participants in the willow–microbes association (Pagé et al., 2015). Information obtained from metatranscriptomics studies on complex systems, such as plants and their associated highly diverse microbial communities, growing in

PHC-contaminated soil could help optimize phytoremediation and enhance their use (Bell et al., 2014b; Yergeau et al., 2014; Pagé et al., 2015; Yergeau et al., 2018).

2.8.3 Genome Sequencing

Due to the decrease in cost and difficulty over the past decade, sequencing, assembly, and annotation of bacterial genomes is becoming a relatively common practice in many fields of microbiology, including environmental microbiology. By sequencing the entire bacterial genome, valuable information can be obtained such as isolate identification, finding important bacterial traits, life style, ecological adaptation, genetic structure, and metabolic pathways.

Over the past few years, many complete and draft genome sequences of bacterial strains, with versatile abilities to degrade PHCs, have been published and are available in public databases (Kotoky et al., 2018). The genome sequences of different PHC-degrading bacterial strains provide structures for sets of genes, operons, and degradative pathways responsible for remediation of PHC-contaminated environments (Kotoky et al., 2018). Some of these bacterial genomes and their importance in rhizoremediation are listed in Table 2.5.

Zhao et al. (2015) reported the complete genome sequence of *Sphingobium yanoikuyae* strain B1 that has versatile abilities to degrade various PHCs pollutants, such as biphenyl, naphthalene, phenanthrene, toluene, and anthracene. The 5,200,045 bp genome of this bacterium contains 35 dioxygenases or putative dioxygenases genes, including catechol 1,2-dixoygenase, biphenyl 2,3-dioxygenase, and biphenyl-2,3-diol 1,2-dioxygenase (Zhao et al., 2015). Additionally, the genome of *S. yanoikuyae* strain B1 contains 48 ABC transporter-related genes and 82 TonB-dependent receptors, which may be involved in PAH transportation (Zhao et al., 2015). Such valuable information can provide clues about the genetic versatility of *Sphingobium* strains and the mechanisms of PAHs biodegradation, which might potentially aid in rhizoremediation applications (Zhao et al., 2015).

Table 2.5 Recent genomes of bacterial strains capable of degrading PHCs.

Bacterial Strains	Importance in Bioremediation	Isolation Source	PGPR Features	Genome Size	Reference
Pseudomonas veronii strain VI4T1	degradation of aromatic and aliphatic hydrocarbons	long-term oil field-polluted soil	IAA, siderophore	7.15 Mb	(Imperato et al., 2019)
Halomonas sp. strain G11	degradation of alkanes and polyaromatic hydrocarbons	hypersaline sediment	Salt-tolerance, biosurfactant production	3.96 Mb	(Neifar et al., 2019)
Pseudomonas aeruginosa strain DN1	fluoranthene degradation	PHC-contaminated soil	N/D	6.6 Mb	(He et al., 2018)
Alcaligenes aquatilis strain BU33N	degradation of n-alkanes and phenanthrene	PHC-polluted sediments	biosurfactant production; heavy metals resistance	3.8 Mb	(Mahjoubi et al., 2019)
Gordonia paraffinivorans strain MTZ052	degradation of n-hexadecane	composting pile	N/D	4.8 Mb	(Silva et al., 2019)
Klebsiella pneumoniae strain AWD5	degradation of xenobiotic compounds	PAH-contaminated soil	siderophore production	4.8 Mb	(Rajkumari et al., 2017)
Bacillus licheniformis strain VSD4	degradation of diesel fuel	leaves of *Hedera helix* plants growing at a high-traffic city center	IAA, siderophore	4.19 Mb	(Stevens et al., 2017)
Pseudomonas putida strain BS3701	degradation of crude oil and PAHs	soil contaminated with coke by-product waste	N/D	6.3 Mb	(Filonov et al., 2020)

2.9 Concluding Remarks and Future Perspectives

In spite of the remarkable progress detailed above, rhizoremediation remains a marginal choice for in situ soil decontamination. Given the important role of the rhizosphere microbiome in phytoremediation, future efforts to optimize this technology should include (i) selection of the right plant host, which can alter the function of the rhizosphere microbiome to benefit rhizoremediation activities. Special emphasis should be placed on selecting native plants that show tolerance toward PHCs. Using such plants could offer economic and environmentally sustainable solutions to remediate PHC-contaminated soil. (ii) Modern microbial ecology omics- tools should be used not only to better understand the structure and function of the rhizosphere microbiome associated with plants but also to recommend more efficient management strategies and predict the clean-up time of rhizoremediation. (iii) The effect of novel microbiome inocula combining PGPR and hydrocarbon-degrading bacteria should be tested under large-scale field experiments. (iv) The complicity of the rhizosphere environment and the influence of many biotic and abiotic factors on the composition and function of rhizosphere microbiome should be taken into account, which might subsequently affect rhizoremediation efficiency. Therefore, it would be important to characterize biotic and abiotic parameters in PHC-contaminated sites prior to application of rhizoremediation strategies.

2.10 ACKNOWLEDGEMENTS

We acknowledge funding for this work by the GenoRem Project, which is primarily financed by Genome Canada and Genome Quebec, and by NSERC discovery grants to M.S.-A. and M.H., F.A. was also supported by a grant from King Saud University. The GenoRem project contains several industrial partners, but these partners have in no way influenced or modified this manuscript.

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

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This chapter was published in:

Fahad Alotaibi, Soon-Jae Lee, Marc St-Arnaud and Mohamed Hijri. 2021. *Salix purpurea* and *Eleocharis obtusa* Rhizospheres Harbor a Diverse Rhizospheric Bacterial Community Characterized by Hydrocarbons Drgradation Potentials and Plant Growth-Promoting Properties. **Plants**. 2021. 10, 1987-2008. (https://doi.org/10.3390/plants10101987).

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.

3.1 Preface:

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 contamination. Thus, it is therefore interesting to understand the composition of their bacterial microbiome and the ability of these bacterial species to contribute to the ability of the plants to perform in a contaminated environment by the degradation or detoxification of hydrocarbons or the stimulation of plant growth.

3.2 Abstract:

Phytoremediation, a method of 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 obusta* 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.

3.3 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 (Alotaibi et al., 2021a). 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) (Gkorezis et al., 2016; Pandey et al., 2016). 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) (Pilon-Smits and Freeman, 2006). Environmental contamination with PHCs products has adversely affected various ecosystems, including soils, causing damage to natural habitats with serious economic consequences (Brzeszcz and Kaszycki, 2018).

Concerns regarding soil pollution with PHCs have initiated the development of several remediation technologies, including biological, chemical and physical methods (Khan et al., 2004; Gkorezis et al., 2016). 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 (Pilon-Smits, 2005; Thijs et al., 2016). 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 (Pulford and Watson, 2003; Bell et al., 2014a; Marchand et al., 2018; Dagher et al., 2019; Robichaud et al., 2019).

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. (Bell et al., 2014a; Yergeau et al., 2014;

Gkorezis et al., 2016; Thijs et al., 2016; Dagher et al., 2019). *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 (Newman and Reynolds, 2004; Kuzovkina and Volk, 2009; Gkorezis et al., 2016; Correa-García et al., 2018). 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 (Bell et al., 2014a; Hassan Sel et al., 2014; Yergeau et al., 2015; Gonzalez et al., 2018).

More recently, a promising strategy that includes the screening and identification of native plants growing spontaneously on PHCs-contaminated soils has been adopted (Desjardins et al., 2014; Lumactud et al., 2016; Pawlik et al., 2017; Iqbal et al., 2019). This is the reason we chose *Eleocharis obtusa* (Willd), which dominated the vegetation at the site of study (Desjardins et al., 2014). *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., 2016) 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 (Pérez-Jaramillo et al., 2016). 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 (Escaray et al., 2012). Additionally, native plants have been shown to develop more close relationships with local rhizosphere microbiota than introduced plants (Johnson, 2010; Dagher et al., 2019), 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 (Backer et al., 2018; Kotoky et al., 2018). Plants growth under stress such as PHCs contamination is expected to be lower than it would be under optimal conditions (Glick and Stearns, 2011). 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 (Khan et al., 2013; Jambon et al., 2018; Singha et al., 2018). PGPR are soil microbes within the rhizomicrobiome with phenotypes that benefit plant growth (Backer et al., 2018). 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 (Hardoim et al., 2008; Glick and Stearns, 2011) degrading a broad range of PHCs compounds (Gkorezis et al., 2016).

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 (Iffis et al., 2014; Marchand et al., 2017). 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.

3.4 Materials and Methods

3.4.1 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◦430 N, 73◦220 W) (for details on the site, see (Bell et al., 2014a) and (Desjardins et al., 2014)). The petrochemical plant was fully operated from 1953 until it was closed in 2008 (Fortin Faubert et al., 2021). 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 (Bell et al., 2014a), 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. (2014a) 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. purpurea* rhizosphere soil samples, *E. 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. obtusa* rhizosphere samples and 1400 mg/kg for the *S. purpurea* rhizosphere samples. Other soil chemical and physical parameters are listed in Table 3.1.

3.4.2 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_2PO_4, 1 g K_2HPO_4, 1 g NH_4NO_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 (Bushnell and Haas, 1941). One percent filter-sterilized (0.2 µm pore size membrane) diesel was added to the Bushnell-Hass 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_2PO_4, 6 g Na_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 2.0 g glucose, 2 g gluconic acid, 2 g citric acid, 0.1 mL of trace elements solution (10 mg H3BO3, 11.19 mg $MnSO_4{\cdot}H_2O$, 124.6 mg $ZnSO_4{\cdot}7H_2O$, 78.22 mg $CuSO_4{\cdot}5H_2O$, and 10 mg MoO_3), 0.1 mL of $FeSO_4{\cdot}7H_2O$ 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

Table 3.1 Chemical and physical characteristics of soils used in this study.

	pH (1:1)	CEC (meq/100g)	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 rhizosphere	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

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-Hass plates and 1/10 TSA plates were incubated at 28 ◦C for 14 days or 3 days, respectively (Daane et al., 2001; Yousaf et al., 2010).

For the isolation of ACC deaminase-producing PGPR, an ACC deaminase enrichment culture method was used as described by Penrose and Glick (Penrose and Glick, 2003). 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_2HPO_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 $(NH_4)_2SO_4$ was added as 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.

3.4.3 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') (Lane, 1991) and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Turner et al., 1999). 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 MgCl2, 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) (Stefani et al., 2015).

3.4.4 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 (Altschul et al., 1997). The partial 16S rRNA gene sequences obtained from the bacterial isolates have been deposited in GenBank under the accession numbers (MZ430069-MZ430506).

3.4.5 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., 2006).

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 (Wrenn and Venosa, 1996). 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 (Wrenn and Venosa, 1996). The experiments were repeated twice, each with three replicates for each bacterial isolate.

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

3.4.6.1 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 (De Freitas et al., 1997). The PDYA medium was prepared in three separate solutions including PDYA-calcium phosphate (CaP) as described by De Freitas et al. (1997). Bacterial cultures were grown in half-strength TSB medium at 28 ˚C 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 ˚C. 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.

3.4.6.2 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 (1981). 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 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 ˚C 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.

3.4.6.3 ACC Deaminase Activity

1-Aminocyclopropane-1-carboxylate (ACC)-deaminase activity was assessed by mentoring 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}) (Penrose and Glick, 2003). Bacterial cultures were grown in half-strength TSB at 28 ˚C 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.

3.4.6.4 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. (1991) and modified by Ribeiro and Cardoso (2012). 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 micro-titre plate wells followed by the addition of 100 µL of Salkowski's reagent (150 mL of 98% H_2SO_4, 7.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$, 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 (Bric et al., 1991). An autoclaved bacterial culture was used as a negative control. The experiments were repeated twice, each with three replicates for each isolate.

3.4.6.5 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 (1991). 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 (1991). 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.

3.5 Results

3.5.1 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 S3.1–S3.3, 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 3.2).

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 3.2).

The phylum Actinobacteria corresponded to 29% of the bacterial isolates, and was represented by 18 genera. The most abundant genera were *Streptomyces* (7.3%), *Microbacterium* (5.2%), *Arthrobacter* (4.5%), *Rhodococcus* (3%), *Nocardioides* (1.8%), *Mycobacterium* (1.3%) and *Gordonia* (1%) (Table 3.2). 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 3.2).

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 3.2). The sub phylum Alphaproteobacteria correspond to 5% of the total isolates, including 11 genera, with *Rhizobium* (1.1%) as the dominant genus (Table 3.2). The phylum Bacteroidetes represents 3.5% of the entire bacterial collection (Table 3.2).

The *E. obtusa* rhizosphere was dominated by Gammaproteobacteria, followed by Actinobacteria and Alpha- and Betaproteobacteria, Bacteroidetes, Firmicutes and

Table 3.2 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	*Amycolatopsis*	0	0	2
7	*Ancylobacter*	1	0	0
8	*Arthrobacter*	0	0	20
9	*Azorhizobium*	2	0	0
10	*Bacillus*	8	16	17
11	*Bosea*	2	0	0
12	*Brevibacillus*	0	1	0
13	*Brevundimonas*	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	*Delftia*	3	0	0
20	*Dyella*	1	3	0
21	*Empedobacter*	1	2	0
22	*Enterobacter*	1	3	3
23	*Exiguobacterium*	2	2	0
24	*Flavihumibacter*	0	1	0
25	*Flavobacteriaceae*	1	0	0
26	*Georgenia*	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	*Mycolicibacterium*	2	3	2
39	*Myroides*	3	2	0

Table 3.2 Continued.

Serial #	*Genera*	*Eleocharis* rhizosphere	Bulk soil	*Salix* rhizosphere
40	*Nocardia*	0	0	2
41	*Nocardioides*	0	0	9
42	*Paenarthrobacter*	0	0	2
43	*Paenibacillus*	0	0	1
44	*Pantoea*	1	1	1
45	*Phycicoccus*	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
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.

unidentified bacteria (Figure 3.1). The *S. purpurea* rhizosphere was dominated by Actinobacteria, followed by Gammaproteobacteria, Firmicutes, Alpha- and Betaproteobacteria (Figure 3.1). In the polluted bulk soil, Gammaproteobacteria was the predominant phyla, followed by Actinobacteria, Alphaproteobacteria, Bacteroidetes, Firmicutes and unidentified bacteria (Figure 3.1).

Dominants families within the *E. obtusa* rhizosphere were *Xanthobacteraceae*, *Comamonadaceae*, *Microbacteriaceae*, *Flavobacteriaceae*, *Bacillaceae* *Xanthomonadaceae* and *Enterobacteriaceae* (Figure 3.2). *S. purpurea* rhizosphere was dominated by *Enterobacteriaceae*, *Micrococcaceae*, *Nocardioidaceae*, *Nocardiaceae* and *Bacillaceae* (Figure 3.2). Several families predominate the bulk soil including *Sphingomonadaceae*, *Flavobacteriaceae*, *Enterobacteriaceae*, *Microbacteriaceae*, *Bacillaceae* and *Xanthomonadaceae* (Figure 3.2). Notably, several families with importance in petroleum hydrocarbons degradation were present in all the three environmental habitats, including *Comamonadaceae*, *Enterobacteriaceae*, *Microbacteriaceae*, *Sphingomonadaceae*, *Bacillaceae, Xanthomonadaceae*, *Gordoniaceae* and *Nocardiaceae* (Figure 3.2). Interestingly, 11 bacterial genera were shared between the *S. purpurea* rhizosphere, *E. obtusa* rhizosphere and the bulk soil (Figure 3.3).

3.5.2 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 3.4). Additionally, 229 bacterial strains (52%) were able to utilize pyrene (4-rings PAH-compound) as a sole carbon source (Figure 3.4).

Regarding *n*-alkanes degrading bacteria, our results showed 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 3.4).

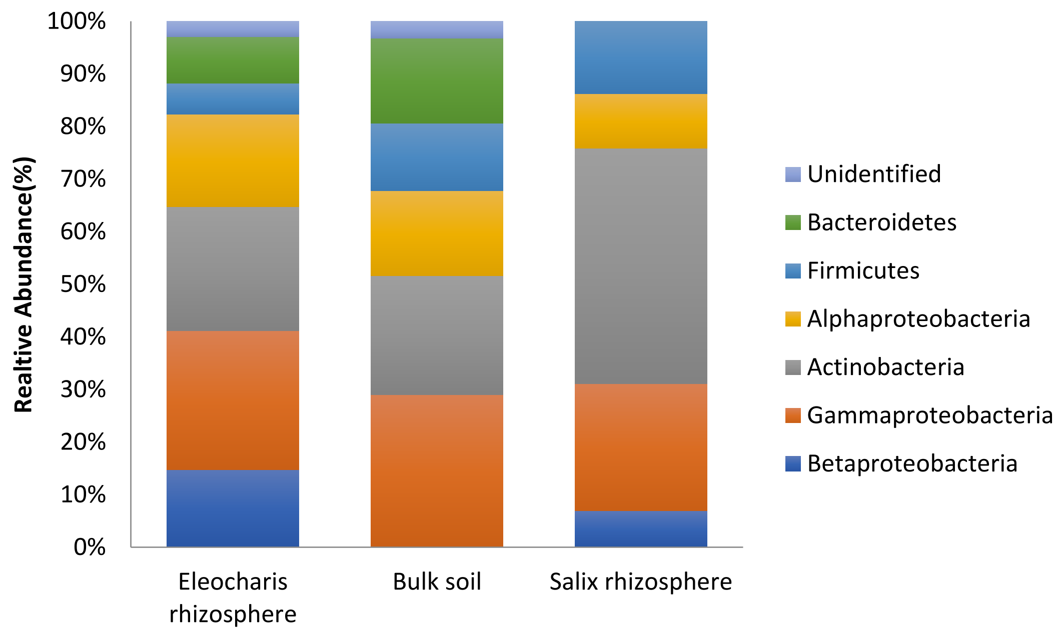

Figure 3.1 Taxonomic breakdown and relative abundance of bacterial isolates at the phylum level.

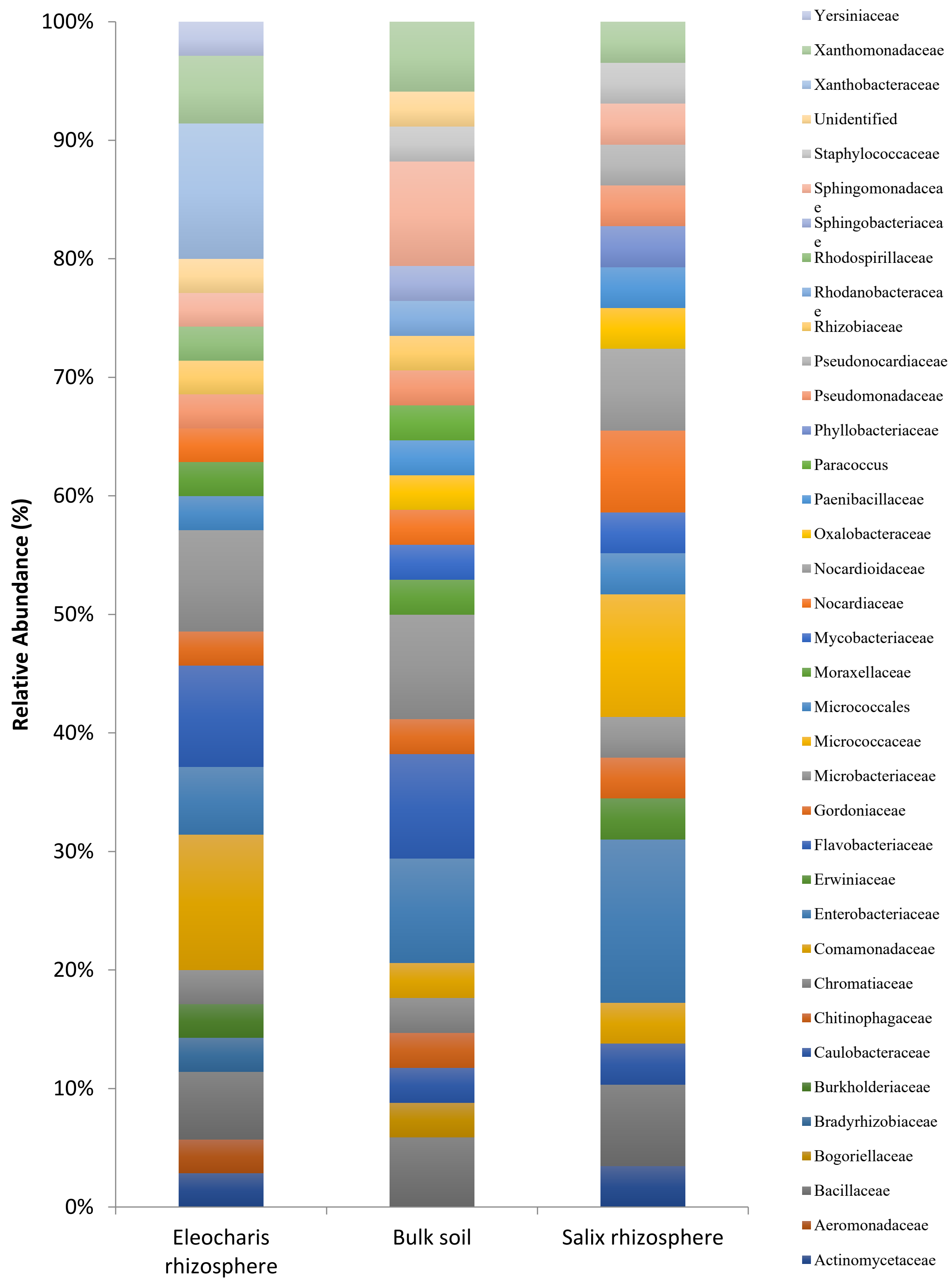

Figure 3.2 Taxonomic breakdown and relative abundance of bacterial isolates at the family level.

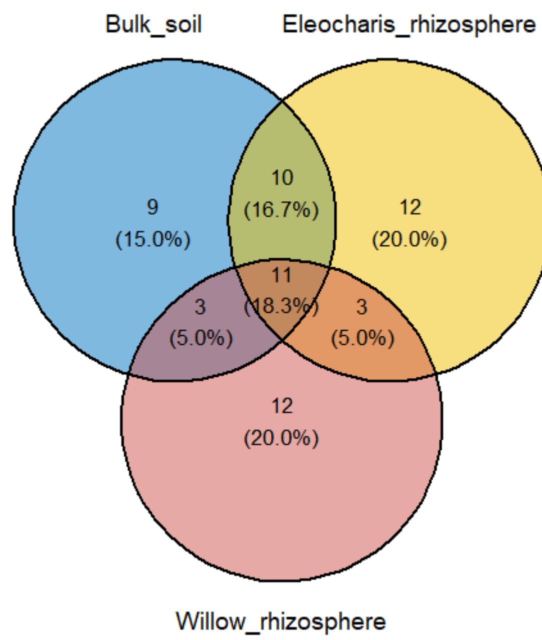

Figure 3.3 Identity of bacterial isolates at the genus level shared among the three environmental niches.

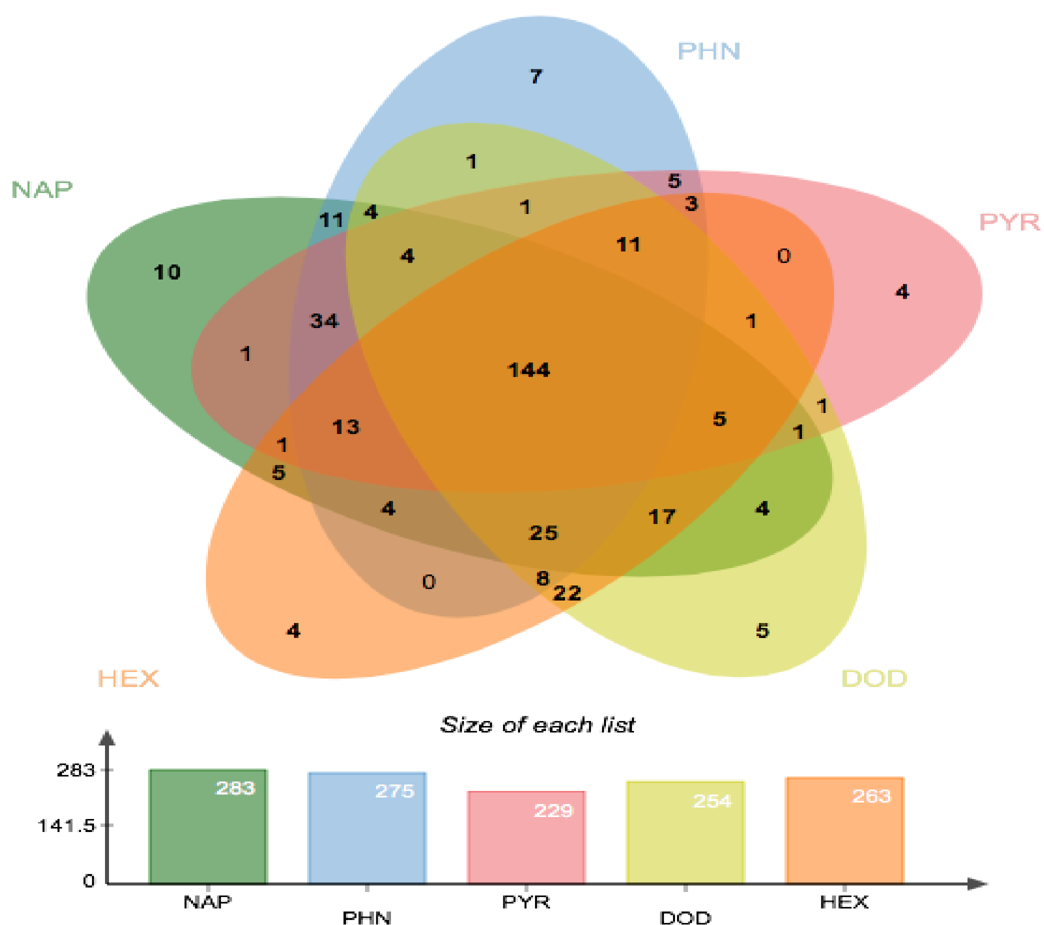

Figure 3.4 Bacterial isolates that were able to grow with various petroleum hydrocarbons as the only carbon source, showing degradation potential. (Above) Venn-diagram showing the number of isolates that grew on one or many of the five (PHC) compounds. (Below) Bar graph indicating the absolute numbers of bacterial isolates that grew on each of the (PHC) compounds under investigation.

About 32% of isolates were able to utilize all 5-hydrocarbon compounds tested in this study (Figure 3.5). 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 3.5).

Isolates that were able to utilize 4 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%), *Micrococcaceae* (4%), *Moraxellaceae* (4%) and *Sphingomonadaceae* (4%) (Figure 3.5).

3.5.3 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 3.3 and Figure 3.6). 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 3.6), 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 3.6).

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 3.7).

Isolates that were positive for at least four PGP traits represented (12%) of total bacterial collection, with *Pseudomonadaceae* being the predominant family (33%) followed by *Enterobacteriaceae* (32%), *Moraxellaceae* (12%), *Comamonadaceae* (5%) and *Microbacteriaceae* (4%) (Figure 3.7).

Table 3.3 List of bacterial isolates that possessed all five-plant growth-promoting traits that were tested in this study.

Isolate	Closest NCBI relative	Environmental Niche	Isolation medium
SB41	*Acinetobacter calcoaceticus*	Bulk soil	B-H_amended diesel
SB55	*Acinetobacter calcoaceticus*	Bulk soil	B-H_amended diesel
SB60	*Acinetobacter* sp.	Bulk soil	B-H_amended diesel
ET27	*Pseudomonas plecoglossicida*	*Eleocharis* rhizosphere	TSA
ET43	*Serratia* sp.	*Eleocharis* rhizosphere	TSA
ET45	*Pseudomonas fluorescens*	*Eleocharis* rhizosphere	TSA
ET46	*Serratia* sp.	*Eleocharis* rhizosphere	TSA
ET50	*Pseudomonas putida*	*Eleocharis* rhizosphere	TSA
ET52	*Serratia* sp.	*Eleocharis* rhizosphere	TSA
ET57	*Pseudomonas monteilii*	*Eleocharis* rhizosphere	TSA
ET60	*Azomonas macrocytogenes*	*Eleocharis* rhizosphere	TSA
EB3	*Pseudomonas songnenensis*	*Eleocharis* rhizosphere	B-H_amended diesel
EB31	*Stenotrophomonas pavanii*	*Eleocharis* rhizosphere	B-H_amended diesel
WT4	*Pseudomonas mandelii*	*Salix* rhizosphere	1/10TSA
WT8	*Streptomyces atriruber*	*Salix* rhizosphere	1/10TSA
WT17	*Pseudomonas kilonensis*	*Salix* rhizosphere	1/10TSA
WT22	*Pseudomonas frederiksbergensis*	*Salix* rhizosphere	1/10TSA
WT32	*Bacillus megaterium*	*Salix* rhizosphere	1/10TSA
WT50	*Pseudomonas frederiksbergensis*	*Salix* rhizosphere	1/10TSA
WT56	*Pseudomonas frederiksbergensis*	*Salix* rhizosphere	1/10TSA
WB31	*Pseudomonas putida*	*Salix* rhizosphere	B-H_amended diesel
EA21	*Pantoea agglomerans*	*Eleocharis* rhizosphere	ACCD

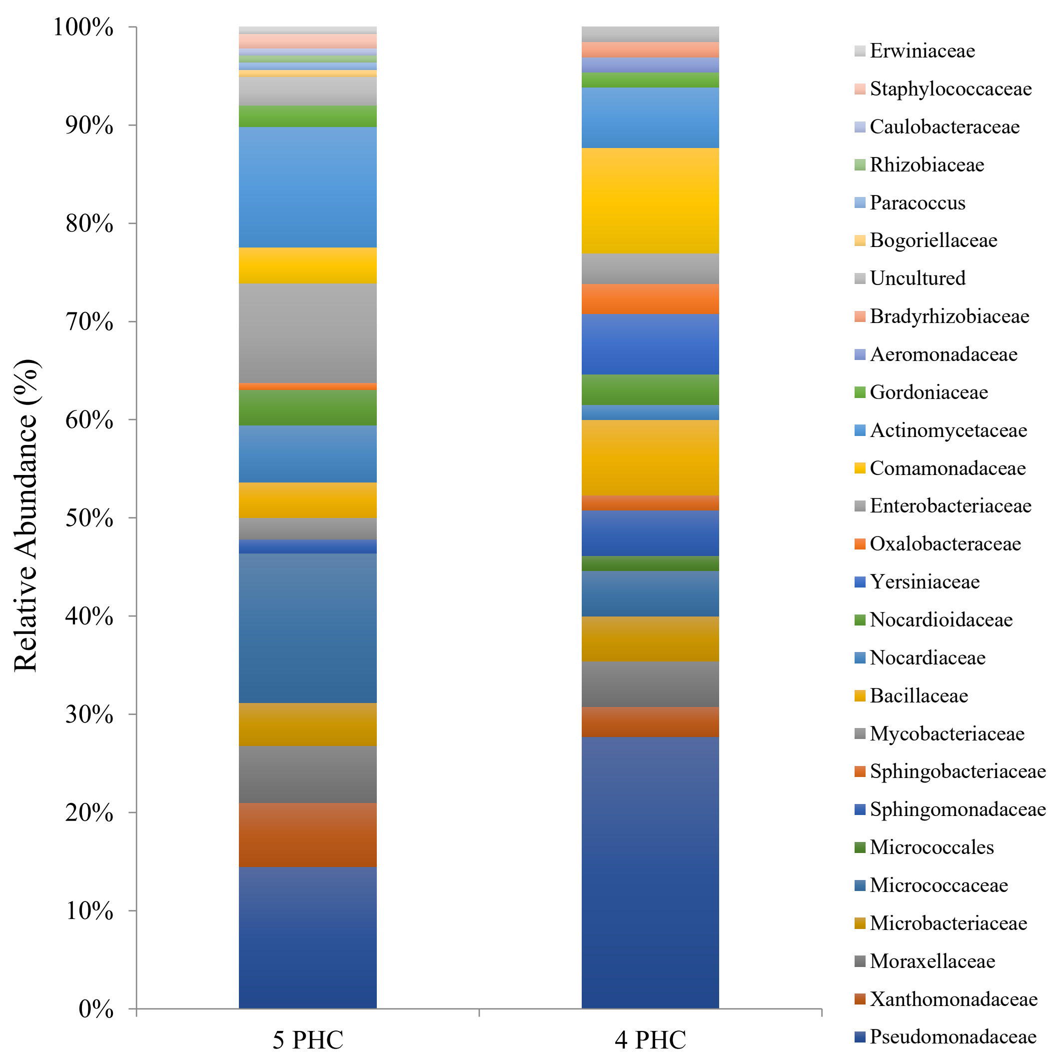

Figure 3.5 Bars indicate the relative abundance of genera among isolates, which are able to degrade five and four different petroleum hydrocarbons compounds (PHC) *in vitro*.

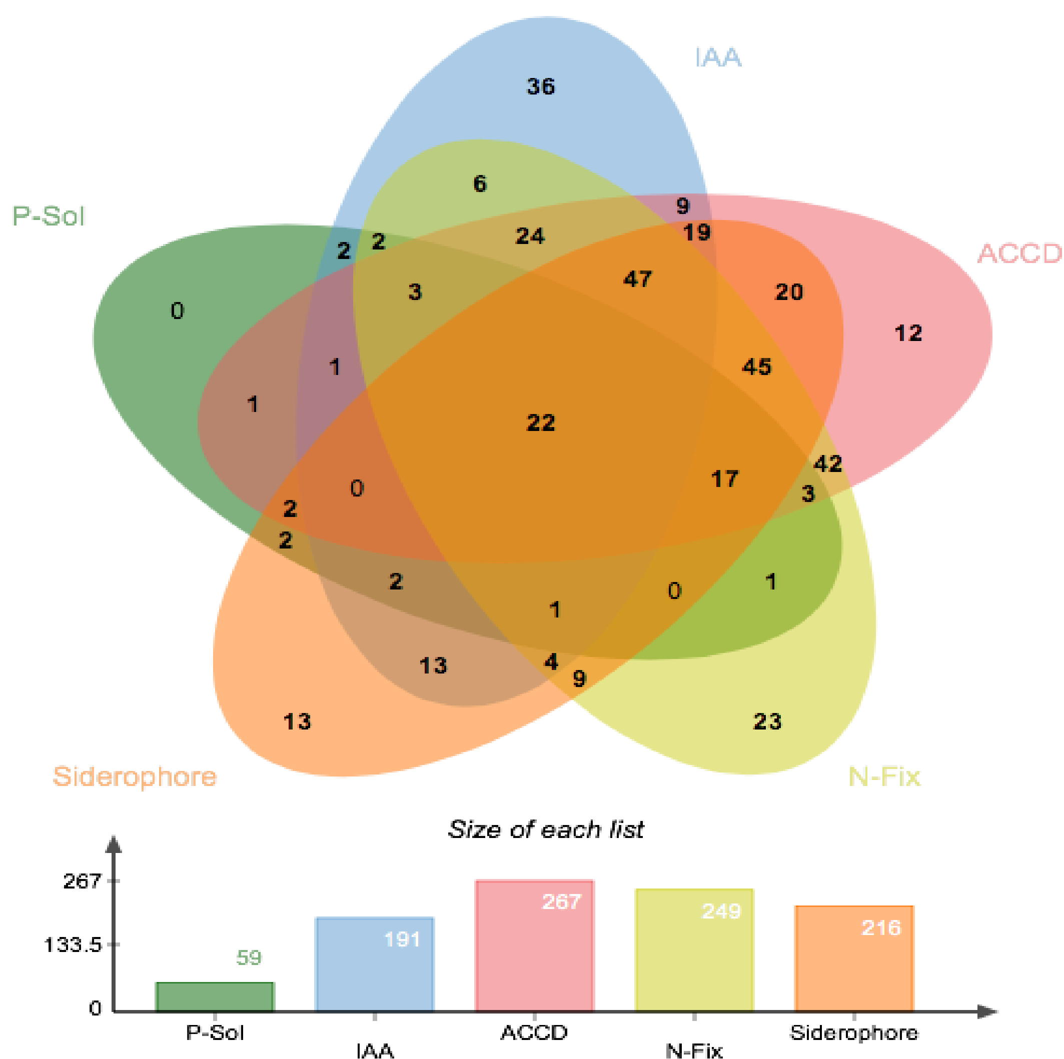

Figure 3.6 Bacterial isolates with plant growth-promoting (PGP) properties. (Above) Venn diagram showing the number of isolates that possesses one or many of the five PGP traits. (Below) Bar graph indicating the absolute numbers of bacterial isolates having each of the PGP traits under investigation (over 438 isolates tested).

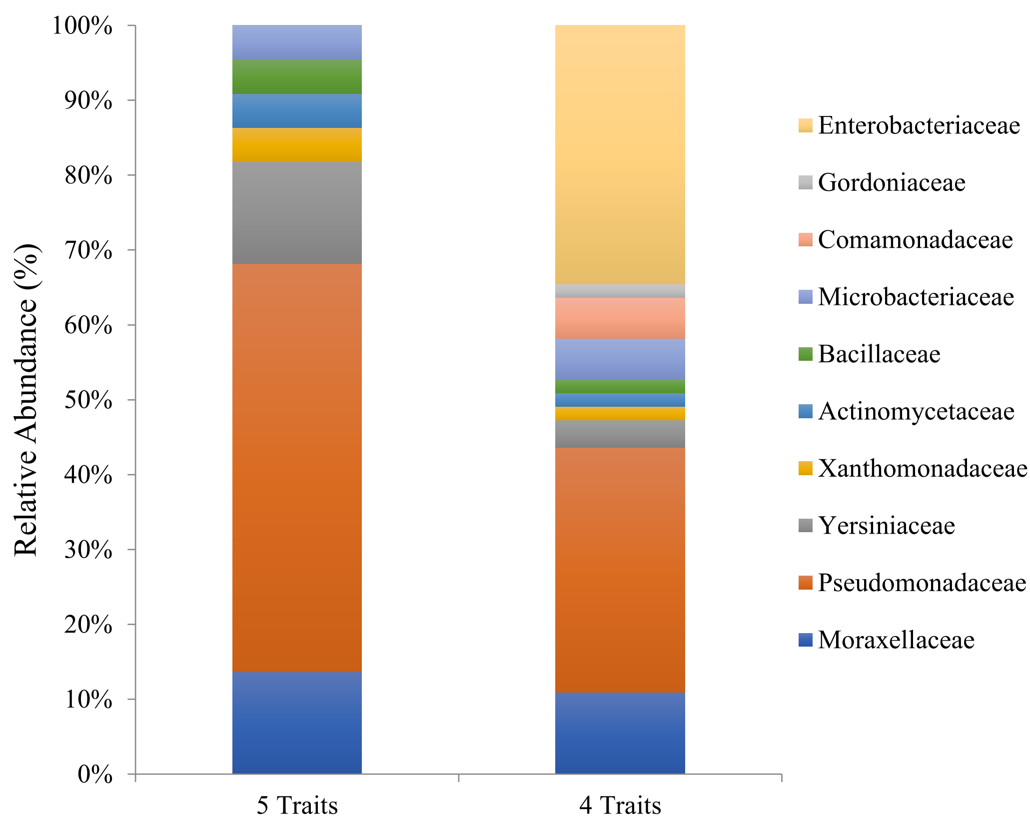

Figure 3.7 Bars indicate the relative abundance of genera among isolates presenting four or five different PGP-associated traits *in vitro*.

3.6 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 (Iffis et al., 2014; Lee et al., 2020), next generation-targeted amplicon sequencing (Bell et al., 2014a; Marchand et al., 2018; Dagher et al., 2019) and metatranscriptomics (Yergeau et al., 2014; Yergeau et al., 2018). This study used 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 on contaminated soils (Baek et al., 2004; Iqbal et al., 2019). For example, the growth rate of corn and red bean plants were reduced at 10000 mg/kg of crude oil (Baek et al., 2004). Similarly, (Chaineau et al., 1997) 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 (Liu et al., 2012; Lumactud et al., 2016). Similarly, (Desjardins et al., 2014) reported three indigenous plant species (*Alisma triviale*, *Eleocharis obtusa* 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 (Desjardins et al., 2014). 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 (Bell et al., 2014a; Hassan Sel et al., 2014; Yergeau et al., 2014).

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. obtusa* 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 3.1), encompassing a fairly diverse collection of bacterial genera (62 genera) (Table 3.2), including *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Chitinimonas*, *Enterobacter*, *Gordonia*, *Klebsiella*, *Microbacterium*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcus*, *Serratia*, *Sphingomonas*, *Stenotrophomonas*, *Streptomyces* and *Variovorax* (Table 3.2). Several of these genera have previously been shown to hold promising petroleum-hydrocarbons degradation potential and plant growth-promoting activities (Lugtenberg and Kamilova, 2009; Glick and Stearns, 2011; Schlaeppi and Bulgarelli, 2015; Ghosal et al., 2016; Xu et al., 2018). 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 (Bai et al., 2015).

Our study revealed that culturable rhizospheric bacteria associated with *S. purpurea* rhizosphere mainly belonged to Actinobacteria and Gammaproteobacteria (Figure 3.1). In contrast to our results, (Bell et al., 2014a) 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., 2014a), all bacteria were potentially amplified and sequenced. In agreement with our explanation, Ferrera-Rodríguez et al. (2013) 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 3.2). 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 (Mitter et al., 2017) and herbaceous plants growing near natural oil seep fields (Lumactud and Fulthorpe, 2018). Endophytic bacteria are thought to be a subset of the larger rhizosphere microbiota (Hardoim et al., 2008) 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 *Micrococcaceae*, *Nocardioidaceae* and *Nocardiaceae* (Figure 3.2), which have been shown to possess strong petroleum hydrocarbon degradation capabilities (Ghosal et al., 2016; Xu et al., 2018).

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 3.1). The Dominant families were *Comamonadaceae*, *Xanthomonadaceae* and *Microbacteriaceae* (Figure 3.2). 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 (Ghosal et al., 2016; Xu et al., 2018). Genera belonging to the phyla Gammaproteobacteria, Actinobacteria are also known to contain bacterial species with efficient petroleum hydrocarbon degradation potentials (Ghosal et al., 2016; Xu et al., 2018) such as *Pseudomonas*, *Streptomyces* and *Rhodococcus* (Dwivedi et al., 2019; Viesser et al., 2020).

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 (Das and Chandran, 2011). 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 3.4). 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 (Marchand et al., 2017; Wolińska et al., 2018; Xu et al., 2018). 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 (Larkin et al., 2005; Martínková et al., 2009). 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 (Larkin et al., 2005; Martínková et al., 2009; Viesser et al., 2020).

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 (Correa-García et al., 2018). PGPR with the capacity to produce the phytohormones IAA, which plays a role in inducing the formation of lateral roots (Lugtenberg and Kamilova, 2009), would further stimulate plant growth in PHC-contaminated soils. In this study, 43% of bacterial isolates synthesized IAA (Supplementary Figure S3.1), which were mostly affiliated to the genera *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Arthrobacter* and *Microbacterium* (Supplementary Figure S3.2). 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 (Hynes et al., 2008; Dutta et al., 2015; Pawlik et al., 2017).

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 (Lugtenberg and Kamilova, 2009; Glick and Stearns, 2011). Stressed plants induce the production of the phytohormone ethylene to bolster their defense. However, ethylene also inhibits plant growth (Glick, 2005).

Certain PGPR can inhibit ethylene biosynthesis via the production of ACC deaminase which cleaves the ethylene precursor ACC into alpha-ketobutyrate and ammonia (Glick, 2005; Glick and Stearns, 2011). In this study, 60% of bacterial isolates were found to produce ACC deaminase (Supplementary Figure S3.1). Most isolates that could catabolize ACC reported in this work belonged to genera such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Stenotrophomonas* and *Microbacterium* (Supplementary Figure S3.2). 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 (Grichko and Glick, 2001; Arshad et al., 2007; Thijs et al., 2014).

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 (Supplementary Figure S3.1). Nitrogen fixation by diazotrophic bacteria is an important trait of PGPR that benefits the plant, especially when growing in nutrients-deficient soils (Vessey, 2003). Diazotrophic bacteria isolated in this study belonged mainly to genera such as *Pseudomonas*, *Klebsiella*, *Bacillus*, *Enterobacter*, *Acinetobacter* and *Variovorax* (Supplementary Figure S3.2). Low levels of soluble P in soils can restrict the growth and development of plants (Lugtenberg and Kamilova, 2009). Some PGPR solubilize inorganic forms of P and convert it to plant-available forms, thereby facilitating plant growth (Kim et al., 1997; Rodríguez and Fraga, 1999). Our study found that the majority of isolates are able to solubilize inorganic P belonged to the genera *Pseudomonas*, *Acinetobacter*, *Bacillus* and *Serratia* (Supplementary Figure S3.2). Another essential nutrient for plant growth is iron, even if it is present in soils in the highly insoluble form $Fe3+$ (Crowley and Kraemer, 2007). Some PGPR produce low molecular-weight organic compounds, siderophores, that chelate $Fe3+$ ions and render them available for reduction to the soluble $Fe2+$ form preferred by plants (Crowley and Kraemer, 2007). The majority of isolates reported in this study that were able to produce siderophores belonged to the genera *Pseudomonas*, *Acinetobacter*, *Microbacterium*, *Rhodococcus* and *Stenotrophomonas* (Supplementary Figure S3.2). 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 (Supplementary Figures S3.3–S3.5): *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 (Nogales et al., 2017; Ansari and Ahmad, 2019; Dwivedi et al., 2019). These bacterial taxa are candidates to look for in follow-up experiments.

3.7. Conclusions

S. purpurea and *E. 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.

3.8 ACKNOWLEDGEMENTS

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.

Chapter Four: In-Depth Characterization of Plant Growth Promotion Potentials of Selected Alkanes-Degrading Plant Growth-Promoting Bacterial Strains

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This chapter was published in:

Fahad Alotaibi, Marc St-arnaud and Mohamed Hijri. 2022. In-Depth Characterization of Plant Growth Promotion Potentials of Selected Alkanes-Degrading Plant Growth-Promoting Bacterial Strains. **Frontiers in Microbiology** 13: 863702. (https://doi.org/10.3389/fmicb.2022.863702).

Author Contributions: F.A., M.S.-A. and M.H. conceived and initiated the study; F.A. performed the experiments and analyzed the data; 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 submitted version of the manuscript.

4.1 Preface:

In the previous chapter, 438 morphologically different bacteria were isolated from bulk soil and the rhizosphere of *Salix purpurea* and *Eleocharis obtusa* plants growing in a site highly polluted with petroleum-hydrocarbons. These isolates showed multiple PHCs degradative activities and PGP features. In this chapter, 50 bacterial strains were selected, among the 438 isolates, based on the fact that they covered a wide phylogenetic affiliation range, their ability to possess various PGP activities and their wide-spectrum hydrocarbon degradation potential. These 50 strains were screened qualitatively and quantitively for PGP traits, followed by identification of genes involved in alkane degradation and assessed their plant growth promotion potential using gnotobiotic under normal and stressed conditions conditions.

4.2 ABSTRACT:

The use of plant-growth-promoting rhizobacteria (PGPR) as a bioremediation enhancer in plant-assisted phytoremediation requires several steps, consisting of the screening, selection and characterization of isolates. A subset of 50 bacterial isolates representing a wide phylogenetic range were selected from 438 morphologically different bacteria that were originally isolated from a petroleum hydrocarbon (PHC)-polluted site of a former petrochemical plant. Selected candidate bacteria were screened using six conventional plant-growth-promoting (PGP) traits, complemented with the genetic characterization of genes involved in alkane degradation, as well as other pertinent functions. Finally, the bacterial isolates were subjected to plant growth promotion tests using a gnotobiotic approach under normal and stressed conditions. Our results indicated that 35 bacterial isolates (70%) possessed at least 4 PGP traits. Twenty-nine isolates (58%) were able to utilize *n*-hexadecane as a sole carbon source, whereas 43 isolates (86%) were able to utilize diesel as the sole carbon source. The presence of catabolic genes related to hydrocarbon degradation was assessed using endpoint PCR, with the alkane monooxygenase (*alkB*) gene found in 34 isolates, the cytochrome P450 hydroxylase (*CYP153*) gene found in 24 isolates and the naphthalene dioxygenase (*nah1*) gene found to be present in 33 isolates. Thirty-six strains (72%) promoted canola root elongation in the growth pouch assay. After several rounds of screening, seven bacterial candidates (individually or combined in a consortium) were tested for canola root and shoot growth promotion in substrates amended by different concentrations of *n*-hexadecane (0%, 1%, 2%, 3%) under gnotobiotic conditions. Our results showed that *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27), *Stenotrophomonas pavanii* (EB31) and *Gordonia amicalis* (WT12) significantly increased the root length of canola grown in 3% *n*-hexadecane compared with the control treatment, whereas *Nocardia* sp. (WB46) and *Bacillus megaterium* (WT10) significantly increased shoot length compared to control treatment at the same concentration of *n*-hexadecane. The consortium had a significant enhancement effect on root length compared to all isolates inoculated individually or to the control. This study demonstrates that the combination of PGPR traits and the petroleum hydrocarbon degradation potential of bacteria can result in an enhanced beneficial effect in phytoremediation management, which could lead to the development of innovative bacterial inoculants for plants to remediate PHC-contaminated soils.

Keywords: 1-aminocyclopropane-1-carboxylate deaminase (ACCD); alkanes; bioinoculants; plant growth promotion; PGPR; rhizoremediation.

4.3 INTRODUCTION

Human activities related to the petroleum and gas industry, such as exploration, extraction, refining, storage and shipping, are polluting soil and water environments with petroleum hydrocarbons (PHCs) (Alotaibi et al., 2021a). Aliphatic hydrocarbons (alkanes) are saturated hydrocarbons, representing the main constituents of crude oil, and are major soil contaminants (Chénier et al., 2003; Stroud et al., 2007). Alkanes are major soil pollutants, characterized by low chemical activity, low water solubility and higher activation energies (Labinger and Bercaw, 2002; Rojo, 2009). Hexadecane ($C_{16}H_{34}$) is present in the aliphatic fraction of crude oil and is a main component of diesel fuel (Chénier et al., 2003). Hexadecane has been used as a model compound to study alkane biodegradation because of its presence in many diesel-contaminated soils and its well-characterized biodegradability (Chénier et al., 2003; Tara et al., 2014; Shiri et al., 2015; Balseiro-Romero et al., 2017a; Garrido-Sanz et al., 2019).The presence of these compounds in the environment adversely affects plant, animal and human health (Arslan et al., 2014). Thus, the remediation of alkane-contaminated environments is a primary goal in the field of environmental biotechnology.

The use of the usual physical and chemical methods to cope with PHC contamination have shown many limitations (Alotaibi et al., 2021a). These conventional approaches are very expensive, only work for specific organic compounds and do not often result in the complete degradation of the contaminants; in addition, they are not environmentally friendly as they contribute to greenhouse gas emissions (Kuiper et al., 2004; Pilon-Smits, 2005; Behera, 2014). On the other hand, a biological method such as phytoremediation that relies on the plant–microbe partnership is a promising strategy for the remediation of soils contaminated with aliphatic hydrocarbons. Phytoremediation requires less maintenance effort, minimizes site disturbances and is a cost-effective and less destructive approach (Alotaibi et al., 2021a).

Plants, due to their exudates, metabolite diversity and enzymatic machinery, can adapt and alleviate stressful conditions such as the presence of hydrocarbons in soil (Pilon-Smits, 2005). However, plant growth and biomass production are often limited under such harsh conditions and subsequently phytoremediation efficiency is reduced and plant mortality is increased (Glick and Stearns, 2011). Plant growth-promoting rhizobacteria (PGPR) can be used to enhance plant growth in stressful conditions, thus

enhancing phytoremediation efficiency (Tara et al., 2014; Balseiro-Romero et al., 2017a; Oleńska et al., 2020). PGPR can alleviate stress in plants and reduce the phytotoxicity of hydrocarbons via many mechanisms, such as reducing soil nutrient deficiencies (fixing nitrogen, solubilizing phosphorus and enhancing iron uptake), synthesizing plant-growth-promoting (PGP) hormones, suppressing ethylene production via 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD) activity (Schlaeppi and Bulgarelli, 2015; Backer et al., 2018; Oleńska et al., 2020), and by virtue of their pollutant-degrading pathways and metabolic activities (Arslan et al., 2014; Xu et al., 2018).

The development of a database and collection of bacterial isolates characterized for PGP traits and hexadecane degradation potential can assist in the selection of the most promising strains for further advancement as bioaugmentation inoculants in phytoremediation strategies for diesel-contaminated soils. In this study, 50 bacterial strains were selected from 438 morphologically different bacteria that were isolated from bulk and rhizosphere soils of plants growing in a petrochemical site contaminated with petroleum hydrocarbons (Alotaibi et al., 2021b). The strains were selected based on the fact that they covered a wide phylogenetic affiliation range, their ability to possess various PGP activities and their wide-spectrum hydrocarbon degradation potential. We hypothesize that combined traits of PGP and hexadecane degradation potential occur in bacteria isolated from an aged petroleum-hydrocarbon polluted site. The specific objectives of this study were: to define and compare the plant-growth-promoting traits of selected bacterial strains qualitatively and quantitatively; to test their ability to utilize 1% (v/v) sterilized *n*-hexadecane and 1% (v/v) diesel as a sole carbon source; to screen for the presence of the stress tolerance gene (*acdS*) encoding ACCD and the dinitrogenase reductase gene (*nifH*) encoding (nitrogen fixation); to search for the presence of hydrocarbon-degrading genes (*alkB, CYP153, nah*); and to assess their plant growth promotion potential under gnotobiotic conditions in growth pouches. Our results indicated that several bacterial strains such as *Nocardia* sp. (WB46), *Pseudomonas plecoglossicida* (ET27), *Stenotrophomonas pavanii* (EB31) and *Gordonia amicalis* (WT12) which possess multiple PGP and hexadecane degradation potentials were able to enhance plant growth under contamination stress. These results may aid in selection of the best hydrocarbon degraders with PGP traits for improving the efficiency of phytoremediation of PHC-contaminated soils. The outcome of this

study can be an effective approach for the developing integrated microbial inoculants for bioremediation biotechnology applications.

4.4 MATERIALS AND METHODS

4.4.1 Bacterial Strains

The 50 bacterial strains used in this study are a subset of a larger collection of 438 morphologically different bacteria isolated from bulk soil and the rhizosphere of *Salix purpurea* and *Eleocharis obtusa* plants growing in a site highly polluted with petroleum-hydrocarbons (Alotaibi et al., 2021b). This site is in Varennes, Quebec, Canada (45°43 N, 73°22 W), with an allocated area of approximately 5000 m^2 (Bell et al., 2014a; Alotaibi et al., 2021b) These bacterial strains were selected based on their phylogenetic affiliations to cover major bacterial lineages in order to increase taxonomic, genetic and functional diversities The identification of bacterial strains was performed using Sanger sequencing of the 16S rRNA gene, as described in Alotaibi et al. (2021b). The isolation source and the species affiliations of bacterial strains used in this study are summarized in Table 4.1. The bacterial strains were stored at −80 °C. Stock cultures were preserved in 20% glycerol at −80 °C. When reviving bacteria, isolates were cultured in 50 mL of 1/2 strength Trypticase Soy Broth (TSB) (Difco Laboratories Inc. Detroit, Michigan, USA) at room temperature for 48 h with continuous agitation at 150 rpm.

Table 4.1 List of bacterial isolates used in this study.

Isolation code	Environmental niche	Isolation medium	Phylum/Family	NCBI taxonomic identity (Accession #)
ST4	Bulk soil	1/10TSA	Actinobacteria/ Nocardiaceae	*Rhodococcus ruber* (MZ430450)
ST15	Bulk soil	1/10TSA	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas* sp. (MZ430461)
ST25	Bulk soil	1/10TSA	Gammaproteobacteria/ Xanthomonadaceae	*Stenotrophomonas nitritireducens* (MZ430471)
ST45	Bulk soil	1/10TSA	Actinobacteria/ Gordoniaceae	*Gordonia amicalis* (MZ430491)
SB26	Bulk soil	B-H_amended diesel	Actinobacteria/ Rhodobacteraceae	*Paracoccus* sp. (MZ430412)
SB32	Bulk soil	B-H_amended diesel	Actinobacteria/ Microbacteriaceae	*Microbacterium hatanonis* (MZ430418)
SB36	Bulk soil	B-H_amended diesel	Gammaproteobacteria/ Moraxellaceae	*Acinetobacter pittii* (MZ430422)
SB38	Bulk soil	B-H_amended diesel	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas stutzeri* (MZ430424)
SB39	Bulk soil	B-H_amended diesel	Actinobacteria/ Microbacteriaceae	*Microbacterium oxydans* (MZ430425)
SB41	Bulk soil	B-H_amended diesel	Gammaproteobacteria/ Moraxellaceae	*Acinetobacter* sp. (MZ430427)
SB45	Bulk soil	B-H_amended diesel	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas mosselii* (MZ430431)
SB49	Bulk soil	B-H_amended diesel	Betaproteobacteria/ Oxalobacteraceae	*Massilia oculi* (MZ430435)
SB50	Bulk soil	B-H_amended diesel	Alphaproteobacteria/ Sphingomonadaceae	*Sphingobium yanoikuyae* (MZ430436)
ET5	*Eleocharis* rhizosphere	1/10TSA	Actinobacteria/ Microbacteriaceae	*Microbacterium testaceum* (MZ430211)
ET10	*Eleocharis* rhizosphere	1/10TSA	Alphaproteobacteria/ Rhizobiaceae	*Rhizobium selenitireducens* (MZ430216)
ET25	*Eleocharis* rhizosphere	1/10TSA	Firmicutes/ Bacillaceae	*Bacillus marisflavi* (MZ430231)
ET27	*Eleocharis* rhizosphere	1/10TSA	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas plecoglossicida* (MZ430233)
ET33	*Eleocharis* rhizosphere	1/10TSA	Betaproteobacteria/ Comamonadaceae	*Delftia lacustris* (MZ430239)
ET46	*Eleocharis* rhizosphere	1/10TSA	Gammaproteobacteria/ Yersiniaceae	*Serratia* sp. (MZ430252)
ET49	*Eleocharis* rhizosphere	1/10TSA	Gammaproteobacteria/ Enterobacteriaceae	*Enterobacter bugandensis* (MZ430255)
EB6	*Eleocharis* rhizosphere	B-H_amended diesel	Betaproteobacteria/ Burkholderiaceae	*Chitinimonas taiwanensis* (MZ430152)
EB26	*Eleocharis* rhizosphere	B-H_amended diesel	Gammaproteobacteria/ Aeromonadaceae	*Aeromonas hydrophila* (MZ430172)
EB31	*Eleocharis* rhizosphere	B-H_amended diesel	Gammaproteobacteria/ Xanthomonadaceae	*Stenotrophomonas pavanii* (MZ430177)
EB35	*Eleocharis* rhizosphere	B-H_amended diesel	Betaproteobacteria/ Comamonadaceae	*Comamonas odontotermitis* (MZ430181)
EB37	*Eleocharis* rhizosphere	B-H_amended diesel	Actinobacteria/ Microbacteriaceae	*Lysinimonas* sp. (MZ430183)

Table 4.1 Continued.

Isolation code	Environmental niche	Isolation medium	Phylum/Family	NCBI taxonomic identity (Accession #)
EB43	*Eleocharis* rhizosphere	B-H_amended diesel	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas entomophila* (MZ430189)
WT8	*Salix* rhizosphere	1/10TSA	Actinobacteria/ Streptomycetaceae	*Streptomyces atriruber* (MZ430334)
WT10	*Salix* rhizosphere	1/10TSA	Firmicutes/ Bacillaceae	*Bacillus megaterium* (MZ430336)
WT12	*Salix* rhizosphere	1/10TSA	Actinobacteria/ Gordoniaceae	*Gordonia amicalis* (MZ430338)
WT17	*Salix* rhizosphere	1/10TSA	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas kilonensis* (MZ430343)
WT19	*Salix* rhizosphere	1/10TSA	Actinobacteria/ Micrococcaceae	*Pseudarthrobacter siccitolerans* (MZ430345)
WT34	*Salix* rhizosphere	1/10TSA	Actinobacteria/ Micrococcaceae	*Arthrobacter* sp. (MZ430360)
WT39	*Salix* rhizosphere	1/10TSA	Actinobacteria/ Streptomycetaceae	*Streptomyces atratus* (MZ430365)
WB17	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Micrococcaceae	*Paenarthrobacter nitroguajacolicus* (MZ430283)
WB23	*Salix* rhizosphere	B-H_amended diesel	Betaproteobacteria/ Comamonadaceae	*Variovorax paradoxus* (MZ430289)
WB25	*Salix* rhizosphere	B-H_amended diesel	Alphaproteobacteria/ Sphingomonadaceae	*Sphingomonas sanxanigenens* (MZ430291)
WB31	*Salix* rhizosphere	B-H_amended diesel	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas frederiksbergensis* (MZ430297)
WB40	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Micrococcaceae	*Pseudarthrobacter siccitolerans* (MZ430306)
WB46	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Nocardiaceae	*Nocardia* sp. (MZ430312)
WB48	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Nocardiaceae	*Streptomyces umbrinus* (MZ430314)
WB49	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Nocardioidaceae	*Nocardioides alpinus* (MZ430315)
WB51	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Gordoniaceae	*Nocardia asteroides* (MZ430317)
WB54	*Salix* rhizosphere	B-H_amended diesel	Actinobacteria/ Intrasporangiaceae	*Phycicoccus bigeumensis* (MZ430320)
SA7	Bulk soil	ACCD	Gammaproteobacteria/ Enterobacteriaceae	*Pantoea agglomerans* (MZ430101)
EA5	*Eleocharis* rhizosphere	ACCD	Gammaproteobacteria/ Enterobacteriaceae	*Klebsiella oxytoca* (MZ430073)
EA9	*Eleocharis* rhizosphere	ACCD	Gammaproteobacteria/ Enterobacteriaceae	*Enterobacter cancerogenus* (MZ430077)
EA21	*Eleocharis* rhizosphere	ACCD	Actinobacteria/ Microbacteriaceae	*Curtobacterium* sp. (MZ430089)
WA8	*Salix* rhizosphere	ACCD	Gammaproteobacteria/ Erwiniaceae	*Raoultella terrigena* (MZ430128)
WA19	*Salix* rhizosphere	ACCD	Gammaproteobacteria/ Enterobacteriaceae	*Citrobacter* sp. (MZ430139)
WA25	*Salix* rhizosphere	ACCD	Gammaproteobacteria/ Pseudomonadaceae	*Pseudomonas thivervalensis* (MZ430145)

4.4.2 Screening for *in vitro* PGP Characteristics

4.4.2.1 *Phosphate Solubilization*

The inorganic phosphate solubilization activity of bacterial isolates was determined using both qualitative and quantitative assays as described in Nautiyal (1999). In the qualitative assay using solid agar plates, fresh pure bacterial isolates were grown in half-strength TSB at 28 °C for 48 h with continuous agitation at 150 rpm in a rotary shaker. Then, 10 µL of growing bacterial culture were spot-inoculated into the center of NBRIP (the National Botanical Institute's phosphate growth medium) agar plates containing tri-calcium phosphate as the sole inorganic phosphate source (Nautiyal, 1999). The NBRIP agar plates were incubated at 28 °C for 14 days and a clear zone around inoculated colonies indicated the solubilization of inorganic phosphate. The test was replicated three times.

In the quantitative liquid assay, a loopful of pure bacterial isolates growing on 1/10 Trypticase Soy Agar (TSA) plates were inoculated into 125-mL Erlenmeyer flasks containing 50 mL freshly sterilized liquid NBRIP medium supplemented with tri-calcium phosphate as the sole inorganic phosphate source. The cultures were grown at 28 °C under continuous agitation at 150 rpm in a rotary shaker for up to 14 days. Five-milliliter aliquots were centrifuged at 10000 g for 10 min and the supernatant were filtered through a 0.2 µm Millipore filter and used for soluble P determination using ammonium-molybdate reagent (Fiske and Subbarow, 1925). The resultant blue-colored compound was measured by reading the absorbance at 650 nm using a multimode microplate spectrophotometer against a standard curve KH_2PO_4. The test was replicated three times.

4.4.2.2 *Indole-3-acetic Acid (IAA) Production*

The production of IAA by bacterial isolates was determined using both qualitative and quantitative assays as described in Patten and Glick (2002). Bacterial isolates were first cultured overnight in 5 mL of DF salts minimal medium, and then 20 µL aliquots were transferred into 15 mL Falcon tubes containing 5 mL of DF salts minimal medium supplemented with tryptophan (1 mg mL^{-1}) as auxin precursor. Cultures were grown in a shaker (120 rpm) for 48 h at 28 °C. One-milliliter aliquots of bacterial cultures were then centrifuged at 9500 g for 2 min and 100 µL of supernatant were added to a 96-well plate, followed by the addition of 100 µL of Salkowski's

reagent, and the 96-well 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. To quantify IAA produced by bacterial isolates, the absorbance was measured at 535 nm using a multimode microplate spectrophotometer against a standard curve of commercial IAA (Sigma-Aldrich, USA). The test was replicated three times.

4.4.2.3 *Siderophore Syntheses*

Siderophore production by bacterial isolates was determined qualitatively using the Chrome-Azurol S (CAS) assay as described in Alexander and Zuberer (1991). Pure bacterial isolates 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 the growing bacterial culture were spot-inoculated into the centers of CAS-agar plates. The CAS-agar plates were incubated at 28 °C for 72 h and bacterial isolates showing an orange halo were considered positive for siderophore synthesis (Schwyn and Neilands, 1987). The test was replicated three times.

Siderophore production quantification was estimated using the CAS-Shuttle assay performed in high-throughput mode using a 96-well format, as described in Payne (1994). Briefly, bacterial strains were inoculated into an iron-deficient MM9 medium to induce siderophore production and grown at 28 °C under continuous agitation. After 48 h, 100 µL of cell-free supernatant was mixed with 100 µL of CAS dye and 2 µL of shuttle solution. The 96-well plate was incubated in the dark for 15 min and the absorbance was measured at 630 nm using a multimode microplate spectrophotometer. The test was replicated three times.

4.4.2.4 *Ammonia Production*

The ammonia production by bacterial isolates was evaluated in both qualitative and quantitative assays as described in Cappuccino (1992) and as outlined in Dutta et al. (2015). The qualitative estimation of ammonia production was carried out by inoculating fresh bacterial isolates into 10 mL test tubes of peptone water (peptone 10 g. L^{-1}; NaCl 5 g. L^{-1}; 1 L dH_2O) and bacterial cultures were incubated for 72 h at 28 °C. Then, 1 mL aliquots of bacterial culture were transferred to 2 mL tubes and 50 µL of Nessler's reagent (10% HgI_2; 7% KI; 50% aqueous solution of NaOH (32%)) were added to each tube. A color change of the mix to yellow indicates ammonia production,

with a weak yellow indicating of small amount of production and a deep yellow being a sign of the maximum capacity of ammonia production (Marques et al., 2010). To quantify ammonia production by bacterial isolates, the absorbance was measured at 450 nm against a standard curve of ammonium sulphate using a multimode microplate spectrophotometer. The test was replicated three times.

4.4.2.5 *ACCD Activity*

ACCD activity was assessed by monitoring the bacterial isolate's ability to grow on DF minimal salts medium containing ACC as a sole nitrogen source (Penrose and Glick, 2003). Pure bacterial isolates were grown in half-strength TSB at 28 °C for 48 h under continuous agitation at 150 rpm in a rotary shaker. A loopful of each bacterial isolate growing in liquid culture was streaked into a DF minimal salts agar plate containing 3 mM ACC solution, which was spread into the agar plate immediately prior to use. Plates were incubated at 28 °C for up to one week. The presence of growth in the DF-ACC agar plates was considered positive. Bacterial strains were classified using a rating scale as follows: −, no growth; +, slightly growth; ++, moderate growth; +++, heavy growth. The test was replicated three times.

ACCD- activity was also confirmed via PCR amplification of the *acdS* gene (Blaha et al., 2006). More details regarding procedure and PCR conditions are given below.

4.4.2.6 *Nitrogen Fixation*

Bacterial isolates were evaluated regarding their capacity to grow on an N-deficient combined carbon medium (Rennie, 1981). Bacterial cultures were grown in half-strength TSB at 28 °C for 48 h under continuous agitation at 150 rpm in a rotary shaker. 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 °C for up to one week. The presence of growth in the agar plates was considered positive. Bacterial strains were classified using a rating scale as follows: −, no growth; +, slightly growth; ++, moderate growth; +++, heavy growth. The test was replicated three times.

Nitrogen fixation activity was also confirmed using PCR amplification of the *nifH* gene (Rösch et al., 2002). More details regarding the procedure and PCR conditions are given below.

4.4.3 Catabolic Gene Detection using PCR Amplifications

Polymerase chain reaction (PCR) analysis was used to assess the presence of hydrocarbon-degrading genes and PGPR genes in bacterial isolates selected in this study. Primers used to detect the presence of genes and PCR conditions are presented in Table S1.

PCR reactions for the analysis of the *alkB* gene were performed in a reaction volume of 25 µL, which consisted of 1× PCR buffer (Qiagen, Toronto, Canada), 0.8 µM of each primer, 0.2 mM of dNTP mix, 0.5 mM of $MgCl_2$, 0.2 mg mL^{-1} of BSA (Amersham Biosciences, Mississauga, Canada), 1.25 U of *Taq* DNA polymerase (Qiagen, Toronto, Canada) and 1 µL purified genomic DNA (Kloos et al., 2006). For the detection of the *CYP153* gene, PCR reactions were prepared as for the *alkB* gene (Wang et al., 2010b). In addition, PCR *nah* gene detection was conducted as previously described in Baldwin et al. (2003). Briefly, PCR analyses were performed in a reaction volume of 25 µL, which consisted of 1× PCR buffer, 0.5 µM of each primer, 1 µL of dNTP (Qiagen, Toronto, Canada), 0.5 mM of $MgCl_2$, 0.2 mg mL^{-1} of BSA, 1U of *Taq* DNA polymerase and 1 µL purified genomic DNA. For the detection of the *acdS* gene, PCR reactions were prepared as for the *nah* gene (Blaha et al., 2006). Finally, for the detection of the *nifH* gene, PCRs were performed in a reaction volume of 25 µL of 1× PCR buffer, 0.5 µM of each primer, 0.5 µL of dNTP, 0.5 µL of $MgCl_2$, 0.2 mg mL^{-1} of BSA, 1U of *Taq* DNA polymerase and 1 µL purified genomic DNA (Rösch et al., 2002).

The presence and length of PCR products were verified by electrophoresis with GelRed-stained 1.5% agarose gels using the Gel-Doc system (Bio-Rad Laboratories, Mississauga, Canada).

4.4.4 PGP Potential of Bacterial Isolates under Gnotobiotic Conditions

4.4.4.1 *Inoculum Preparation*

Bacterial isolates (Table 4.1) were first grown in fresh 1/10 TSA plates and incubated for 72–96 h at 28 °C. Then, pure colonies of each isolate were inoculated separately into a 500 mL Erlenmeyer flask containing 250 mL half-strength TSB medium. Bacterial isolates were incubated on a rotary shaker (150 rpm) at 28 °C for 48 h (except for the following isolates, which were grown for up to 120 h at 28 °C: *Rhodococcus ruber* ST4, *Sphingobium yanoikuyae* SB50, *Lysinimonas* sp. EB37,

Gordonia amicalis WT12, *Sphingomonas* sp. WB25, *Nocardioides alpinus* WB49, *Gordonia* sp. WB51 and *Phycicoccus* sp. WB54). The optical density (OD) of bacterial cells was measured and adjusted to an OD_{600} value of 1. Bacterial suspensions were harvested via centrifugation (15 min at 5000 g), washed three times in phosphate buffer saline (PBS; Difco Laboratories, Detroit, USA) and resuspended in 20 mL sterile tap water. Serial dilutions were then prepared in PBS and spread on 1/10 TSA plates, and incubated at 28 °C for 72 h. This yielded cell densities of approximately 10^9 (colony-forming units) cfu mL^{-1}.

4.4.4.2 ***Seed Inoculation***

Canola (cv. 4187 RR) seeds were surface-sterilized by washing with ethanol (95% $v.v^{-1}$) for 30 s, followed by soaking in NaClO (2.5% $v.v^{-1}$) for 10 min under constant gentle shaking (Hynes et al., 2008). Seeds were rinsed with sterile distilled water 10 times in order to remove excess sodium hypochlorite. The seeds were then air-dried by placing them in a biosafety cabinet for 24 h. Sub-samples of surface-sterilized seeds were picked randomly and placed onto 1/10-strength TSA plates and incubated at 28 °C for 24 h to further check for any contamination. Surface-sterilized seeds were soaked in 5 mL of bacterial suspension for 4 h with gentle shaking in a rotary shaker to allow the bacteria to penetrate into the seeds. For the control treatment (without bacterial inoculum), seeds were soaked in 5 mL autoclaved distilled H_2O.

4.4.4.3 ***Root Elongation Assay***

The root elongation assays were conducted under gnotobiotic conditions using growth pouches as previously described in Lifshitz et al. (1987). Seed growth pouches (16.5 × 18 cm) containing chromatographic filter paper (Mega International, Minneapolis, U.S.A) were filled with 10 mL of sterile half-strength N-free Hoagland's nutrient solution, wrapped in aluminum foil and sterilized at 121 °C for 20 min prior to seeding. Ten seeds soaked in the bacterial suspension were aseptically sown inside the growth pouches and five replicate pouches were used for each treatment and for the control. After seed germination, pouches were thinned to five seeds per pouch. The pouches were wrapped with Saran plastic wrap to minimize the loss of moisture and covered with aluminum foil to prevent light from reaching plant roots. The moisture content was kept constant during the time course of the experiment via additions of sterile distilled water and half-strength N-free Hoagland nutrient solution, on an

alternative day's basis, using aseptic techniques. The seeds were grown in growth pouches for 7 days at 28 °C, with a 16/8 h day/night cycle, before the root measurements were taken.

4.4.5 Growth of Bacterial Isolates in MSM with (1%) *n*-hexadecane and (1%) Diesel

Bacterial isolates were tested for their ability to utilize either 1% (v/v) filter-sterilized *n*-hexadecane or 1% (v/v) diesel in mineral salt medium (MSM) by measuring the cell density at 600 nm. Bacterial isolates were first grown in half-strength TSB at 28 °C for 48 h with continuous agitation at 150 rpm. Then, cells were collected via centrifugation, washed three times with PBS and resuspended in sterile dH_2O, and 10 μL were used to inoculate MSM amended with 1% *n*-hexadecane or 1% diesel. The assay was carried out in 125 mL Erlenmeyer flasks containing 50 mL sterile MSM and the *n*-hexadecane or diesel as carbon source, incubated at 28 °C under continuous agitation at 150 rpm on a rotary shaker. After a week, cells growth was measured at 600 nm and compared with a control containing no carbon source. The experiment was carried out in triplicate.

4.4.6 Gnotobiotic Assay under Alkane Stress Conditions

Seven candidates that performed well in different assays were selected and tested gnotobiotically for their plant growth promotion potential under different concentrations of *n*-hexadecane (0%, 1%, 2%, 3%). The growth of these isolates was also determined on MSM medium containing different concentrations of the compound (1%, 2%, 3%).

The bacterial strains tested in this experiment were *Acinetobacter* sp. strain SB41, *Pseudomonas putida* strain ET27, *Stenotrophomonas maltophilia* strain EB31, *Bacillus megaterium* strain WT10, *Gordonia amicalis* strain WT12, *Arthrobacter* sp. strain WT19 and *Nocardia* sp. strain WB46. The bacterial inoculum was prepared as described above.

The experiment was performed utilizing growth pouches as described above, with modifications. The ability of bacterial strains to enhance plant growth under alkane stress conditions was tested by including different concentrations of *n*-hexadecane (0%,

1%, 2%, 3%) in the sterile half-strength N-free Hoagland nutrient solution in the growth pouches. Briefly, ten seeds soaked in the bacterial suspension were aseptically sown inside the growth pouches and five replicated pouches were used for each treatment and control. After seed germination, pouches were thinned to five seeds per pouch. The pouches were wrapped with Saran plastic wrap to minimize the loss of moisture and covered with aluminum foil to prevent light from reaching the plant roots. The seeds were grown in growth pouches for 7 days at 28 °C, with a 16/8 h day/night cycle, before the root and shoot measurements were made.

4.4.7 *Growth of Bacterial Isolates on Different Concentrations of n-hexadecane*

The growth of bacterial strains was measured in sterile 50 mL MSM medium containing different concentrations of *n*-hexadecane (1%, 2%, 3%) as the carbon source in 125 mL Erlenmeyer flasks. Bacterial cultures were grown as described in above and then the bacterial cultures were incubated at 28 °C under continuous agitation at 150 rpm on a rotary shaker. Bacterial growth was determined by measuring the cell density at 600 nm every day for up to 7 days. Non-inoculated control treatments were included at each concentration. The experiment was carried out in triplicate.

4.4.8 Statistical Analyses

The growth pouch study was carried out in a completely randomized design. In the first growth pouch study, the data were presented as means and standard deviations, and the difference between treatments compared to the control were analyzed using Dunnett's test ($P = 0.05$). In the second growth pouch experiment, the differences between treatments were analyzed using one-way ANOVA at a 5% significance level with Tukey's post hoc test, using JMP software (SAS Institute Inc. Cary, NC, USA.). A Venn diagram was generated using *InteractiVenn* software (Heberle et al., 2015).

4.5 RESULTS

4.5.1 PGP Traits of Bacterial Isolates

A total of 50 bacterial isolates were screened for six different PGP traits and the presence of two genes encoding nitrogenase (*nifH*) and ACCD (*acdS*). The results of screening tests are shown in Table 4.2. Fourteen strains (28%) were able to solubilize calcium phosphate in the liquid medium (Table 4.2). Among these, several strains showed excellent P solubilization ability. *Bacillus megaterium* strain WT10 showed the highest solubilization activity with 690.86 µg mL^{-1} calcium phosphate (Table 4.2), followed by *Gordonia amicalis* strain WT12, *Curtobacterium* sp. strain EA21 and *Pseudomonas fluorescens* strain WT17, which were able to solubilize 567.12 µg mL^{-1}, 525.4 µg mL^{-1} and 476.48 µg mL^{-1} calcium phosphate, respectively (Table 4.2).

Out of 50 bacterial strains, 34 strains (68%) were able to produce IAA after 48 h of incubation with a 1 mg mL^{-1} supplement of tryptophan as an auxin precursor (Table 4.2). Three bacterial strains showed the highest IAA production among all the strains, specifically *Rhizobium* sp. strain ET10, *Curtobacterium* sp. strain EA21 and *Klebsiella* sp. EA5, which produced 44.31 µg mL^{-1}, 44.13 µg mL^{-1} and 31.32 µg mL^{-1} IAA, respectively (Table 4.2).

Twenty-four bacterial strains (48%) were able to synthesize siderophores (Table 4.2). The maximum production of siderophores by bacterial strains were observed in *Pseudomonas putida* strain ET27, *Enterobacter* sp. strain EA9, *Pseudomonas stutzeri* strain SB38, *Enterobacter cancerogenus* strain ET49 and *Pseudomonas fluorescens* strain WT17, which produced around 29% or above of siderophore units (Table 4.2).

Most of the bacterial strains under investigation were able to produce ammonia (Table 4.2). Most bacterial isolates produced ammonia in the range of 5.00 to 10.50 µmol mL^{-1} (Table 4.2). Four bacterial strains showed the maximum ammonia production among the strains, namely, *Comamonas* sp. strain EB35, *Chitinimonas* sp. strain EB6, *Microbacterium oxydans* strain SB39 and *Stenotrophomonas maltophilia* strain EB31 (Table 4.2).

All bacterial isolates were further screened qualitatively for ACCD and N fixation, of which 34 isolates (68%) demonstrated ACCD activity and also showed the presence of an ACCD gene (*acdS*) (Table 4.2). Additionally, 28 isolates (56%) showed the ability to fix atmospheric N_2 and the presence of the N fixation gene (*nifH*) (Table 4.2).

Table 4.2 Screening of selected bacterial strains for plant-growth-promoting traits.

Strain code	Identity	IAA production [a] (μg mL^{-1})	Phosphate solubilization (μg mL^{-1})	Siderophore production (%)	Ammonia production (μmol mL^{-1})	ACC deaminase [b]	Nitrogen fixation [c]	PCR [d]	
								acdS	*nifH*
ST4	*Rhodococcus ruber*	25.93	-	-	9.59	+	+	+	+
ST15	*Pseudomonas* sp.	11.51	-	13.4	7.35	++	+	-	+
ST25	*Stenotrophomonas nitritireducens*	21.48	-	7.6	9.81	+++	-	+	-
ST45	*Gordonia amicalis*	-	-	-	-	++	+++	+	+
SB26	*Paracoccus* sp.	-	-	-	9.47	-	-	-	-
SB32	*Microbacterium hatanonis*	-	-	9.16	7.39	++	+++	-	-
SB36	*Acinetobacter pittii*	-	420.36	22.3	-	+++	+	+	+
SB38	*Pseudomonas stutzeri*	13.78	-	32.1	8.11	-	-	+	-
SB39	*Microbacterium oxydans*	6.51	-	8.2	12.51	++	-	-	-
SB41	*Acinetobacter* sp.	4.39	369.28	-	7.25	+++	++	-	+
SB45	*Pseudomonas mosselii*	19.10	-	12.3	6.42	+++	+++	+	+
SB49	*Massilia oculi*	7.67	-	17.1	7.36	-	-	-	-
SB50	*Sphingobium yanoikuyae*	15.34	-	-	5.93	++	++	+	+
ET5	*Microbacterium testaceum*	11.01	-	-	8.23	-	-	-	-
ET10	*Rhizobium selenitireducens*	44.31	-	26.7	9.05	-	+++	-	+
ET25	*Bacillus marisflavi*	-	-	-	8.61	+++	-	-	-
ET27	*Pseudomonas plecoglossicida*	11.80	424.68	36.4	7.07	+++	+++	+	+
ET33	*Delftia lacustris*	5.05	-	-	-	+++	-	+	-
ET46	*Serratia* sp.	13.47	381.51	24.8	7.06	++	+	-	+
ET49	*Enterobacter bugandensis*	9.18	-	31.1	8.59	++	++	-	+
EB6	*Chitinimonas taiwanensis*	-	-	-	13.05	++	-	-	-
EB26	*Aeromonas hydrophila*	1.67	-	-	10.50	-	-	-	-
EB31	*Stenotrophomonas pavanii*	29.44	380.79	19.6	11.46	++	+	-	+
EB35	*Comamonas odontotermitis*	-	-	14.9	13.95	+	-	+	+
EB37	*Lysinimonas* sp.	-	-	-	8.64	-	-	-	-
EB43	*Pseudomonas entomophila*	0.45	-	-	8.89	++	++	-	+
WT8	*Streptomyces atriruber*	0.53	72.16	-	9.16	-	-	-	-
WT10	*Bacillus megaterium*	-	690.86	-	9.27	-	-	-	-
WT12	*Gordonia amicalis*	0.45	567.12	-	7.97	++	+++	+	+
WT17	*Pseudomonas kilonensis*	8.41	476.48	29. 3	6.41	+++	++	+	+

Table 4.2 Continued.

Strain code	Identity	IAA production [a] (µg mL^{-1})	Phosphate solubilization (µg mL^{-1})	Siderophore production (%)	Ammonia production (µmol mL^{-1})	ACC deaminase [b]	Nitrogen fixation [c]	PCR [d]	
								acdS	*nifH*
WT19	*Pseudarthrobacter siccitolerans*	11.56	-	10.1	6.70	-	+	-	+
WT34	*Arthrobacter* sp.	10.32	-	-	10.34	-	-	-	-
WT39	*Streptomyces atratus*	-	-	-	5.0	-	-	-	-
WB17	*Paenarthrobacter nitroguajacolicus*	-	-	12.1	6.86	+++	++	+	+
WB23	*Variovorax paradoxus*	-	-	-	-	++	+++	+	+
WB25	*Sphingomonas sanxanigenens*	1.14	-	8.3	9.10	++	-	-	-
WB31	*Pseudomonas frederiksbergensis*	9.68	449.86	22.8	7.19	+++	++	+	+
WB40	*Pseudarthrobacter siccitolerans*	7.88	-	-	7.28	-	-	-	-
WB46	*Nocardia* sp.	1.46	-	8.2	6.67	-	-	-	-
WB48	*Streptomyces umbrinus*	-	-	-	9.05	++	+++	+	-
WB49	*Nocardioides alpinus*	8.49	-	-	9.71	-	-	-	-
WB51	*Nocardia asteroides*	-	-	-	8.60	++	++	+	+
WB54	*Phycicoccus bigeumensis*	0.24	72.16	-	9.35	-	-	-	-
SA7	*Pantoea agglomerans*	19.37	-	29.1	8.42	+++	-	+	-
EA5	*Klebsiella oxytoca*	31.32	-	18.7	7.85	+++	+++	+	+
EA9	*Enterobacter cancerogenus*	10.58	-	36.4	8.25	+++	+	+	+
EA21	*Curtobacterium* sp.	44.13	525.4	-	8.60	+++	+++	-	+
WA8	*Raoultella terrigena*	13.54	-	-	7.97	+++	++	+	+
WA19	*Citrobacter* sp.	-	338.35	-	8.42	+++	+	+	+
WA25	*Pseudomonas thivervalensis*	-	324.68	19.3	7.11	+++	+++	+	+

Values are means of three replicates ± SD.

a. Indole-3-acetic acid

b. 1-aminocyclopropane- 1-carboxylate deaminase " –" means showed no growth on agar plates, "+" means showed low growth on agar plates, "++" means showed medium growth on agar plates and "+++" means showed heavy growth on agar plates.

c. The presence of growth in the agar plates was considered positive for nitrogen fixation " –" means showed no growth on agar plates, "+" means showed low growth on agar plates, "++" means showed medium growth on agar plates and"+++" means showed heavy growth on agar plates.

d. " –" indicates the absence of PCR products and "+" indicates the presence of PCR products for the functional genes—*acdS*: ACCD gene, *nifH:* nitrogen fixation gene.

4.5.2 Root Elongation Assay

The bacterial isolates were tested on canola root elongation under gnotobiotic conditions. The results indicated that the highest canola root elongation effect was induced by the following bacterial isolates: *Curtobacterium* sp. EA21, *Bacillus megaterium* WT10 and *Gordonia* sp. ST45, which significantly increased ($P \leq 0.05$) canola root elongation by 118%, 98% and 86%, respectively, compared with the control treatment (Figure 4.1). Other isolates that significantly increased ($P \leq 0.05$) canola root length included *Citrobacter* sp. WA19, *Pseudomonas thivervalensis* WA25, *Microbacterium oxydans* SB39, *Pseudomonas mosselii* SB45, *Pseudomonas stutzeri* SB38, *Acinetobacter pittii* SB36, *Pseudomonas putida* ET27, *Stenotrophomonas maltophilia* EB31, *Gordonia amicalis* WT12, *Arthrobacter* sp. WB40 and *Klebsiella* sp. EA5 (Figure 4.1). In contrast, bacterial isolates *Arthrobacter* sp. WT34, *Pseudomonas* sp. WB31 and *Gordonia* sp. WB51 tended to inhibit canola root elongation, although this effect was not significant at $P = 0.05$ (Figure 4.1).

4.5.3 *n*-hexadecane and Diesel Degradation Potential, and Presence of *alkB, CYP153* and *nahA* Genes in Bacterial Isolates

n-hexadecane was used as the sole carbon source for the growth of 29 isolates (58%) (Table 4.3). Some isolates, such as *Gordonia amicalis* ST45, *Comamonas odontotermitis* EB35, *Pseudomonas fluorescens* WT17, *Nocardia* sp. WB46, *Nocardia asteroides* WB51 and *Phycicoccus bigeumensis* WB54, showed high growth rates when they were cultivated in MSM medium supplemented with *n*-hexadecane as the sole source of carbon and energy (Table 4.3).

Similarly, 43 bacterial isolates (86%) were able to utilize diesel as the sole carbon source (Table 4.3). Isolates *Rhodococcus ruber* ST4, *Gordonia amicalis* ST45, *Comamonas odontotermitis* EB35, *Bacillus megaterium* WT10, *Gordonia amicalis* WT12, *Pseudomonas kilonensis* WT17, *Paenarthrobacter nitroguajacolicus* WB17, *Sphingomonas sanxanigenens* WB25, *Nocardia* sp. WB46, *Nocardia asteroides* WB51 and *Enterobacter cancerogenus* EA9 showed the highest growth rate when they were cultivated in MSM medium supplemented with diesel as the sole source for carbon and energy (Table 4.3).

The detection of the presence of functional genes related to PHC-degradation—*alkB, CYP153* and *nah1*—was used to evaluate the biodegradation ability of bacterial isolates. The *alkB* gene was detected in 34 isolates (68%) by PCR amplification (Table

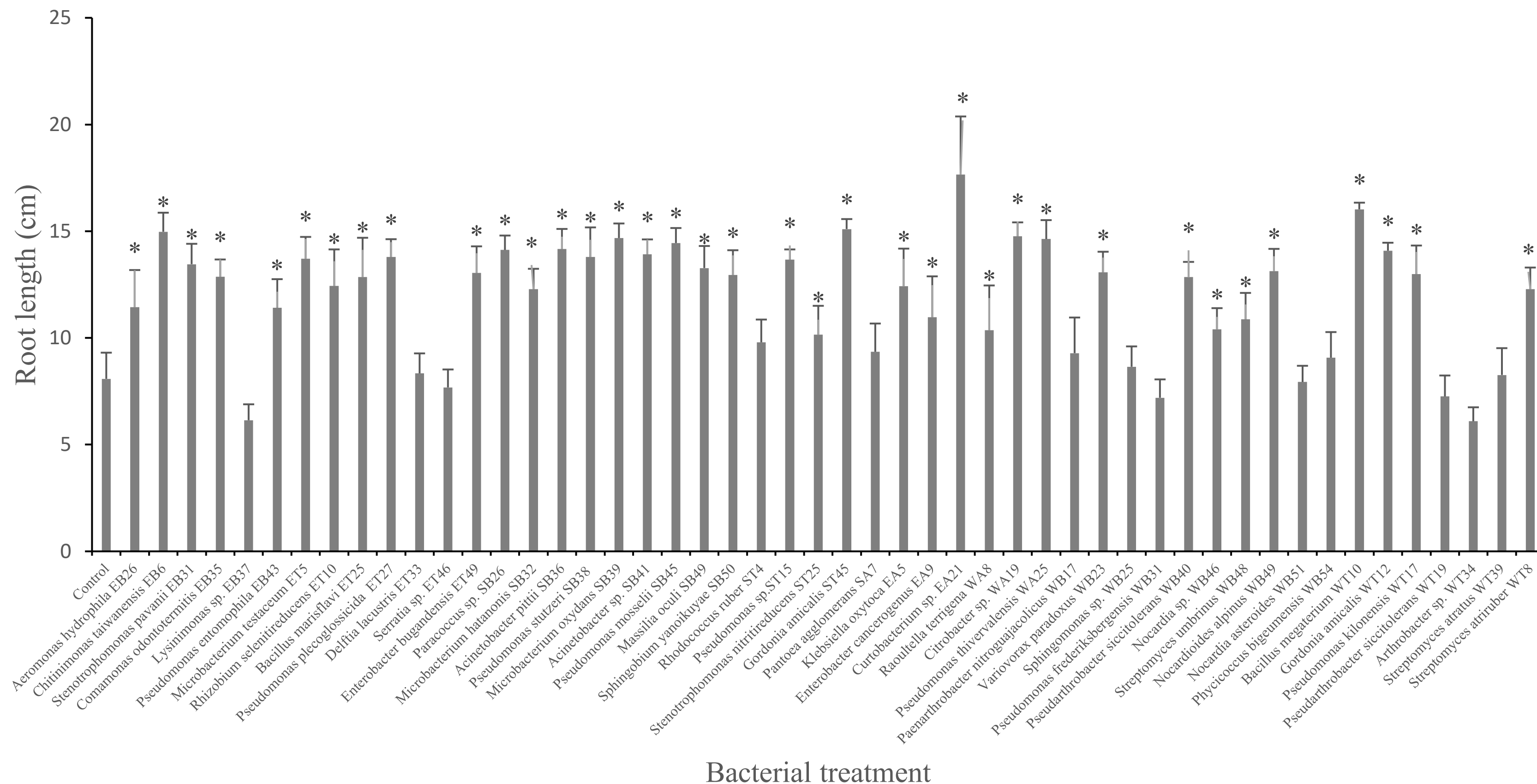

Figure 4.1 Effect of selected PGPR bacterial isolates on root length (cm) of canola plants measured after 7 days of growth. Error bars represent standard deviations and * indicates a significant difference compared to the control according to a Dunnett test, $p \leq 0.05$.

4.3). The *CYP153* gene was also detected in 24 isolates (48%) (Table 4.3), whereas 33 bacterial isolates (66%) possessed the *nah1* gene (Table 4.3).

Notably, five hexadecane-degrading bacterial isolates possessed all PGP traits under investigation (Figure 4.2) specifically, *Pseudomonas putida* ET27, *Serratia* sp. ET46, *Stenotrophomonas maltophilia* EB31, *Pseudomonas fluorescens* WT17 and *Pseudomonas* sp. WB31 (Figure 4.2).

4.5.4 Canola Growth Promotion under *n*-hexadecane Gradient

Further screening tests were performed on bacterial isolates in order to assess their plant-growth-promoting activity using canola plants in growth pouches amended or not with a gradient of *n*-hexadecane concentrations ranging from 0% as a control to 3%. Under the conditions of no hydrocarbon stress (0% *n*-hexadecane), the consortium significantly increased ($P \leq 0.05$) root length compared to all isolates inoculated individually and to the control (Figure 4.3). All bacterial isolates, except *Pseudarthrobacter siccitolerans* WT19, significantly increased ($P \leq 0.05$) their root length compared to the control treatment (Figure 4.3). The highest growth promotion was observed in inoculations with *Stenotrophomonas pavanii* EB31 and *Gordonia amicalis* WT12 (Figure 4.3). Similarly, all bacterial treatments significantly increased ($P \leq 0.05$) the shoot lengths of canola plants compared to the control (Figure 4.3). The highest growth promotion was observed in the consortium treatment (Figure 4.3).

When canola seedlings were grown in the presence of different concentrations of *n*-hexadecane (1%, 2%, 3%), the resultant hydrocarbon stress caused a decrease in both root length and shoot length among most of the treatments (Figures 4.3, 4.4, 4.5 and 4.6). For example, canola seedlings treated with *Stenotrophomonas pavanii* EB31, *Gordonia amicalis* WT12 and *Pseudomonas plecoglossicida* ET27 grown in the presence of 3% *n*-hexadecane stress showed shoot lengths that were decreased by up to 60% compared to seedlings inoculated with the same strains grown in the absence of the hydrocarbon (Figures 4.3 and 4.6). However, under 3% *n*-hexadecane amendment, all bacteria treatments significantly ($P \leq 0.05$) increased their shoot length when compared with the control treatment (Figure 4.6). The highest shoot enhancement was induced by the isolate *Nocardia* sp. WB46 and the consortium treatment (Figure 4.6).

Unlike shoot length, canola seedlings grown under different concentrations of *n*-hexadecane showed an almost 16% decrease in root length when compared with

Table 4.3 Ability of bacterial isolates to grow on aliphatic compounds and to possess hydrocarbon degradation genes.

Strain	Identity [a]	Isolation medium	Growth in diesel [b]	Growth in hexadecane [c]	PCR[d]		
					alkB	*CYP153*	*nah*
ST4	*Rhodococcus ruber*	1/10TSA	+++	+++	+	+	+
ST15	*Pseudomonas* sp.	1/10TSA	+	-	-	-	+
ST25	*Stenotrophomonas nitritireducens*	1/10TSA	++	-	+	-	-
ST45	*Gordonia amicalis*	1/10TSA	++++	++++	+	+	-
SB26	*Paracoccus* sp.	B-H_amended diesel	+	-	-	+	+
SB32	*Microbacterium hatanonis*	B-H_amended diesel	++	-	+	-	+
SB36	*Acinetobacter pittii*	B-H_amended diesel	-	++	+	-	+
SB38	*Pseudomonas stutzeri*	B-H_amended diesel	++	+	-	+	+
SB39	*Microbacterium oxydans*	B-H_amended diesel	+	-	+	-	-
SB41	*Acinetobacter* sp.	B-H_amended diesel	+++	++	+	-	+
SB45	*Pseudomonas mosselii*	B-H_amended diesel	++	-	-	+	+
SB49	*Massilia oculi*	B-H_amended diesel	-	+	+	-	+
SB50	*Sphingobium yanoikuyae*	B-H_amended diesel	+++	+	+	-	+
ET5	*Microbacterium testaceum*	1/10TSA	+	-	-	-	+
ET10	*Rhizobium selenitireducens*	1/10TSA	+	-	+	+	+
ET25	*Bacillus marisflavi*	1/10TSA	++	-	-	-	+
ET27	*Pseudomonas plecoglossicida*	1/10TSA	+++	+	+	+	+
ET33	*Delftia lacustris*	1/10TSA	-	+	+	-	+
ET46	*Serratia* sp.	1/10TSA	-	+	+	-	+
ET49	*Enterobacter bugandensis*	1/10TSA	+++	-	+	+	+
EB6	*Chitinimonas taiwanensis*	B-H_amended diesel	++++	+	-	+	+
EB26	*Aeromonas hydrophila*	B-H_amended diesel	+	+	+	-	-
EB31	*Stenotrophomonas pavanii*	B-H_amended diesel	++++	+++	+	+	+
EB35	*Comamonas odontotermitis*	B-H_amended diesel	+	++++	+	-	+
EB37	*Lysinimonas* sp.	B-H_amended diesel	+	-	+	-	+
EB43	*Pseudomonas entomophila*	B-H_amended diesel	+	+	+	-	-

Table 4.3 Continued.

Strain	Identity	Isolation medium	Growth in diesel	Growth in hexadecane	PCR		
					alkB	*CYP153*	*nah*
WT8	*Streptomyces atriruber*	1/10TSA	-	-	-	-	+
WT10	*Bacillus megaterium*	1/10TSA	+++	+	+	-	-
WT12	*Gordonia amicalis*	1/10TSA	++++	++++	+	+	+
WT17	*Pseudomonas kilonensis*	1/10TSA	+++	+	-	+	-
WT19	*Pseudarthrobacter siccitolerans*	1/10TSA	+	++	+	-	+
WT34	*Arthrobacter* sp.	1/10TSA	++	+	+	-	-
WT39	*Streptomyces atratus*	1/10TSA	+	-	-	-	-
WB17	*Paenarthrobacter nitroguajacolicus*	B-H_amended diesel	++++	+	+	+	+
WB23	*Variovorax paradoxus*	B-H_amended diesel	++	+	+	-	+
WB25	*Sphingomonas sanxanigenens*	B-H_amended diesel	+++	-	+	-	-
WB31	*Pseudomonas frederiksbergensis*	B-H_amended diesel	+	+++	-	+	-
WB40	*Pseudarthrobacter siccitolerans*	B-H_amended diesel	++	++	-	-	+
WB46	*Nocardia* sp.	B-H_amended diesel	++++	++++	+	-	+
WB48	*Streptomyces umbrinus*	B-H_amended diesel	++	+	+	+	-
WB49	*Nocardioides alpinus*	B-H_amended diesel	+	-	-	+	+
WB51	*Nocardia asteroides*	B-H_amended diesel	+++	++++	+	+	+
WB54	*Phycicoccus bigeumensis*	B-H_amended diesel	-	++++	+	-	-
SA7	*Pantoea agglomerans*	ACCD	+	-	+	-	-
EA5	*Klebsiella oxytoca*	ACCD	+	-	-	+	-
EA9	*Enterobacter cancerogenus*	ACCD	+++	+	+	+	+
EA21	*Curtobacterium* sp.	ACCD	+	-	-	+	-
WA8	*Raoultella terrigena*	ACCD	++	-	+	+	+
WA19	*Citrobacter* sp.	ACCD	-	+	-	+	-
WA25	*Pseudomonas thivervalensis*	ACCD	++	+	+	+	+

a. Indicates 16S rDNA identity of bacterial strains with their closest type strains in GenBank.
b. Indicates growth capability of bacterial strains on 1% (v:v) diesel in MSM. ++++, +++, ++, + and -, indicating the growth capability from strong to weak with diesel as a sole carbon and energy source, measured by optical density at 600 nm, after one week incubation at 28 °C. ++++, growth ($OD_{600} > 1$); +++, growth ($OD_{600} > 0.6$); ++, growth ($0.6 > OD_{600} > 0.2$); +, growth ($OD_{600} < 0.2$); -, no growth.
c. Indicates growth capability of bacterial strains on 1% (v:v) *n*-hexadecane in MSM. ++++, +++, ++, + and -, indicating the growth capability from strong to weak with *n*-hexadecane as a sole carbon and energy source, measured by optical density at 600 nm, after one week incubation at 28 °C. ++++, growth ($OD_{600} > 1$); +++, growth ($OD_{600} > 0.6$); ++, growth ($0.6 > OD_{600} > 0.2$); +, growth ($OD_{600} < 0.2$); -, no growth.
d. Indicates "-" absence of PCR products and "+" presence of PCR products for the functional genes: *alkB*: alkane monooxygenase, *CYP153*: cytochrome P450 hydroxylase and *nah*: naphthalene dioxygenase.

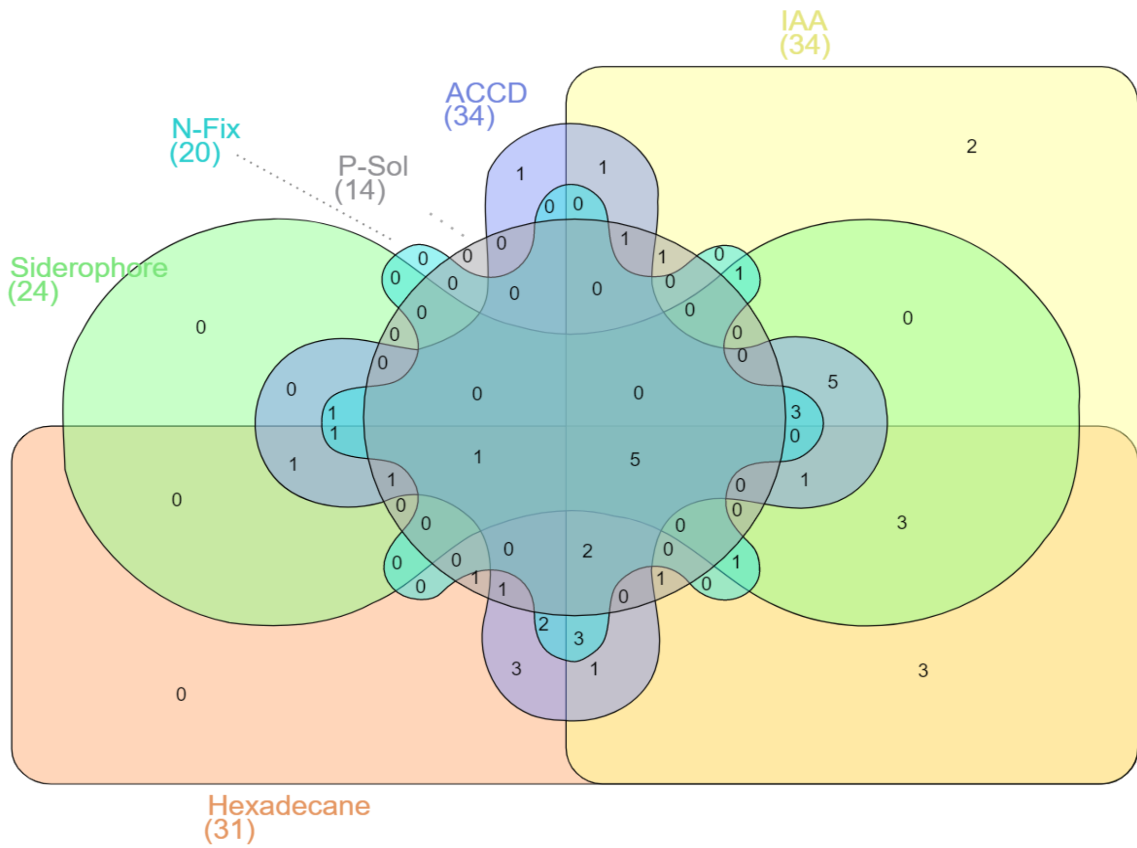

Figure 4.2 Venn diagram representation of 50 rhizobacterial strains, showing positive results for hexadecane degradation potential and different PGP traits (with 5 strains showing positive results for all the traits under investigation).

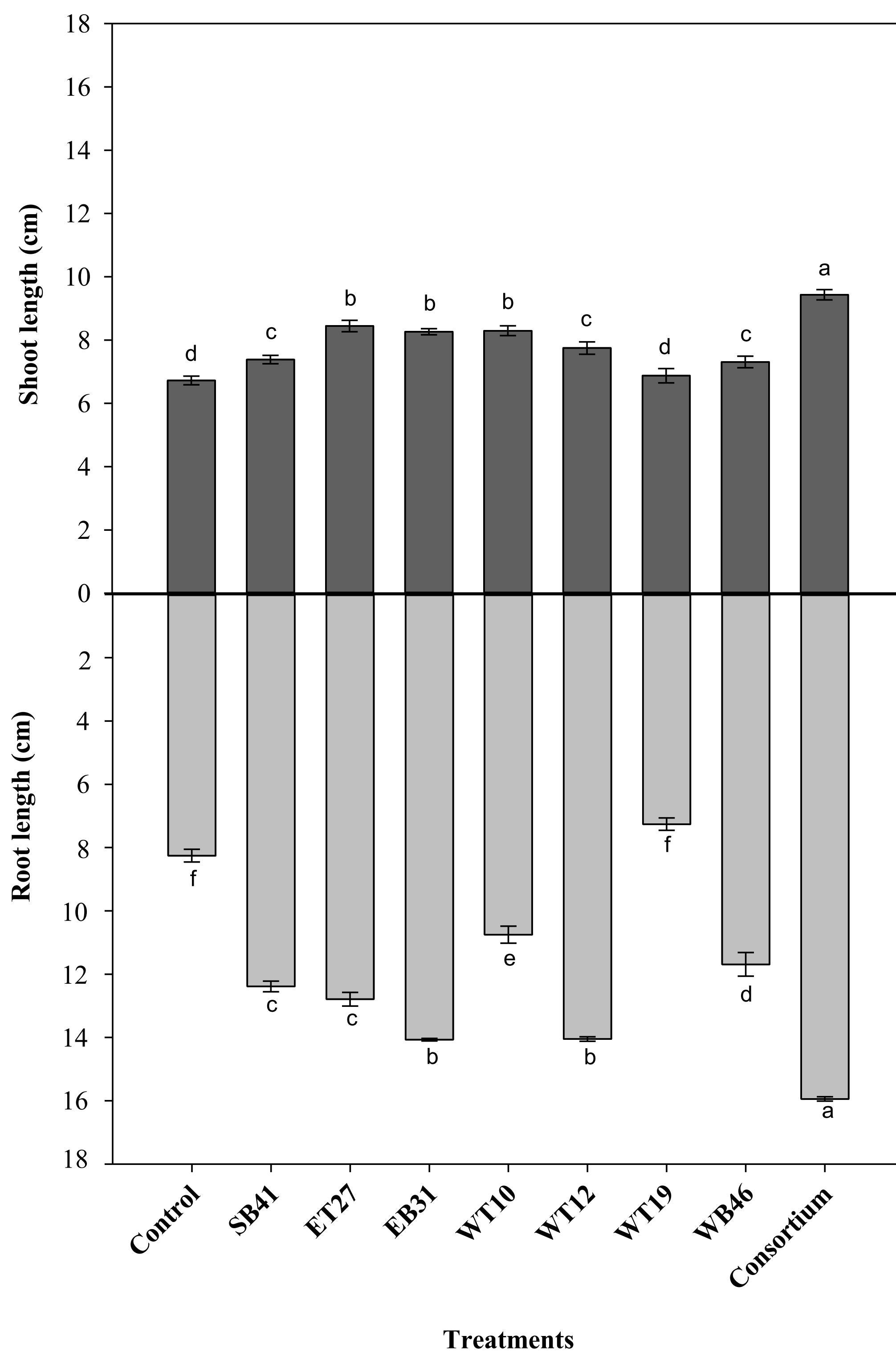

Figure 4.3 Effect of selected PGPR bacterial strains on root and shoot length (cm) of canola plants measured after 7 days of growth in the presence of 0% *n*-hexadecane. Error bars represent standard deviations and different letters indicate significance according to Tukey's post hoc test at $p \leq 0.05$.

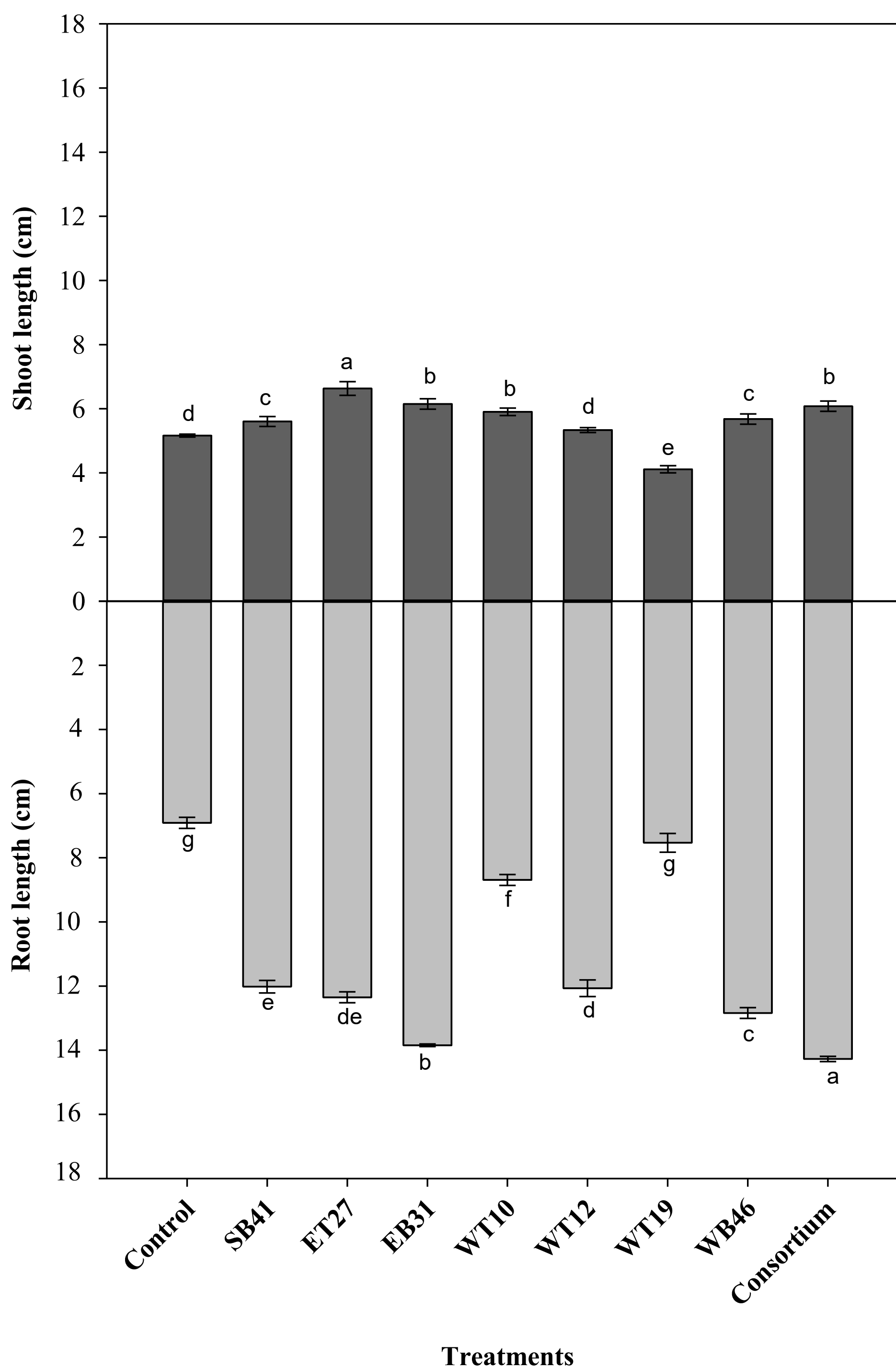

Figure 4.4 Effect of selected PGPR bacterial strains on root and shoot length (cm) of canola plants measured after 7 days of growth in the presence of 1% *n*-hexadecane. Error bars represent standard deviations and different letters indicate significance according to Tukey's post hoc test at $p \leq 0.05$.

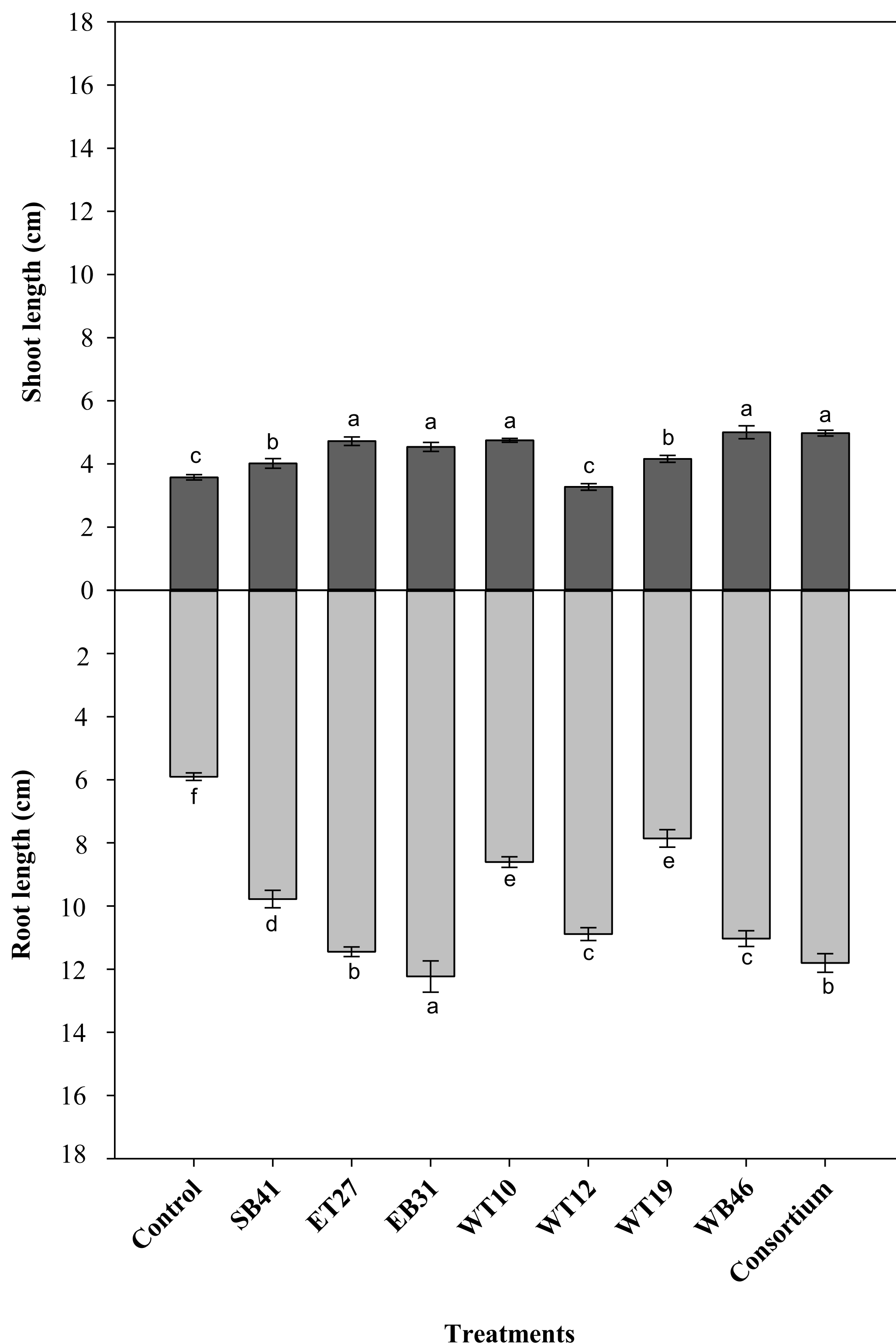

Figure 4.5 Effect of selected PGPR bacterial strains on root and shoot length (cm) of canola plants measured after 7 days of growth in the presence of 2% *n*-hexadecane. Error bars represent standard deviations and different letters indicate significance according to Tukey's post hoc test at $p \leq 0.05$.

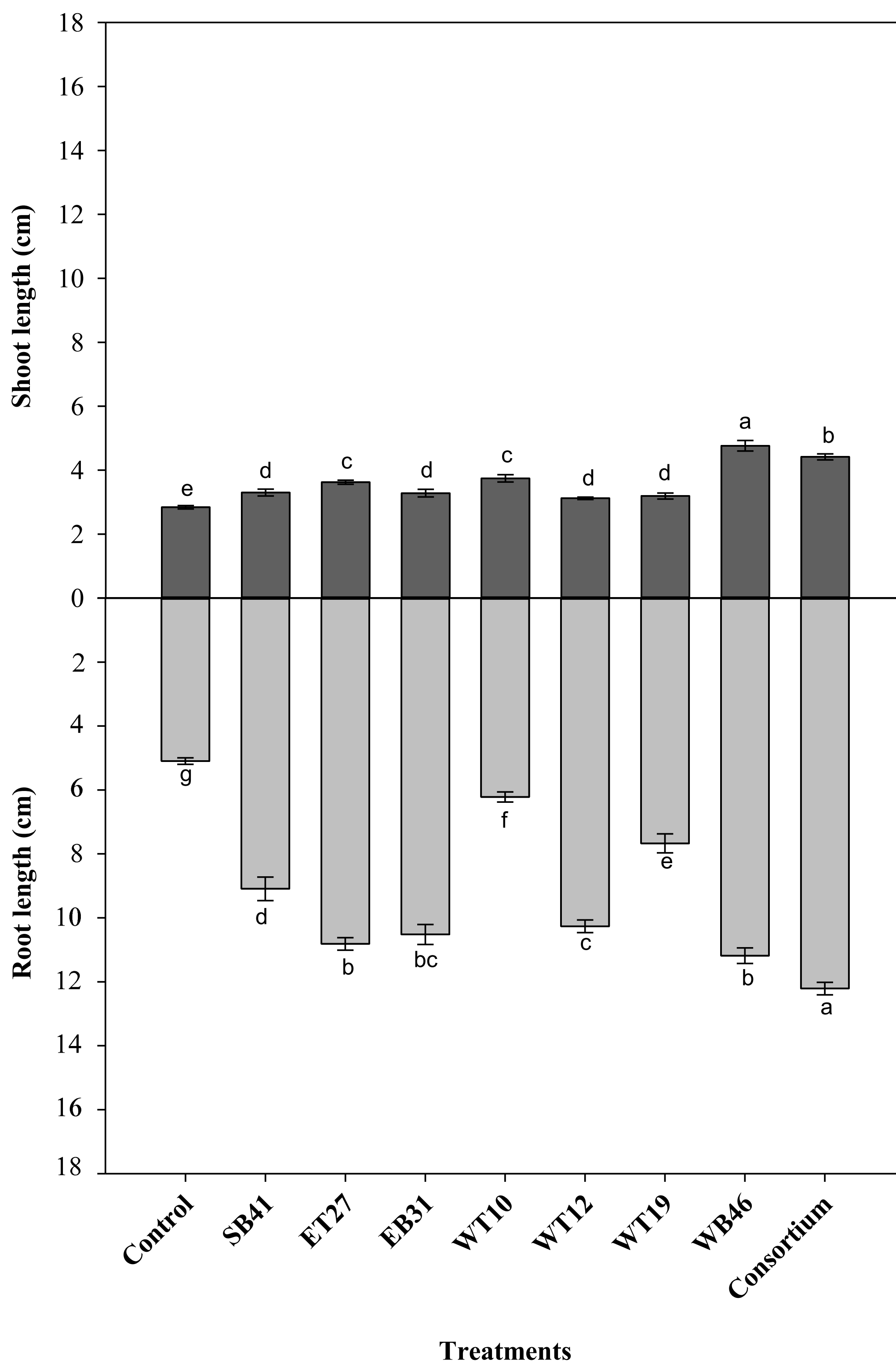

Figure 4.6 Effect of selected PGPR bacterial strains on root and shoot length (cm) of canola plants measured after 7 days of growth in the presence of 3% *n*-hexadecane. Error bars represent standard deviations and different letters indicate significance according to Tukey's post hoc test at $p \leq 0.05$.

seedlings grown in the absence of the hydrocarbon (Figures 4.3, 4.4, 4.5 and 4.6). In the presence of 3% *n*-hexadecane stress, all bacteria treatments significantly ($P \leq 0.05$) increased root length when compared with the control treatment (Figure 4.6). The highest root growth promotion was induced by the consortium treatment of *Nocardia* sp. WB46, *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31 (Figure 4.6).

4.5.5 Growth of Bacterial Isolates in Different Concentrations of *n*-hexadecane

Bacterial isolates were grown in different concentrations of *n*-hexadecane (1%, 2%, 3%) to determine the effect of increasing concentrations on bacterial growth. The results of this experiment indicated that when the concentration of *n*-hexadecane increased, the growth rate of some hexadecane-degrading bacteria was inhibited (Figure 4.7). For example, *Bacillus megaterium* WT10 has an OD of 0.470 in 1% *n*-hexadecane, whereas this value decreased to 0.230 in the presence of the 3% concentration of *n*-hexadecane (Figure 4.7). Similar trends were observed for *plecoglossicida* ET27, *Stenotrophomonas pavanii* EB31 and *Acinetobacter* sp. SB41. In contrast, *Nocardia* sp. WB46 and *Pseudarthrobacter siccitolerans* WT19 showed increased bacterial growth as the *n*-hexadecane concentrations increased (Figure 4.7). For example, *Nocardia* sp. WB46 had an OD of 1.6 with 1% *n*-hexadecane, whereas its growth rate increased to 2.3 with 3% *n*-hexadecane (Figure 4.7).

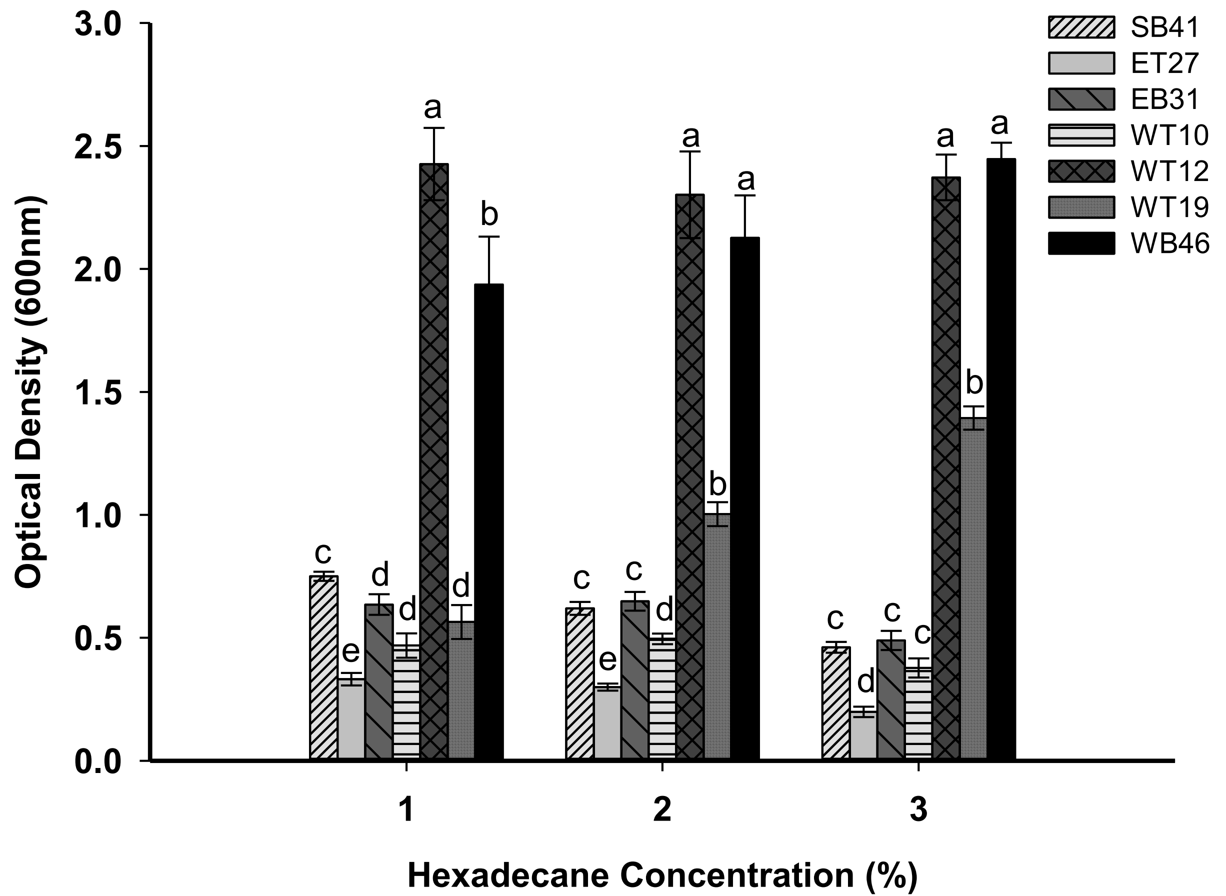

Figure 4.7 Effect of different concentrations of *n*-hexadecane (1%, 2%, 3%) on bacterial growth of selected bacterial strains. All strains were grown for 7 days. Error bars represent standard deviations and different letters indicate significance according to Tukey's post hoc test at $p \leq 0.05$.

4.6 DISCUSSION

The use of plants in combination with bacteria possessing the ability to degrade petroleum hydrocarbons and to promote plant growth is an efficient and environmentally sustainable strategy to remediate diesel-contaminated soils. High concentrations of PHCs can have phytotoxic effects on plants growing on contaminated soils (Baek et al., 2004). Therefore, the use of bacterial strains with multiple PGP and hydrocarbon degradation capabilities have crucial advantages for plants growing in such hostile environments.

To select bacterial isolates for the phytoremediation of diesel-contaminated soil, it is important to consider characteristics such as high degradation potential, the presence of alkane-degrading genes and a robust substrate affinity, as well as multiple PGP traits, such as the production of plant growth regulator substances and the ability to improve nutrient acquisition, which may enhance plant growth under contamination stress (Balseiro-Romero et al., 2017a).

We previously isolated and identified 438 PHC-degrading bacteria with multiple PGP characteristics from rhizosphere soil of *Salix purpurea* and *Eleocharis obtusa* plants growing in a highly PHC-contaminated site (Alotaibi et al., 2021b). In this study, we selected and characterized 50 bacterial isolates in depth based on their taxonomic and functional diversities and they were tested for their alkane degradation potential, PGP traits and plant growth promotion potential under normal and stressed conditions using growth pouch assays.

PGPR regulate plant growth via diverse sets of mechanisms (Lugtenberg and Kamilova, 2009; Schlaeppi and Bulgarelli, 2015). In the present work, bacterial isolates exhibited several PGP traits, including P solubilization, IAA production, siderophore synthesis, ammonia production, ACCD activity and N-fixation (Table 2). Ammonia production may play a role in enhancing plant growth through the accumulation of N and subsequently increasing biomass production (Marques et al., 2010). Ammonia production was the most common PGP trait observed among the strains. Similar results were reported in Dutta and Thakur (2017) in the characterization of 48 bacterial strains isolated from different tea cultivars in India. This suggest that ammonia production is among the mechanisms used by PGPR to stimulate plant growth. It has been shown that ammonia produced by PGPR supplies N to their host plants and thus promotes root and shoot elongation (Marques et al., 2010; Bhattacharyya et al., 2020).

The ability to fix nitrogen would provide a selective advantage for hydrocarbon-degrading bacteria used in phytoremediation applications, particularly in N-limited soils (Foght, 2018). Our results indicated that majority of diazotrophic bacteria belonged to *Gammaproteobacteria* (Table 2). In line with our findings, several reports indicate that diazotrophic bacteria predominate in PHCs-contaminated environments were affiliated to *Gammaproteobacteria* (Church et al., 2008; Radwan et al., 2010; Do Carmo et al., 2011) including taxa such as *Acinetobacter*, *Pseudomonas*, *Azotobacter*, *Stenotrophomonas* and *Klebsiella* (Eckford et al., 2002; Dashti et al., 2009; Do Carmo et al., 2011; Foght, 2018; Alotaibi et al., 2021b). One of the constrains limiting biodegradation activities of microbial communities in PHC-contaminated soils is the lack of sufficient nutrients especially nitrogen. Thus, application of diazotroph could offer a sustainable and efficient approach to enhance bioaugmentation and phytoremediation of PHC-contaminated soils (Dashti et al., 2009; Foght, 2018)

Bacteria capable of solubilizing inorganic forms of P may promote plant growth by improving the nutrient uptake of plants. The majority of bacterial isolates that showed P solubilization activity in this study belonged to *Proteobacteria* (Table 2). This corroborates previous reports about the ability of many bacterial strains isolated from plants growing in agricultural and contaminated soils and belonging to this phylum to have P solubilization abilities (Chowdhury et al., 2017; Pawlik et al., 2017; Lumactud and Fulthorpe, 2018). Iron-chelating siderophores are another important factor for PGP. PGPR produce siderophores that bind Fe^{3+} and render it available for reduction to Fe^{+2}, a preferred form for plant roots uptake (Oleńska et al., 2020). Similar results were reported previously in Príncipe et al. (2007) in the characterization of PGPR isolates from saline soils. In a recent study, Eze et al. (2022) isolated a PGP and diesel-degrading bacterial consortium, dominated by *Alphaproteobacteria*, that led to a 66% increase in *Medicago sativa* biomass and resulted in 91% removal of diesel hydrocarbons in just 60 days. Functional metagenome analysis of the consortium revealed the presence of several genes responsible for PGP traits, including N-fixation, phosphate solubilization and siderophore production (Eze et al., 2022). The prevalence of PGP genes in the consortium may account for not only the growth promotion of *M. sativa* but also their tolerance of diesel toxicity (Eze et al., 2022).

PGPR capable of lowering levels of ACC, a precursor of ethylene phyto-hormone, may stimulate growth and stress tolerance in plants under normal and stressed

conditions (Glick, 2014). The high percentage of ACCD-producing bacteria in our study is in agreement with previous reports documenting the prevalence of this phenotype in various soil bacteria isolated from many stressed environments (Belimov et al., 2001; Mayak et al., 2004; Sandhya et al., 2010; Ali and Kim, 2018). Tara et al. (2014) reported that maximum bacterial population, plant biomass and hydrocarbon degradation activity were achieved for carpet grass plants growing in soil spiked with diesel and inoculated with bacterial strains (*Pseudomonas* sp. ITRH25, *Pantoea* sp. BTRH79 and *Burkholderia* sp. PsJN) possessing both alkane-degradation and ACCD activity, compared to bacterial strains possessing only alkane degradation activity.

IAA produced by PGPR is responsible for increasing root elongation, lateral root formation and root hairs, thus enhancing the water and nutrient uptake efficiency of the plant root system (Lugtenberg and Kamilova, 2009). Some recent studies have shown that rhizobacteria isolated from PHC-contaminated soil including strains of *Arthrobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Rhodococcus* sp., *Pantoea* sp., *Pseudomonas* sp., *Stenotrophomonas* sp. and *Streptomyces* sp. are also PGPR-producing IAA (Balseiro-Romero et al., 2017a; Pawlik et al., 2017; Lumactud and Fulthorpe, 2018; Kidd et al., 2021). In a recent study, Li et al. (2021) reported a significant plant growth enchantment of ryegrass growing in PHC-contaminated soils and co-inoculated with bacterial strains *Arthrobacter pascen* and *Bacillus cereus,* possessing both IAA production and fluoranthene (Flu) degradation traits. Additionally, the Flu concentration was enhanced in the roots and shoots of inoculated plants (Li et al., 2021). The increase in the absorption and transport of Flu into plant tissues was attributed to the effect of IAA-producing bacteria on plants growth. IAA producing microbes would increase plant growth, which may increase the production of extra root exudates and lead to a higher transpiration rate, thus improving the rate of mineralization, solubility and transport of Flu into the plant tissues (Técher et al., 2011; Li et al., 2021).

Plant-related factors in the rhizosphere, such as the production of organic compounds in root exudates, might affect the survival and colonization of PGPR and their ability to express many PGP activities (Drogue et al., 2012; Alemneh et al., 2021). Therefore, we used a plant-based strategy for screening PGPR regarding their plant-growth-promoting potential. In our study, numerous bacterial strains significantly increased the root elongation of canola plants (Figure 1). Our results are in line with the findings of Asghar et al. (2004), who screened the effect of 100 rhizobacterial strains

on the promotion of canola root growth under gnotobiotic conditions and found that 58% enhanced root growth. Several studies have suggested that the PGPR isolates that most effectively promote plant growth produce both IAA and ACCD (Glick, 2014; Balseiro-Romero et al., 2017b; Kang et al., 2019). In our study, the highest root-growth promotion effect was observed with *Curtobacterium* sp. strain EA21. This strain produced the highest amount of IAA among all strains (44.13 µg mL^{-1}; Table 2) and produced ACCD, as demonstrated by the plate assay and the positive PCR amplification result (Table 2). The cross-talk between IAA and ACCD is fundamental for PGPR to enhance root growth (Glick, 2014). Several studies have reported that PGPR producing IAA higher than 40 µg mL^{-1} inhibited root growth and seed germination (Pawlik et al., 2017; Alemneh et al., 2021) due to the stimulation of ethylene caused by the higher amount of IAA (Glick, 2014). However, if the bacterium has both IAA and ACC deaminase activities, then the ACCD would mediate the decreasing of ethylene production, thus permitting IAA synthesis, which could continue to enhance root growth (Glick, 2014; Kang et al., 2019; Alemneh et al., 2021). Several other PGPR in our study that promoted root growth and produced both IAA and ACC deaminase included strains such as *Microbacterium oxydans* strain SB39, *Pseudomonas mosselii* strain SB45, *Pseudomonas stutzeri* strain SB38, *Pseudomonas plecoglossicida* strain ET27, *Stenotrophomonas pavanii* strain EB31, *Gordonia amicalis* strain WT12 and *Klebsiella oxytoca* strain EA5 (Figure 1). Interestingly, *Bacillus megaterium* strain WT10, which was unable to express ACCD and IAA, significantly enhanced the root growth of canola plants compared to the control treatment (Figure 1). Therefore, the positive effect of this strain might be related to its ability to solubilize inorganic phosphate up to 690.86 µg mL^{-1} and its ammonia production (Table 2). Similar results were reported in Alemneh et al. (2021), who observed that several strains belonging to *Bacillus* spp. enhanced the growth and nodulation of chickpeas under gnotobiotic conditions. The improvement of plant growth was mainly related to the ability of these strains to express PGP traits other than IAA and ACCD (Alemneh et al., 2021). Interestingly, bacterial strains tested in this experiment showed growth-promoting potential for canola plants despite being isolated from different plant species. This fact suggests that these PGPR strains are non-host-specific, thus having huge potential as inoculants to promote plant growth in phytoremediation, as well as in organic agriculture.

Degradative bacteria can enhance the removal of alkanes and reduce the phytotoxicity of pollutants in soils due to their capability to possess hydrocarbon-degrading enzymes (van Beilen and Funhoff, 2007; Arslan et al., 2014). In our study, numerous bacterial strains had the potential to utilize aliphatic hydrocarbons (Table 3). Several authors have reported both large populations and high diversities of alkane-degrading bacteria in various habitats, ranging from marine environments to polar soils (Whyte et al., 2002a; Yakimov et al., 2007; Jurelevicius et al., 2013; Lumactud et al., 2016; Pawlik et al., 2017).

Alkane hydroxylases (AHs) genes are responsible for the aerobic biodegradation of alkanes by bacteria (van Beilen and Funhoff, 2007). In our study, various bacterial strains harbored *AlkB* and *CYP153*-related AHs. These two AHs genes demonstrate a complementary substrate range. *AlkB* is involved in the degradation of medium-chain alkanes (C10-C20), whereas *CYP153* catalyzes the biodegradation of short-chain alkanes (C5-C16) (Rojo, 2009; Ji et al., 2013a; Wang and Shao, 2013). Similar results were reported by Pawlik et al. (2017)**,** who screened 26 bacterial strains isolated from *Lotus corniculatus* and *Oenothera biennis* plants growing in a long-term polluted site, and found that 50% of these strains were equipped with *CYP153* genes.

Previous research has shown that AH genes are often associated with *Betaproteobacteria* and *Gammaproteobacteria*, particularly the *Pseudomonas* genus (van Beilen and Funhoff, 2007; Liu et al., 2014; Garrido-Sanz et al., 2019; Eze et al., 2021). In our study, AH-degrading genes were also found in strains that belonged to *Betaproteobacteria* and *Gammaproteobacteria*; in addition, members of the *Actinobacteria*, such as *Gordonia*, *Arthrobacter*, *Nocardia*, *Rhodococcus* and *Rhodococcus*, were found to harbor these genes (Table 3). The wider taxonomic affiliations of bacterial strains capable of metabolizing alkanes demonstrate the potential of this culture collection for the remediation of diesel-contaminated soils.

Interestingly, several strains tested in this study had multiple *AlkB* and *CYP153* genes coexisting together (Table 3). The co-occurrence of multiple AHs has been reported previously in several bacterial strains, such as *Acinetobacter* sp. ADP1 (Barbe et al., 2004), *Dietzia* sp. DQ12-45-1b (Nie et al., 2011) and *Amycolicicoccus subflavus* DQS3-9A1 (Nie et al., 2014c). Undoubtedly, the coexistence of multiple AH genes in one bacterium would extend the alkane substrate range, thus enhancing the adaptation ability and subsequently the degradation potential of the host bacterium (Sun et al., 2018).

Under contaminant stress conditions (the second growth pouch experiments with various *n*-hexadecane concentrations), the inoculation of canola seeds with the selected PGPR strains either alone or in consortia generally provoked a significant increase in both the root length and shoot length of canola seedlings when compared with control plants (Figures 3, 4, 5 and 6). This indicates that PGPR inoculants exert a positive effect on plant growth under such stressful conditions. In agreement with our results, Balseiro-Romero et al. (2017b) reported that the inoculation of *Cytisus striatus* L. and *Lupinus luteus* L plants, grown in 1.25% diesel-contaminated soil in a pot experiment, with diesel-degrading bacterial strains with multiple PGP activities significantly improved plant growth. In the present experiment, selected hexadecane-degrading strains were evaluated for their ability to promote the growth of canola plants under increasing hexadecane concentrations (Table 3). Additionally, these selected hexadecane-degrading strains possessed multiple PGP traits (Table 2). Among the hexadecane-degrading strains, after the consortium treatments, the actinobacterium *Nocardia* sp. strain WB46 was found to be the best plant growth promoter among all the strains assessed (Figure 6). This strain showed robust growth on hexadecane as a sole energy source (Figure 7). Genome analyses revealed that *Nocardia* sp. strain WB46 contains three copies of the *alkB* gene (unpublished data). *AlkB* is a class of alkane hydroxylase enzymes that is responsible for the microbial degradation of oil and fuel additives, as well as many other compounds (van Beilen and Funhoff, 2007; Nie et al., 2014b). *Nocardia* sp. strain WB46 was also shown to possess several PGP activities, such as IAA, siderophore and ammonia production (Table 2). IAA is a phytohormone responsible for increasing root elongation and the formation of lateral root and root hairs, thus enhancing the water and nutrient uptake efficiency of plant root systems (Lugtenberg and Kamilova, 2009), whereas the production of siderophores and ammonia play a role in enhancing plant growth by increasing the nutrient acquisition efficiency of Fe^{+2} and N, respectively (Lugtenberg and Kamilova, 2009; Marques et al., 2010).

Other hexadecane-degrading isolates, specifically *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31, exhibited excellent plant growth promotion potential (Figure 6). Although these two isolates do not utilize hexadecane as efficiently as *Nocardia* sp. WB46 (Figure 7), they were shown to possess strong PGP capabilities (Table 2). *Pseudomonas plecoglossicida* ET27 was able to produce all PGP traits under investigation in this study. In agreement with our results,

Balseiro-Romero et al. (2017b) reported the characterization of *Pseudomonas* strain 12, which was isolated from the rhizosphere of poplar plants growing in a diesel-contaminated site. In their study, *Pseudomonas* stain 12 was able to solubilize P, produce siderophore, synthesize IAA and produce ACCD, as well as promoting plant growth when used as an inoculum to enhance the growth of plants growing in diesel-contaminated soils (Balseiro-Romero et al., 2017b). *Stenotrophomonas pavanii* EB31 was also shown to possess all PGP features. Several recent studies highlighted the potential of the members of genus *Stenotrophomonas* having multiple PGP traits to be used as inoculants in the bioremediation of PHC-contaminated soils (Pawlik et al., 2017; Lumactud and Fulthorpe, 2018; Mitter et al., 2019; Alotaibi et al., 2021b).

Interestingly, *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31 contain genes for N-fixation and alkane degradation. Earlier studies reported that other N-fixers such as *Frankia* spp. were found to harbored alkane genes in addition *nifH* gene (Rehan et al., 2016). Diazotroph capable of coupling N-fixation to hydrocarbon degradation represent a key strategy to promote plant growth in N-limited marginal lands such as PHC-contaminated soils (Foght, 2018). Thus, enhancing the efficiency of phytoremediation of PHC-contaminated soils.

Our results along with previous reports (Tara et al., 2014; Balseiro-Romero et al., 2017a, b; Kidd et al., 2021), support our hypothesis that bacteria with multiple PGP and pollutant degradation characteristics performed better than strains with only one of these traits. In addition, hexadecane-degrading activity could be considered itself a PGP feature, because pollutants have a harmful effect on plant growth and development.

4.7 CONCLUSIONS

In conclusion, the results of our study suggest that the screening of rhizobacteria for *in vitro* PGP activities, aliphatic hydrocarbon degradation potential and root growth promotion under gnotobiotic conditions is an effective approach for the selection of efficient PGPR candidates for bioremediation biotechnology applications. After several rounds of screenings, bacterial strains *Nocardia* sp. WB46, *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31 showed the highest growth stimulation when grown under the presence of 3% *n*-hexadecane. These isolates originated from a unique site with high concentration of PHC pollution, scored positive for PGP traits and hexadecane degradation potentials, indicating the potential to serve as inoculants for assisting the phytoremediation of diesel-contaminated soils. Additionally, with this culture collection in hand, a better understanding of the role of plant growth promotion in the phytoremediation of PHC-contaminated soils can be achieved through additional phenotypic and in planta characterization, whole genome sequencing and the construction of bacterial consortia for field applications.

4.8 ACKNOWLEDGEMENTS

This study was supported by Funds from Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to MH (Grant number: RGPIN-2018-04178), Genome Quebec, Genome Canada, F.A. was also supported by a grant from King Saud University via the Saudi Arabian Cultural Bureau in Ottawa which are gratefully acknowledged.

Chapter Five: Draft Genome sequencing of *Nocardia* sp. strain WB46 Isolated from *Salix purpurea* Growing in a Chronically Petroleum Hydrocarbon-Contaminated Site

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In preparation for submission to a scientific journal.

Author Contributions: F.A., M.S.-A. and M.H. conceived and initiated the study; F.A. performed the experiments and analyzed the data; 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 submitted version of the manuscript.

5.1 Preface:

In the previous chapter, 50 bacterial strains were characterized in depth using different approaches such as *in vitro* screening using six conventional plant-growth-promoting (PGP) traits, complemented with the characterization of genes involved in alkane degradation, as well as other pertinent functions, and by testing their growth in alkanes compounds. Finally, the bacterial isolates were subjected to plant growth promotion tests using a gnotobiotic approach under normal and stressed conditions. After several rounds of screenings, bacterial strain *Nocardia* sp. WB46 showed the highest plant growth stimulation when grown under the presence of 3% *n*-hexadecane. Therefore, we decided to sequence the genome of this isolate to gain more insight into its genetic versatility and the mechanisms of PHCs biodegradation, PGP traits, which might potentially aid in rhizoremediation applications.

5.2 Abstract:

Nocardia sp. strain WB46 was isolated from the rhizosphere of *Salix* plants growing in soil contaminated with petroleum hydrocarbons. *Nocardia* sp. strain WB46 exhibits a 7.15 Mb (69.55% GC content) draft genome sequence containing 6,387 protein-coding genes, 51 tRNA and 15 rRNA sequences, and many genes responsible for petroleum hydrocarbon degradation such as alkane 1-monooxygenase (*alkB*) and naphthalene dioxygenase (*ndo*). 16S rRNA gene analyses, *in silico* DNA-DNA hybridization (DDH) and average nucleotide identity (ANI) all suggest that *Nocardia* sp. strain WB46 is a new species. Interestingly, the sequence divergence of 16S rRNA gene showed that the region of divergence only occurs in the V2 region. Therefore, the conventional V3-V4 targeting metabarcoding would not be able to assess the diversity related with this new species.

5.3 Introduction:

The genus *Nocardia* belongs to the family *Nocardiaceae* of the order Corynebacteriales, *within the phylum Actinobacteria* (Zhi et al., 2009). Nocardia species are ubiquitous in both aquatic and terrestrial habitats, such as soil, water and animal's decaying fecal deposits (Brown-Elliott et al., 2006), with a prevailing importance in clinical and environmental settings (Luo et al., 2014; Mehta and Shamoo, 2020). Since the first isolation of *Nocardia* sp. by Edmond Nocard in 1888 (Nocard, 1888), more than 119 species have been described so far (http://www.bacterio.net/). Many species of *Nocardia* are opportunistic pathogens for human and animals (Beaman and Beaman, 1994). However, more recently, several species of *Nocardia* were found to produce novel bioactive substances (Schneider et al., 2007; El-Gendy et al., 2008) and to degrade various petroleum hydrocarbons compounds (Brzeszcz and Kaszycki, 2018). Clearly, this genus demonstrates a potential to be harnessed for biodegradation of petroleum hydrocarbons. However, there are still only a few species isolated and confirmed for the biodegradation ability.

Nocardia sp. strain WB46 was isolated from the rhizosphere of *Salix* plants growing in soil contaminated with petroleum hydrocarbons, from an abandoned petrochemical plant located at Varennes, Québec, Canada (Alotaibi et al., 2021b). *In vitro* analyses indicated that this bacterium can utilizes a wide range of petroleum hydrocarbons compounds, as a sole source of carbon to grow and reproduce, including aliphatic and aromatic hydrocarbons (Table 5.1). *Nocardia* sp. strain WB46 also showed positive activities for some plant growth-promoting traits such as phosphate solubilization and siderophores production, when tested under *in vitro* conditions, suggesting it can be a useful partner for bioremediation with plants (Table 5.1) (Alotaibi, 2021); see also chapter 3 and 4).

Table 5.1 Hydrocarbon degradation potential and plant growth promoting traits of bacterial strain *Nocardia* sp. WB46[1].

Assays		Activity
Hydrocarbons Degradation Potential	Naphthalene	++
	Phenanthrene	+++
	Pyrene	++
	Dodecane	++
	Hexadecane	+++
Catabolic genes	*alkB*	+
	CYP153	-
	Nah1	+
Cell growth measurement at 600 nm	1% diesel	++++
	1% hexadecane	++++
	2% hexadecane	++++
	3% hexadecane	++++
Plant growth-promoting traits	ACCD[2]	-
	Phosphate solubilization	-
	Siderophore production	+ (8.2 %)
	Nitrogen fixation	-
	IAA production[3]	+ (1.46 µg mL^{-1})
	Ammonia production	+ (2.9 µmol mL^{-1})
Root elongation assay (cm)	0%	13.4
	1%	12.2
	2%	10.1
	3%	8.6

[1] Data presented in this table are taken from Alotaibi et al., (2022). See also Chapter 4 for more information on the methodology used and results.

[2] 1-aminocyclopropane- 1-carboxylate deaminase

[3] indole-3-acetic

5.4 Materials and Methods

Genomic DNA was extracted from stationary-phase cells grown in 1/10 Trypticase Soy Broth (TSB) (Difco Laboratories, Detroit, USA) medium using the DNeasy UltraClean Microbial Kit (Qiagen, Toronto, Canada), according to the manufacturer's instructions. DNA concentration was determined on a Qubit fluorometer (Invitrogen, Thermo Fisher Scientific, USA). The genomic library was prepared with a NEB Ultra II kit (New England BioLabs Inc., Ipswich, USA) and sequenced on an Illumina MiSeq platform with 250-bp paired-end chemistry. Raw paired-end sequences were subjected to quality trimming using SeqMan NGen software (DNAStar Inc.). Genome assembly was also performed using SeqMan NGen software (DNAStar Inc.). Gene annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016). The *in silico* DNA-DNA hybridization (DDH) value was calculated using the Genome-to-Genome distance calculator version 2.1 (GGDC) (http://ggdc.dsmz.de/ggdc_background.php#) (Meier-Kolthoff et al., 2013). The average nucleotide identity (ANI) analyses were determined between *Nocardia* sp. strain WB46 and closely related strains using the NCBI's PGAP –taxcheck option (Tatusova et al., 2016). The 16S rRNA gene sequence (length of 1516 bp) derived from the assembled genome was compared with the available sequences in the Ribosomal Database Project (RDP) database using the SeqMatch tool (https://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp).

5.5 Results and discussion

In total 1,605,568 raw paired-end sequences were subjected to quality trimming using SeqMan NGen software (DNAStar Inc.). From assembling, we obtained 7,150,745 bp in 10 contigs. The genome of *Nocardia* sp. strain WB46 has an average G+C content of 69.55% and includes 6,387 predicted protein coding sequences (CDSs), 15 rRNAs (5S, 16S, 23S), 51 tRNAs, and three noncoding RNAs (ncRNAs) sequences. Detailed genomic information is presented in Tables 5.2 and 5.3, and in Figure 5.1.

In silico DNA-DNA hybridization (DDH), Type (Strain) Genome Server (TYGS), average nucleotide identity (ANI) and 16S rRNA gene analyses all suggested that *Nocardia* sp. strain WB46 is in fact a new species. The *in silico* DNA-DNA hybridization (DDH) value between *Nocardia* sp. strain WB46 and closely related strain *Nocardia asteroides* showed a distance of 63.4%. This value was below the threshold level of 70% recommended by Wayne et al. (1987) for assigning bacterial strains to the same species, thus suggesting that *Nocardia* sp. strain WB46 is a new species. The *Nocardia* sp. strain WB46 was also uploaded to the Type (Strain) Genome Server (TYGS) (https://tygs.dsmz.de) for a whole genome-based taxonomic analysis. *Nocardia* sp. strain WB46 did not belong to any species found in TYGS database, and was tagged as a potential new species. Additionally, the average nucleotide identity (ANI) analyses result predicted *Nocardia* sp. strain WB46 as *Nocardia asteroides*, but the value of 88.63% is below the generally proposed species boundary cut-off of 95-96% (Kim et al., 2014). Additional pairwise genome comparisons between *Nocardia* sp. strain WB46 and *Nocardia asteroides* with other (ANI)'s tools all suggested *Nocardia* sp. strain WB46 being a new species: ChunLab's ANI Calculator (https://www.ezbiocloud.net/tools/ani) (Yoon et al., 2017): OrthoANIu = 88.16%, JSpeciesWS (http://jspecies.ribohost.com/jspeciesws/): ANIb = 87.27%,

Table 5.2 Genome Statistics of bacterial strain *Nocardia* sp. WB46.

Attribute	Value
Genome Size (bp)	7,150,745
Genes (total)	6571
CDSs (total)	6502
Genes (coding)	6387
CDSs (with protein)	6387
RNA genes	69
Complete rRNAs	5 (5S)
Partial rRNAs	5, 5 (16S, 23S)
tRNA genes	51
rRNA genes	5, 5, 5 (5S, 16S, 23S)
ncRNAs	3
Pseudogenes	115
CDSs[1] (without protein)	115
Pseudogenes (frameshifted)	37 of 117
Pseudogenes (incomplete)	82 of 115
Pseudogenes (internal stop)	13 of 115

[1] CDSs: Coding DNA Sequences

Table 5.3 Scaffold (ordered contigs) organization of the genome of bacterial strain *Nocardia* sp. WB46.

Contigs	Length (bp)	Avg. coverage	Notes
Contig_67	727310[1]	112.71	Contig_67 - (16S)
Contig_73	1130037	105.52	(23S-5S) - Contig_73 - (16S)
Contig_5	72035	96.54	(23S-5S) - Contig_5 - (16S)
Contig_26	542152	100.53	(23S-5S) - Contig_26 - (16S)
Contig_3	235293	94.91	(23S-5S) - Contig_3 - (16S)
Contig_100	99836	91.74	(23S-5S) - Contig_100 - (GC-rich region)
Contig_60	131949	95.00	(GC-rich region) - Contig_60
Contig_90	2014676	90.74	Contig_90
Contig_11	90874	93.65	Contig_11
Contig_24	2106583	102.85	Contig_24 Contig_67[†]

[1] The scaffold is circular, with a 111 bp overlap between the end of Contig_24 and the beginning of Contig_67.

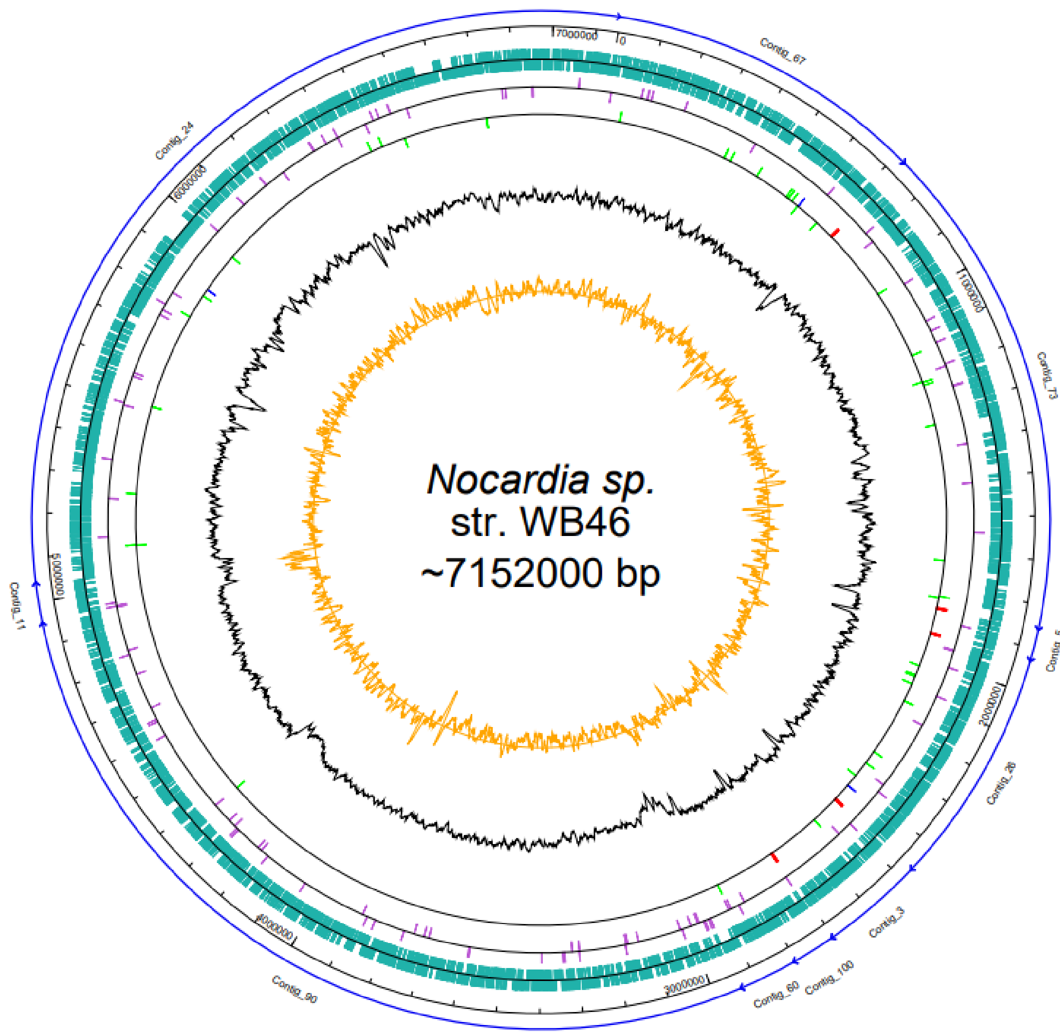


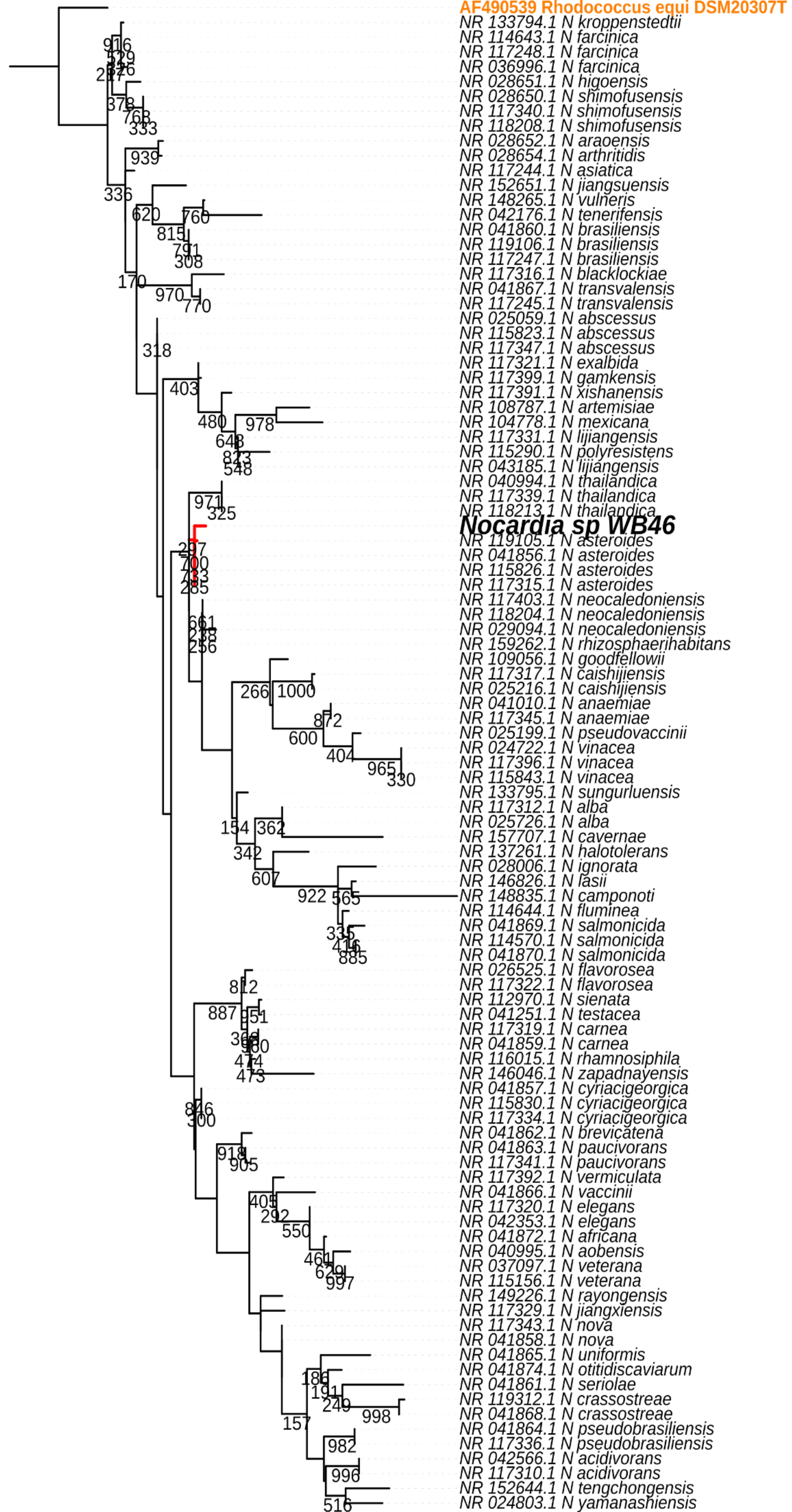
Figure 5.1 Map of the scaffolded contigs of the *Nocardia* strain WB46**.** From outer to inner ring: the individual contigs (blue arrows), scale, coding sequences (green) on forward strand and reverse strand, pseudogenes (purple) on forward strand and reverse strand, RNA genes on forward strand and reverse strand (tRNAs green, rRNAs red, other RNAs blue), G + C content (black), CG-skew (orange).

ANIm = 89.20% and Kostas lab ANI Calculator (http://enve-omics.ce.gatech.edu/ani/): two-way ANI = 88.23%.

Finally, in order to resolve the taxonomic position of *Nocardia* sp. strain WB46, a 16S rRNA gene sequence (length of 1516 bp) derived from the assembled genome was compared with the available sequences in the Ribosomal Database Project (RDP) database. The SeqMatch tool classified the almost complete 16S rRNA gene sequence of strain *Nocardia* sp. WB46 into the genus *Nocardia* with high sequence similarity to a sequence being identified as *Nocardia asteroides* (98.8%). Recently, 16S rRNA gene sequence similarity threshold value in the range of 98.2-99.0% were being widely accepted and used for differentiating two species (Stackebrandt, 2006; Meier-Kolthoff et al., 2013; Kim et al., 2014), instead of the 16S rRNA gene sequence similarity threshold level of 97% previously used (Tindall et al., 2010), thus supporting that *Nocardia* sp. strain WB46 is a potent new species. To understand the evolutionary relatedness of the *Nocardia* sp. strain WB46 with closely related *Nocardia* species, phylogenetic analysis of complete 16S rRNA sequences was conducted. BLASTn was conducted with full length 16S rRNA sequence of *Nocardia* sp. strain WB46 as a query against a 16S rRNA sequence collection (bacteria/archaea) of NCBI. Top 100 hits with E-value above $1E^{-100}$ and percent sequence identity above 90% were retrieved for the phylogenetic analysis. A multiple sequence alignment was established by using MUSCLE v3.5 and further trimmed by Gblock v0.91b (Edgar, 2004). Best nucleotide evolution model was selected with JModelTest2 (Darriba et al., 2012) and the model of GTR + G + I was selected. Maximum-likelihood phylogenetic analysis was conducted with PhyML 3.0 (Guindon et al., 2010) with 1000 bootstrap analysis. The resulting phylogeny was visualized using iTOL (Letunic and Bork, 2021). Interestingly, *Nocardia* sp. strain WB46 did not clustered with any of groups at species level, showing

its 16S rRNA sequence divergence from other publicly available *Nocardia* species in Genbank. The tree topology (Figure 5.2) suggested that *Nocardia* sp. strain WB46 shares a common ancestor with *N. asteroides,* but further diverged from the ancestor of *N. asteroides* (297/1000) to form a monophyletic node. Surprisingly, the divergence of sequence in *Nocardia* sp. strain WB46 only occurred in V2 region (position 108 to 110 bp and 121 bp in the multiple sequence alignment (Figure 5.3). It has been suggested that V3-V4 region of 16S rRNA is informative for understanding bacterial diversity and thus it is widely used in ecological and environmental studies (Fadeev et al., 2021), even though the value of other regions of variance was also emphasized (Bukin et al., 2019). The position of informative sequence divergence in our study is showing that V2 region should be taken into account for capturing the diversity of this ecologically important bacterial taxa.

Genes connected with the degradation of petroleum hydrocarbons were found in the genome of *Nocardia* sp. strain WB46 (Table 5.4). Alkane 1-monooxygenase (*alkB*) and cytochrome P450 hydroxylase (*CYP153*) are important alkane hydroxylases responsible for microbial aerobic alkane degradation in oil-polluted environments. These enzymes hydroxylate alkanes to alcohols, which are further oxidized to fatty acids and catabolized via the bacterial β-oxidation pathway (Ji et al., 2013b). Previous studies showed that the gene repertoire of *alkB* and *CYP153* are diverse among species of Nocardia. For instance, *Nocardia cyriacigeorgica* GUH-2 has 2 copies of *alkB* and also 2 copies of *CYP153*, while *Nocardioidaceae bacterium* Broad-1 has 2 copies of *alkB* but only 1 copy of *CYP153* (Nie et al., 2014a). It has been reported that almost all the Actinobacteria genomes containing *CYP153* genes had also *alkB* genes, implying a potential link between the CYP153 and alkB genes in the Actinobacteria (Nie et al., 2014a; Nie et al., 2014c). Interestingly, the genome of *Nocardia* sp. strain WB46 has



Tree scale: 0.01
AF490539 Rhodococcus equi DSM20307T
NR 133794.1 N kroppenstedtii
NR 114643.1 N farcinica
NR 117248.1 N farcinica
NR 036996.1 N farcinica
NR 028651.1 N higoensis
NR 028650.1 N shimofusensis
NR 117340.1 N shimofusensis
NR 118208.1 N shimofusensis
NR 028652.1 N araoensis
NR 028654.1 N arthritidis
NR 117244.1 N asiatica
NR 152651.1 N jiangsuensis
NR 148265.1 N vulneris
NR 042176.1 N tenerifensis
NR 041860.1 N brasiliensis
NR 119106.1 N brasiliensis
NR 117247.1 N brasiliensis
NR 117316.1 N blacklockiae
NR 041867.1 N transvalensis
NR 117245.1 N transvalensis
NR 025059.1 N abscessus
NR 115823.1 N abscessus
NR 117347.1 N abscessus
NR 117321.1 N exalbida
NR 117399.1 N gamkensis
NR 117391.1 N xishanensis
NR 108787.1 N artemisiae
NR 104778.1 N mexicana
NR 117331.1 N lijiangensis
NR 115290.1 N polyresistens
NR 043185.1 N lijiangensis
NR 040994.1 N thailandica
NR 117339.1 N thailandica
NR 118213.1 N thailandica
Nocardia sp WB46
NR 119105.1 N asteroides
NR 041856.1 N asteroides
NR 115826.1 N asteroides
NR 117315.1 N asteroides
NR 117403.1 N neocaledoniensis
NR 118204.1 N neocaledoniensis
NR 029094.1 N neocaledoniensis
NR 159262.1 N rhizosphaerihabitans
NR 109056.1 N goodfellowii
NR 117317.1 N caishijiensis
NR 025216.1 N caishijiensis
NR 041010.1 N anaemiae
NR 117345.1 N anaemiae
NR 025199.1 N pseudovaccinii
NR 024722.1 N vinacea
NR 117396.1 N vinacea
NR 115843.1 N vinacea
NR 133795.1 N sungurluensis
NR 117312.1 N alba
NR 025726.1 N alba
NR 157707.1 N cavernae
NR 137261.1 N halotolerans
NR 028006.1 N ignorata
NR 146826.1 N iasii
NR 148835.1 N camponoti
NR 114644.1 N fluminea
NR 041869.1 N salmonicida
NR 114570.1 N salmonicida
NR 041870.1 N salmonicida
NR 026525.1 N flavorosea
NR 117322.1 N flavorosea
NR 112970.1 N sienata
NR 041251.1 N testacea
NR 117319.1 N carnea
NR 041859.1 N carnea
NR 116015.1 N rhamnosiphila
NR 146046.1 N zapadnayensis
NR 041857.1 N cyriacigeorgica
NR 115830.1 N cyriacigeorgica
NR 117334.1 N cyriacigeorgica
NR 041862.1 N brevicatena
NR 041863.1 N paucivorans
NR 117341.1 N paucivorans
NR 117392.1 N vermiculata
NR 041866.1 N vaccinii
NR 117320.1 N elegans
NR 042353.1 N elegans
NR 041872.1 N africana
NR 040995.1 N aobensis
NR 037097.1 N veterana
NR 115156.1 N veterana
NR 149226.1 N rayongensis
NR 117329.1 N jiangxiensis
NR 117343.1 N nova
NR 041858.1 N nova
NR 041865.1 N uniformis
NR 041874.1 N otitidiscaviarum
NR 041861.1 N seriolae
NR 119312.1 N crassostreae
NR 041868.1 N crassostreae
NR 041864.1 N pseudobrasiliensis
NR 117336.1 N pseudobrasiliensis
NR 042566.1 N acidivorans
NR 117310.1 N acidivorans
NR 152644.1 N tengchongensis
NR 024803.1 N yamanashiensis

Figure 5.2 Phylogenetic analysis of *Nocardia* sp. strain WB46 with other species in the *Nocardia* genus, using complete 16S rRNA sequences (1358bp). Maximum likelihood of nucleic acid sequences of 16S rRNA sequences was analyzed with the GTR + I+G (with four distinct gamma categories) phylogenetic model which showed the lowest AIC value. The tree was rooted by using *Rhodococcus equi* as an outgroup (coloured orange), following the previous publication of *Nocardia* phylogeny (Roth et al., 2003). The numbers at branches correspond to bootstrap support values generated with 1000 bootstrap replicates. The branches of a clade, which suggested to share the most direct common ancestor with Nocardia sp. WB46 with more than 200/1000 bootstrap supporting, was colored red.

Nocardia_sp_WB46/1-1427	ATACCGGATATGACCACAGGATGCATGTCTTGTGGTGGAAAGATTTATCGGTACGAGA
NR_041856.1_Nocardia_asteroides/1-1427	ATACCGGATATGACCTTCGGATGCATGTCTGAGGGTGGAAAGATTTATCGGTACGAGA
NR_119105.1_Nocardia_asteroides_DSM43757/1-1427	ATACCGGATATGACCTTCGGATGCATGTCTGAGGGTGGAAAGATTTATCGGTACGAGA
NR_117315.1_Nocardia_asteroides_NBRC15531/1-1427	ATACCGGATATGACCTTCGGATGCATGTCTGAGGGTGGAAAGATTTATCGGTACGAGA
NR_115826.1_Nocardia_asteroides_NBRC15531/1-1427	ATACCGGATATGACCTTCGGATGCATGTCTGAGGGTGGAAAGATTTATCGGTACGAGA

Figure 5.3 Region of 16S rRNA sequence divergence in multiple sequence alignment of *Nocardia* sp. strain WB46 and *Nocardia asteroides* isolates. Within full length 16S rRNA sequence, there were 6 nucleotide sequences different in *Nocardia* sp. strain WB46 from other *Nocardia asteroides* isolates which did not have any sequence divergence. Diverged sequences were shown without background colour.

Table 5.4 Genes related to PHCs degradation and PGP found in the genome of *Nocardia* sp. strain WB46.

Gene	Candidate	Similar to	NCBI Reference Sequence (RefSeq)
alkane 1-monooxygenase	pgaptmp_005297	alkane 1-monooxygenase [*Rhodococcus ruber*]	WP_017682157.1
	pgaptmp_001119	alkane 1-monooxygenase [*Actinobacteria*]	WP_019048757.1
	pgaptmp_001123	alkane 1-monooxygenase [*Nocardia brasiliensis*]	WP_014987826.1
Phosphate solubilization	pgaptmp_001057	acid phosphatase [*Actinobacteria*]	WP_019048697.1
Naphthalene dioxygenase	pgaptmp_004770	aromatic ring-hydroxylating dioxygenase subunit alpha [*Frankia inefficax*]	WP_013424580.1
	pgaptmp_004772	aromatic ring-hydroxylating dioxygenase subunit alpha [*Gordonia rhizosphera*]	WP_006331860.1
Siderophore utilization	pgaptmp_000075	SIP domain-containing protein [*Nocardia* sp. MH4]	WP_218717909.1
	pgaptmp_004292	SIP domain-containing protein [*Nocardia* sp.]	WP_218719407.1

no *CYP153* gene but has 3 copies of *alkB* genes. So, the alkane-degrading capability of this strain might be associated mainly with the *alkB* activity. Additionally, 2 naphthalene dioxygenase (*ndo*) genes that are responsible for biodegrading polycyclic aromatic hydrocarbons (PAHs) (Peng et al., 2008a) were also present in the genome of *Nocardia* sp. strain WB46. Further, genes related to plant growth-promoting characteristics were also detected, including phosphate solubilization and siderophore utilization (Table 5.4). The genome sequence data of *Nocardia* sp. strain WB46 will enhance our understanding of the metabolic capabilities of *Nocardia* strains.

5.6 CONCLUSIONS

This study demonstrates the genomic features of isolate *Nocardia* sp. strain WB46 which was isolated from the rhizosphere of *Salix* plants growing in soil contaminated with petroleum hydrocarbons and utilize several PHC compounds as sole source of carbon and have attributes for plant growth-promotion. The draft genome sequence of *Nocardia* sp. strain WB46 features genes for such multifarious characteristics relevant PHC degradation, and plant growth-promotion.

Nocardia sp. strain WB46 will be further evaluated as an inoculant to enhance rhizoremediation of soil contaminated with petroleum hydrocarbons, which might lead to biotechnological applications.

Accession number(s):

The datasets presented in this study can be found in online repository at Zenodo under the flowing link: https://zenodo.org/record/6973367#.YxFJ2S294Q8.

5.7 ACKNOWLEDGEMENTS

This study was supported by Funds from Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to MH (Grant number: RGPIN-2018-04178), Genome Quebec, Genome Canada, F.A. was also supported by a grant from King Saud University via the Saudi Arabian Cultural Bureau in Ottawa which are gratefully acknowledged.

Chapter Six: SUMMARY AND CONCLUSIONS

Previous research aiming to develop microbial inoculant for use in phytoremediation of PHC-contaminated soils focus mainly on isolating bacterial candidates with ability to degrade particular contaminates such as alkanes (Balseiro-Romero et al., 2017a; Marchand et al., 2017) or PAHs (Al-Thukair et al., 2020). Over the last decade, research was directed toward utilizing PGPR to enhance the phytoremediation of PHC-contaminated soils. However, most of these researches were focused toward characterizing a single PGP trait such as ACC deaminase (Tara et al., 2014), N-fixation (Chaudhary et al., 2019) or IAA (Li et al., 2021). Here in my thesis research, I took a different approach. We isolated and screened bacteria for both alkanes and PAHs degradation potentials. Additionally, bacteria were screened for five plant-growth-promoting (PGP) traits (ACC deaminase synthesis, IAA production, N-fixation, siderophore production, and P-solubilization). This strategy resulted in generating a structurally and functionally diverse culture collection of PGPR and PHCs-degrading bacteria isolated from the rhizosphere of *Salix purpurea* and *Eleocharis obusta* plants growing in a long-term PHC-polluted site.

Summary of findings

In chapter 3, 438 morphologically distinct bacterial isolates were cultivated from the rhizosphere of *S. purpurea* and *E. obusta* plants as well as from bulk soil using three different isolation strategies: 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. Based on the 16S rRNA gene sequencing, bacterial isolates were classified into 62 genera belonging the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Interestingly, roughly 5% of the total sequences were not assigned to any known bacteria. The most frequently identified genera within this culture collection were *Pseudomonas* (14.3%), *Bacillus* (9%), *Streptomyces* (7.3%), *Microbacterium* (5.2%), *Klebsiella* (5%), *Arthrobacter* (4.5%), *Acinetobacter* (4%), *Pseudoxanthomonas* (3.8%), *Enterobacter* (3%), *Rhodococcus* (3%) and *Variovorax* (3%). These findings are in agreement with previous reports of the prevalence of these bacterial genera in soils contaminated with

PHCs (Glick and Stearns, 2011; Ghosal et al., 2016; Brzeszcz and Kaszycki, 2018; Xu et al., 2018). More importantly, several of these genera are known to contain bacterial species with promising PHC-degradation potentials and PGP activities (Pawlik et al., 2017; Lumactud and Fulthorpe, 2018; Imperato et al., 2019; Iqbal et al., 2019).

These 438 bacterial isolates were also assessed for their ability to utilize five different alkanes or polycyclic aromatic hydrocarbons (PAHS) as the sole carbon source (Chapter 3). Our results indicated that 141 bacterial isolates were able to utilize all 5-hydrocarbon compounds tested. Of those, 16% belonged to the families *Micrococcaceae*, 15% to *Pseudomonadaceae*, 13% to *Actinomycetaceae*, 10% to *Enterobacteriaceae*, 7% to *Xanthomonadaceae*, and 5% to *Moraxellaceae*. Our findings corroborate previous reports confirming the ability of these families to degrade wide range of PHC compounds (Ferrera-Rodríguez et al., 2013; Brzeszcz and Kaszycki, 2018). Several genera within these families, such as *Pseudomonas*, *Acinetobacter, Streptomyces, Bacillus, Arthrobacter, Microbacterium, Rhodococcus, Enterobacter, Gordonia* and *Nocardia*, have previously been shown to hold promising petroleum-hydrocarbons degradation potential (Wolińska et al., 2018; Xu et al., 2018; Viesser et al., 2020).

Our bacterial culture collection (*n*=438) was further screened for traits that are associated with PGP capabilities (Chapter 3). Our results indicated that PGP traits are common among bacterial isolates. For example, 267 isolates (60%) were able to grow on DF-mineral salt medium with ACC as the sole nitrogen source, indicating the presence of ACC deaminase. Similarly, 191 bacterial isolates were able to synthesize the phytohormone IAA. Also, 246, 216, and 59 bacterial isolates were able to fix nitrogen, produce siderophores and solubilize inorganic phosphorus, respectively. Interestingly, 22 bacterial isolates (5%) possess all five PGP traits under investigation. Bacterial isolates positive for all PGP belonged to the genera *Pseudomonas*, *Acinetobacter*, *Serratia*, *Azomonas*, *Bacillus*, *Stenotrophomonas*, *Streptomyces*, and *Pantoea*. Our findings corroborate previous research of the ability of these bacterial genera to hold multiple PGP traits (Lugtenberg and Kamilova, 2009; Schlaeppi and Bulgarelli, 2015; Jambon et al., 2018; Lumactud and Fulthorpe, 2018; Eze et al., 2022).

Interestingly, several bacterial isolates were capable of degrading all five PHCs, and had all five tested PGP traits (Chapter 3), such as *Pseudomonas putida* strain EB3, *Streptomyces* sp. strain WT8 and *Bacillus* sp. strain WT32. These findings support earlier reports which indicate that many isolates from these genera can degrade PHCs and stimulate plant growth (Nogales et al., 2017; Ansari and Ahmad, 2019; Dwivedi et al., 2019). These bacterial taxa were therefore excellent candidates to look for in the following experiments.

The use of PGPR holding PHC-degradation potentials as bioremediation inoculant in plant-assisted phytoremediation requires multiple steps of screening, selection and characterization of candidate bacterial strains. Therefore, in chapter 4, an in-depth characterization of plant growth-promoting potentials of selected PGP and alkanes-degrading bacterial isolates was carried out. Fifty bacterial isolates encompassing a wide phylogenetic affiliation range were selected from the previous experiment (Chapter 3). Selected isolates were screened using six common PGP traits, tested for their ability to utilize diesel and *n*-hexadecane as sole carbon source, complemented with a genetic characterization of genes involved in alkanes degradation as well as in other pertinent functions. Additionally, isolates were tested for their plant growth-promotion potentials using gnotobiotic approach under normal and stressed conditions. Our results showed that 70% of bacterial isolates, assessed qualitatively and quantitatively for PGP traits, exhibited at least four PGP traits. For example, 14 strains (28%) were able to solubilize calcium phosphate in the liquid medium, with *Bacillus megaterium* WT10 showing the highest solubilization activity with 690.86 µg mL^{-1} calcium phosphate; 34 strains (68%) were able to produce IAA after 48 h of incubation with 1 mg mL^{-1} supplement of tryptophan as auxin precursor, with *Rhizobium* sp. ET10 exhibiting the highest IAA production among all the strains (44.31 µg mL^{-1} IAA). Further, 24 bacterial strains (48%) were able to synthesize siderophores and the highest siderophores synthesizing were observed in *Pseudomonas putida* ET27, *Enterobacter* sp. EA9 and *Pseudomonas stutzeri* SB38. Ammonia production was common among the majority of tested isolates. The maximum ammonia production was exhibited by *Comamonas* sp. EB35 (13.95 µmol mL^{-1}). Additionally, bacterial strains were further screened qualitatively for ACC deaminase and N fixation, where 34 strains (68%) demonstrated ACC deaminase activity, and 28 strains (56%) showed the ability to fix

atmospheric N_2. These results were confirmed by the presence of ACC deaminase gene (*acdS*), and N fixation gene (*nifH*) in bacterial isolates.

Furthermore, these 50 bacterial strains (*n*=50) were assessed for their plant growth-promoting potential (Chapter 4). A canola root elongation assay under gnotobiotic conditions revealed that 36 bacterial strains (72%) promoted root growth. The maximum root elongation was induced by the bacterial strains *Curtobacterium* sp. EA21, *Bacillus megaterium* WT10 and *Gordonia* sp. ST45, which significantly increased ($P \leq 0.05$) canola root elongation by 118%, 98%, and 86%, respectively, compared with the control treatment. Earlier studies indicated that the ability to synthesize ACC deaminase and produce IAA are main bacterial mechanisms involved in plant stimulation (Belimov et al., 2001; Arshad et al., 2007; Thijs et al., 2014; Balseiro-Romero et al., 2017b; Kang et al., 2019). However, in our study, bacterial strain *Bacillus megaterium* WT10 did not produce ACC deaminase nor synthesize IAA, indicating that plant stimulation by this strain involved other PGP traits such as phosphate solubilization or by multiple PGP mechanisms which we have not been tested for in the current study. Importantly, despite being isolated from different plant species, many bacterial isolates tested in the current study significantly enhanced canola plant growth, indicating that these PGPR strains are non-host specific, thus having huge potential as bacterial inoculant to stimulate plant growth in phytoremediation as well as in organic agriculture.

In addition to screening for PGP mechanisms and plant growth, bacterial isolates (*n*=50) were further tested for their ability to utilize 1% (v/v) *n*-hexadecane and 1% diesel as sole carbon source (Chapter 4). Our results indicated that 29 strains (58%) could growth on *n*-hexadecane as sole carbon source. Some isolates exhibited robust growth such as *Gordonia amicalis* ST45, *Comamonas odontotermitis* EB35, *Pseudomonas fluorescens* WT17, *Nocardia* sp. WB46, *Nocardia asteroides* WB51 and *Phycicoccus bigeumensis* WB54. Additionally, 43 bacterial strains (86%) could utilize diesel as the sole carbon source. When grown in MSM medium supplemented with diesel, several strains showed the highest growth, such as *Rhodococcus ruber* ST4, *Gordonia amicalis* ST45, *Comamonas odontotermitis* EB35, *Bacillus megaterium* WT10, *Gordonia amicalis* WT12, *Pseudomonas kilonensis* WT17, *Paenarthrobacter nitroguajacolicus* WB17, *Sphingomonas sanxanigenens* WB25, *Nocardia* sp. WB46, *Nocardia asteroides* WB51 and *Enterobacter cancerogenus* EA9. Also, catabolic genes

related to PHC-degradation were detected in isolates and the *alkB* gene was detected in 34 strains (68%), *CYP153* gene was found in 24 strains (48%), while 33 bacterial strains (66%) possess the *nah1* gene. This suggest that the alkane-degradation capabilities of bacterial strains tested in this study might be associated mainly with *alkB* and CYP153 enzymes activities.

After several rounds of screening and characterization, seven bacterial candidates (alone or in consortium) were assessed for their ability to stimulate canola root and shoot growth in substrates amended with a gradient of *n*-hexadecane concentrations, ranging from 0% as control to 3%, under gnotobiotic conditions. Our results indicated that inoculation of canola with these strains either alone or in combination generally provoked a significant increase in both root and shoot length of canola seedlings when compared with control plants. Notably, bacterial strains *Nocardia* sp. WB46, *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31 showed the highest root growth stimulation when grown under the presence of 3% *n*-hexadecane, While bacterial strains *Nocardia* sp. WB46 and *Bacillus megaterium* WT10 significantly increased shoot length when compared with control treatment at the same concentration of *n*-hexadecane. The bacterial consortium formed of *Nocardia* sp. WB46, *Bacillus megaterium* WT10, *Pseudomonas plecoglossicida* ET27 and *Stenotrophomonas pavanii* EB31, *Gordonia amicalis* WT12, *Acinetobacter* sp. SB41 and *Pseudarthrobacter siccitolerans* WT19 exerted the maximum root growth promotion compared to all isolates inoculated alone, or to the control treatment. These finding indicate that PGPR inoculants positively impact plant growth under such stressful conditions. Bacterial isolates used in this experiment exhibited several PGP properties involved in nutrient acquisition, alkanes degradation potentials, stress alleviation and plant hormone modulation that could possibly be responsible for this growth stimulation.

In chapter 4, bacterial strains *Nocardia* sp. WB46, *Pseudomonas plecoglossicida* ET27, *Stenotrophomonas pavanii* EB31 and *Bacillus megaterium* WT10 were found to have a high plant growth promotion potential upon inoculation of canola seeds, under the presence of an increasing gradient of *n*-hexadecane concentrations in gnotobiotic conditions. Therefore, we decided to further characterize the genome of the most promising bacterial isolate using genomics methods to unveil its PGP mechanisms and PHCs-degrading genes. In this thesis, we therefore provide

results from draft genome sequencing of bacterial strain *Nocardia* sp. WB46. Chapter 5 provides a description of the feature of the genome of *Nocardia* sp. WB46. Taxonomic analysis based on the genome sequence data (16S rRNA gene analyses, *in silico* DNA-DNA hybridization (DDH) and average nucleotide identity (ANI)), revealed that *Nocardia* sp. strain WB46 is a new species. Additional phylogenetic analysis confirmed that *Nocardia* sp. strain WB46 did not clustered with any of *Nocardia* at species level, showing its 16S rRNA sequence divergence from other publicly available *Nocardia* species in Genbank. Genome annotation revealed the presence of genes involved in the degradation of different PHC compounds. For example, *Nocardia* sp. strain WB46 has 3 copies of *alkB* genes, and 2 naphthalene dioxygenase (*ndo*) genes responsible for microbial biodegradation of alkanes and PAHs in oil-polluted environments, respectively (Larkin et al., 2005; Pagnout et al., 2007; Wang and Shao, 2013; Nie et al., 2014c). So, these findings might explain the high capacity of this strain to utilize various alkanes and PAHs compounds. Furthermore, genes related to PGP characteristics were also detected in the genome of *Nocardia* sp. WB46, including phosphate solubilization and siderophore utilization. The presence of such PGP traits might contribute to the growth promotion potential of this strain. The genome sequence of *Nocardia* sp. strain WB46 will definitely enhance our understanding of the metabolic abilities of *Nocardia* strains.

Hypothesis revisited

Findings obtained in this thesis, which are summarized in the above section, are in line with our hypothesis 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. Based on the isolation strategy used in this work, 438 bacterial isolates were obtained through growth on different selective and non-selective media, with high genetic diversity, belonging to 62 bacterial genera (Chapter 3). This culture collection of bacterial isolates was not only structurally diverse, but also was found to be functionally diverse. Our results indicated that many isolates were able to utilize different alkanes or polycyclic aromatic hydrocarbons (PAHS) as the sole carbon source as well as to possess various PGP traits (Chapters 3 and 4). Interestingly, our

results indicated that *Nocardia* sp. WB46 possessed multiple PGP and PHCs degradation traits which could be utilized to improve phytoremediation of PHC-contaminated soils.

Concluding Remarks:

Overall, phytoremediation is an environmentally friendly and cost-effective *in-situ* approach for the remediation of PHC-contaminated soils. However, for phytoremediation to be successful, several constrains must be overcome such as pollutant phytotoxicity, proper plant establishment and development, and pollutant bioavailability. This has necessitated the exploration of plant-bacteria associations for the improvement of phytoremediation of PHC-contaminated soils. Therefore, the proper identification, selection and characterization of bacteria capable of promoting phytoremediation through both plant growth-promoting and hydrocarbon-degrading activities is a priority to the success and adoption of such phytotechnologies. In this study, *S. purpurea* and *E. obtusa* plants, which grow in soils chronically contaminated with PHCs, harbored a diverse group of rhizospheric bacteria with multiple PGP and PHCs degradation characteristics. In order to obtain bacterial isolates better suited for bioremediation applications, multiple screening schemes were conducted to characterize and select potential bacterial candidates for future use as inoculants. After several rounds of rigorous screening, seven bacterial candidates were selected based on their ability to possess PGP traits, PHCs degradation potentials and enhancement of plant growth under PHCs stressed conditions. The use of these bacterial isolates, especially *Nocardia* sp. WB46, could be an efficient strategy for enhancing the growth and phytoremediation potential of plants growing in PHC-contaminated soils. With this culture collection in hand, a better understanding of the role of plant growth promotion in the phytoremediation of PHC-contaminated soils can be achieved. More importantly, culture collection of bacteria generated in this study are of interest not only for biotechnological applications aimed at bioremediation of organic contaminants, but also for enhancing crop productivity in agriculture.

Chapter Seven: Future Perspectives

The considerable environmental footprint of conventional remediation interventions, together with slow rate of natural attenuation of PHCs contaminants, have encouraged to harness and exploit biological organisms such as plants and their associated-microbiomes for enhancing bioremediation efficiency. Among plant microbiomes, PGPR have proven their efficiency to assist phytoremediation management which together with their associated plants, form an innovative, environmentally and sustainable approach to increase the efficiency of phytoremediation of PHCs-contaminated soils.

To successfully exploit PGPR as a tool in phytoremediation applications, further investigations are needed. Examples of future research avenues are given below:

1- Bacterial isolates used in this study that promote canola growth under gnotobiotic conditions should be further tested under greenhouse conditions. Such experiments would show the remediation potential of candidate strains in contaminated soils. Ideally, these experiments would involve different plant species (fast growth, moderate tolerance, high biomass) and a range of pollutants and of their concentrations. Hence, future phytoremediation experiments could focus on the utilization of bacterial strains *Nocardia* sp. WB46, *Pseudomonas plecoglossicida* ET27, *Stenotrophomonas pavanii* EB31, *Bacillus megaterium* WT10 and on a consortium of these strains. Field trials should also be undertaken in different conditions to validate efficiency of these trains in outdoor conditions.

2- In addition to the previous point, novel microbiome inocula containing PGPR and PHCs-degrading bacteria should be evaluated under large-scale field conditions. Such field experiments would demonstrate the potential of phytoremediation under real field conditions where many factors control its success, such as competition with indigenous microbes, contaminants level, soil structure, etc. Furthermore, genomic-based tools should be used to understand how bacterial inoculants shape the structure and function of indigenous microbial communities.

3- A clear understanding of the various traits by which PGPR enhance plant growth and degrade PHCs will permit us to better improve the selection of bacterial inoculants from culture collections. In this regard, the use of omics techniques (genomics, transcriptomics, proteomics, etc..) combined with bioinformatics tools will reveal valuable information about the structure, composition, and function of root-associated bacteria. Additional information could be obtained using these tools, such as the interaction of PHC-degrading and PGP bacteria with their host plants, as well as with each other's. For instance, comparative genomics of plant growth-promoting and PHC-degrading bacteria could identify genes responsible for plant growth promotion potentials and PHCs degradations abilities as well as other genes required for adaptations to various stress conditions, competition, and root colonization pattern.

4- The bacterial collection developed in this study represents an untapped reservoir for the discovery of new plant growth-promoting bacteria because they were isolated from plants growing under harsh environmental conditions. Some of these isolates showed plant growth promotion when tested on canola, indicating that they are non-host specific. Therefore, these bacterial isolates should be evaluated regarding their plant growth promotion potentials on other important agronomic crops such as maize, soybean and wheat as biofertilizer and biostimulator agents for agricultural crops. Additionally, these isolates can be exploited to enhance growth of bioenergy crops growing on marginal lands.

Chapter Eight: Bibliography

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APPENDICES

APPENDIX A: Bacterial Isolation Code

Bacterial Isolation code:

Isolate code refers to the environmental niche and isolation medium from which the isolate came. The first letter in the code (W, E and S) indicates that the isolate where from Willow rhizosphere, *Eleocharis* rhizosphere or bulk soil, respectively. The second letter indicates the isolation medium used to isolate the bacteria (B= Bushnell-Haas medium amended with 1 % diesel, as the sole carbon and energy source, T= One-tenth-strength Trypticase Soy Agar (TSA) medium and A= DF-ACC agar. The numbers are a randomly assigned numbers.

APPENDIX B Supporting Information (Chapter 3)

3.1 National Botanical Institute's phosphate growth medium (NBRIP) agar (Nautiyal, 1999)

Glucose	10.0 g L^{-1}
$(NH4)SO_4$	0.1 g L^{-1}
MgCl2.6H2O	5.0 g L^{-1}
$Ca_3(PO4)_2$	5.0 g L^{-1}
KCI	0.2 g $L^{-}1$
MgSO4.7H2O	0.25 g L^{-1}
Agar	15.0 g L^{-1}
pH adjusted to	7.0

3.2 DF salts minimal medium utilized for IAA production (Dworkin and Foster, 1958)

KH_2PO_4	4.0 g L^{-1}
Na_2HPO_4	6.0 g L^{-1}
$MgSO_4.7H_2O$	0.2 g L^{-1}
$FeSO_4.7H_2O$	(0.1ml of stock solution)
Micro nutrients	(0.1ml of stock solution)
Glucose	2.0 g L^{-1}
Gluconic acid (Ksalt)	2.0 g L^{-1}
Citric acid (Tri-Na salt)	2.0 g L^{-1}
$(NH_4)_2SO_4$	2.0 g L^{-1}

Dissolved in 1000 ml of distilled H_2O

Micro nutrients (Stock solution: 0.1ml L^{-1} was added to above DF salts minimal medium)

H_3BO_3	10 mg L^{-1}
$MnSO_4$	11.2 mg L^{-1}
$ZnSO_4$	124.6 mg L^{-1}
$CuSO_4$	78.2 mg L^{-1}
MoO_3	78.2 mg L^{-1}

Dissolved in 1000 ml of distilled H_2O

Salkowski's Reagent (Gordon and Weber, 1951)

150 mL concentrated Sulphuric acid

250 mL distilled water

7.5 mL (0.5M) $FeCl_3.6H_2O$

3.3 CAS-AGAR Media (Alexander and Zuberer, 1991)

Solution 1

a) 2.7 mg of $FeCl_3$ $6H_2O$ + 10 ml of 10 mM HC1

b) 60.5mg CAS + 50 ml of distilled H_2O

c) 72.8 mg HDTMA + 40 ml of distilled H_2O

The **c** solution is slowly added to the **ab** mixture to obtain a dark blue solution

Solution 2

a) 750 ml of distilled H_2O

b) 0.3 g of KH_2PO_4

c) 0.5g of NaCl

d) 30.24 g of PIPES

The **a b c** compounds are dissolved and then PIPES are added. Adjust pH to.6.8 with 50% KOH

e) 15gagar

Adjust the volume of the solution to 800 ml with bi-distilled water

Solution 3

a) 70 ml of distilled H_2O

b) 2 g glucose

c) 2 g mannitol

d) 493 mg $MgSO_4$ 7 H_2O

e) 11 mg $CaCl_2$

f) 1.17mg$MnSO_4$ H_2O

g) 1.4 mg H_3BO3

h) 0.04 mg $CuSO_4$ $5H_2O$

i) 1.2 mg $ZnSO_4$ $7H_2O$

j) 1 mg Na_2MoO_4 2 H_2O

Solution 4 (sterilized by filtration)

a) 3 g casamino acids

b) 30 ml of distilled H_2O

The solution number **3** is autoclaved, cooled at 50°C and added to the **sol. 2** previously
autoclaved also. Then, **sol. 4** previously sterilized by filtration is added. The sol. **1** is the last to be added (sterilized by autoclave also).

3.4 MM9 medium (Payne, 1994, Alexander and Zuberer, 1991

Solution 1

a) 900 ml of distilled H_2O

b) 0.3 g of KH_2PO_4

c) 0.5g of NaCl

d) 30.24 g of PIPES

The **a b c** compounds are dissolved and then PIPES are added. Adjust pH to.6.8 with 50% KOH

Solution 2

a) 70 ml of distilled H_2O

b) 2 g glucose

c) 2 g mannitol

d) 493 mg $MgSO_4$ 7 H_2O

e) 11 mg $CaCl_2$

f) 1.17mg$MnSO_4$ H_2O

g) 1.4 mg H_3BO3

h) 0.04 mg $CuSO_4$ 5H_2O

i) 1.2 mg $ZnSO_4$ 7H_2O

j) 1 mg Na_2MoO_4 2 H_2O

Solution 3 (sterilized by filtration)

a) 3 g casamino acids

b) 30 ml of distilled H_2O

The solution number **2** is autoclaved, cooled at 50°C and added to the **sol. 1** previously
autoclaved also. Then, **sol. 3** previously sterilized by filtration is added.

3.5 Ammonia production (Cappuccino and Sherman, 1992)

Peptone water

Peptone	10 g. L^{-1}
NaCl	5 g. L^{-1}
dH_2O	1000 ml

Nessler's reagent

10% HgI_2

7% KI

50% aqueous solution of NaOH (32%)

3.6 Modified DF salts minimal medium utilized for ACC assay (Dworkin and Foster, 1958)

KH_2PO_4 4.0 g L^{-1}

Na_2HPO_4 6.0 g L^{-1}

$MgSO_4.7H_2O$ 0.2 g L^{-1}

$FeSO_4.7H_2O$ (0.1ml of stock solution)

Micro nutrients (0.1ml of stock solution)

Glucose 2.0 g L^{-1}

Gluconic acid (Ksalt) 2.0 g L^{-1}

Citric acid (Tri-Na salt) 2.0 g L^{-1}

All of the above were dissolved in 1000 ml of distilled H_2O

$FeSO_4$.$7H_2O$ (Stock solution preparation)

100 mg of $FeSO_4$.$7H_2O$ is dissolved in 10ml sterile dH2O and is stored in the refrigerator for up to several months.

Micro nutrients (Stock solution preparation)

$H3BO_3$ 10 mg L^{-1}

$MnSO_4$ 11.2 mg L^{-1}

$ZnSO_4$ 124.6 mg L^{-1}

$CuSO_4$ 78.2 mg L^{-1}

MoO_3 78.2 mg L^{-1}

Dissolved in 1000 ml of distilled H2O

ACC, $(NH4)_2SO_4$ and 0.1M $MgSO_4.H_2O$ (stock solutions)

ACC 30.33 mg in 10 ml of distilled H_2O

$(NH4)_2SO_4$ 13.21 g L^{-1}

$MgSO_4.H_2O$24.64 g L^{-1}

3.7 Nitrogen-Limited Medium: Combined Carbon Medium (Rennie, 1981)

Solution 1:

Sucrose 5 g L^{-1}

Mannitol 5 g L^{-1}

Sodium Lactate (ml, 60%, v/v) 0.5 ml/L^{-1}

K_2HPO_4 0.80 g L^{-1}

KH_2PO_4 0.20 g L^{-1}

NaCl 0.10 g L^{-1}

$Na_2Mo0_4.2H_20$ 25.0 mg L^{-1}

Na_2FeEDTA 28.0 mg L^{-1}

Yeast Extract 100mg L^{-1}

Distilled Water 900ml

Agar, 15 g

Solution 2:

$MgSO_4$. 7 H_20 0.20 g L^{-1}

$CaCl_2$ 0.06 g L^{-1}

Distilled water 100 ml

Autoclave Solution 1 and 2 separately, cool to 50C and mix.

3.8 N-free Hoagland's Nutrient Solution (Hoagland and Boyer, 1936)

Macronutrients Stock solutions

KH_2PO_4 (1M) 136.09 g L^{-1}

K_2SO_4 (0.5M) 87.135 g L^{-1}

$MgSO_4.7H_2O$ (1M) 246.48 g L^{-1}

Micronutrients

Boric acid 1.00 g L^{-1}

Manganous chloride 1.00 g L^{-1}

Zinc sulfate 0.58 g L^{-1}

Cupric sulfate 0.13 g L^{-1}

Sodium molybdate 0.10 g L^{-1}

Iron stock solution: 20 g L^{-1}

The final medium contained:

KH_2PO_4: 2 ml L^{-1} of stock

K_2SO_4: 4 ml L^{-1} of stock

$CaSO_4$: 1 g L^{-1} of stock

$MgSO_4.7H_2O$: 1 ml L^{-1} of stock

Microstock: 1 ml L^{-1} of stock

IRON: 1 ml L^{-1} of stock

The pH was adjusted to 7.0 using 0.5 M KOH and sterilized for 20 minutes at 121°C for15 minutes.

Table S3.1 Taxonomic affiliations of rhizospheric bacteria isolated from *Salix* rhizosphere on different media based on 16S rRNA gene.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
WB1	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	98
WB2	Actinobacteria	*Micrococcaceae*	*Arthrobacter sulfonivorans*	99
WB3	Actinobacteria	*Micrococcaceae*	*Arthrobacter nicotinovorans*	99
WB4	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	98
WB5	Actinobacteria	*Actinomycetaceae*	*Streptomyces ederensis*	99
WB6	Actinobacteria	*Actinomycetaceae*	*Streptomyces ederensis*	99
WB7	Actinobacteria	*Nocardiaceae*	*Nocardia asteroides*	98
WB8	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	99
WB9	Betaproteobacteria	*Comamonadaceae*	*Variovorax ureilyticus*	99
WB10	Betaproteobacteria	*Comamonadaceae*	*Variovorax ureilyticus*	99
WB11	Actinobacteria	*Actinomycetaceae*	*Streptomyces sp.*	99
WB12	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	98
WB13	Actinobacteria	*Micrococcaceae*	*Arthrobacter* sp.	97
WB14	Actinobacteria	*Micrococcaceae*	*Arthrobacter* sp.	99
WB15	Betaproteobacteria	*Comamonadaceae*	*Variovorax boronicumulans*	98
WB16	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	99
WB17	Actinobacteria	*Micrococcaceae*	*Arthrobacter* sp.	97
WB18	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	98
WB19	Actinobacteria	*Actinomycetaceae*	*Streptomyces* sp.	99
WB20	Actinobacteria	*Nocardioidaceae*	*Nocardioides albus*	99
WB21	Actinobacteria	*Pseudonocardiaceae*	*Amycolatopsis speibonae*	99
WB22	Actinobacteria	*Micrococcaceae*	*Arthrobacter pascens*	98
WB23	Betaproteobacteria	*Comamonadaceae*	*Variovorax paradoxus*	99
WB24	Actinobacteria	*Actinomycetaceae*	*Streptomyces canus*	99
WB25	Alphaproteobacteria	*Sphingomonadaceae*	*Sphingomonas sanxanigenens*	97
WB26	Actinobacteria	*Actinomycetaceae*	*Streptomyces umbrinus*	99
WB27	Actinobacteria	*Actinomycetaceae*	*Streptomyces phaeochromogenes*	99
WB28	Actinobacteria	*Nocardioidaceae*	*Nocardioides albus*	99
WB29	Actinobacteria	*Actinomycetaceae*	*Streptomyces chartreusis*	98
WB30	Actinobacteria	*Nocardioidaceae*	*Nocardioides albus*	99
WB31	Gammaproteobacteria	*Pseudomonadaceae*	*Pseudomonas frederiksbergensis*	98
WB32	Actinobacteria	*Nocardioidaceae*	*Kribbella aluminosa*	98
WB33	Actinobacteria	*Micrococcaceae*	*Paenarthrobacter nitroguajacolicus*	97
WB34	Actinobacteria	*Microbacteriaceae*	*Microbacterium oxydans*	99
WB35	Actinobacteria	*Nocardioidaceae*	*Kribbella sindirgiensis*	98
WB36	Actinobacteria	*Micrococcaceae*	*Pseudarthrobacter oxydans*	98
WB37	Actinobacteria	*Nocardioidaceae*	*Kribbella koreensis*	99
WB38	Actinobacteria	*Nocardioidaceae*	*Nocardioides* sp.	98
WB39	Actinobacteria	*Actinomycetaceae*	*Streptomyces umbrinus*	99
WB40	Actinobacteria	*Micrococcaceae*	*Pseudarthrobacter siccitolerans*	98

Table S3.1 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
WB41	Actinobacteria	Actinomycetaceae	*Streptomyces phaeochromogenes*	99
WB42	Firmicutes	Staphylococcaceae	*Staphylococcus warneri*	99
WB43	Actinobacteria	Micrococcales	*Phycicoccus aerophilus*	98
WB44	Actinobacteria	Actinomycetaceae	*Streptomyces umbrinus*	99
WB45	Actinobacteria	Actinomycetaceae	*Streptomyces umbrinus*	99
WB46	Actinobacteria	Actinomycetaceae	*Nocardia asteroides*	98
WB47	Actinobacteria	Actinomycetaceae	*Streptomyces umbrinus*	98
WB48	Actinobacteria	Nocardiaceae	*Nocardia* sp.	98
WB49	Actinobacteria	Nocardioidaceae	*Nocardioides alpinus*	99
WB50	Actinobacteria	Micrococcaceae	*Arthrobacter humicola*	96
WB51	Actinobacteria	Gordoniaceae	*Gordonia* sp.	99
WB52	Actinobacteria	Nocardioidaceae	*Nocardioides albus*	98
WB53	Actinobacteria	Actinomycetaceae	*Streptomyces canus*	98
WB54	Actinobacteria	Micrococcales	*Phycicoccus bigeumensis*	99
WB55	Actinobacteria	Actinomycetaceae	*Streptomyces* sp.	98
WB56	Actinobacteria	Micrococcaceae	*Pseudarthrobacter oxydans*	98
WB57	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	99
WB58	Actinobacteria	Micrococcaceae	*Micromonospora palomenae*	98
WB59	Actinobacteria	Micrococcaceae	*Pseudarthrobacter sulfonivorans*	98
WB60	Actinobacteria	Nocardioidaceae	*Nocardioides albus*	99
WT1	Firmicutes	Bacillaceae	*Bacillus cereus*	99
WT2	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	99
WT3	Firmicutes	Paenibacillaceae	*Paenibacillus polysaccharolyticus*	99
WT4	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mandelii*	98
WT5	Firmicutes	Bacillaceae	*Bacillus cereus*	99
WT6	Firmicutes	Bacillaceae	*Bacillus indicus*	99
WT7	Actinobacteria	Actinomycetaceae	*Streptomyces griseolus*	96
WT8	Actinobacteria	Actinomycetaceae	*Streptomyces atriruber*	97
WT9	Actinobacteria	Micrococcaceae	*Streptomyces umbrinus*	99
WT10	Firmicutes	Bacillaceae	*Bacillus megaterium*	99
WT11	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	98
WT12	Actinobacteria	Gordoniaceae	*Gordonia amicalis*	98
WT13	Actinobacteria	Actinomycetaceae	*Streptomyces pseudovenezuelae*	99
WT14	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	98
WT15	Firmicutes	Bacillaceae	*Bacillus aryabhattai*	99
WT16	Actinobacteria	Micrococcaceae	*Micromonospora halotolerans*	98
WT17	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas kilonensis*	99
WT18	Actinobacteria	Nocardioidaceae	*Nocardioides albus*	96
WT19	Actinobacteria	Micrococcaceae	*Pseudarthrobacter siccitolerans*	96
WT20	Firmicutes	Bacillaceae	*Bacillus indicus*	99

Table S3.1 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
WT21	Alphaproteobacteria	Phyllobacteriaceae	*Mesorhizobium norvegicum*	98
WT22	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas frederiksbergensis*	97
WT23	Actinobacteria	Micrococcaceae	*Pseudarthrobacter siccitolerans*	95
WT24	Actinobacteria	Micrococcaceae	*Pseudarthrobacter defluvii*	97
WT25	Firmicutes	Bacillaceae	*Bacillus simplex*	99
WT26	Actinobacteria	Actinomycetaceae	*Streptomyces griseolus*	99
WT27	Actinobacteria	Actinomycetaceae	*Streptomyces umbrinus*	99
WT28	Firmicutes	Bacillaceae	*Bacillus aryabhattai*	98
WT29	Firmicutes	Bacillaceae	*Bacillus cereus*	98
WT30	Firmicutes	Bacillaceae	*Bacillus indicus*	98
WT31	Firmicutes	Bacillaceae	*Lysinibacillus xylanilyticus*	98
WT32	Firmicutes	Bacillaceae	*Bacillus megaterium*	99
WT33	Actinobacteria	Micrococcaceae	*Mycolicibacterium vanbaalenii*	99
WT34	Actinobacteria	Micrococcaceae	*Pseudarthrobacter oxydans*	96
WT35	Firmicutes	Bacillaceae	*Bacillus thuringiensis*	98
WT36	Firmicutes	Bacillaceae	*Bacillus indicus*	98
WT37	Actinobacteria	Micrococcaceae	*Arthrobacter* sp.	98
WT38	Actinobacteria	Micrococcaceae	*Arthrobacter* sp.	97
WT39	Actinobacteria	Actinomycetaceae	*Streptomyces atratus*	98
WT40	Actinobacteria	Micrococcaceae	*Paenarthrobacter nitroguajacolicus*	96
WT41	Betaproteobacteria	Oxalobacteraceae	*Massilia suwonensis*	97
WT42	Actinobacteria	Actinomycetaceae	*Streptomyces pseudovenezuelae*	98
WT43	Actinobacteria	Actinomycetaceae	*Streptomyces pseudovenezuelae*	98
WT44	Firmicutes	Bacillaceae	*Bacillus simplex*	99
WT45	Alphaproteobacteria	Caulobacteraceae	*Caulobacter rhizosphaerae*	96
WT46	Actinobacteria	Nocardiaceae	*Rhodococcus degradans*	95
WT47	Firmicutes	Bacillaceae	*Bacillus indicus*	99
WT48	Actinobacteria	Micrococcaceae	*Arthrobacter* sp.	98
WT49	Actinobacteria	Gordoniaceae	*Gordonia amicalis*	99
WT50	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas frederiksbergensis*	98
WT51	Firmicutes	Bacillaceae	*Bacillus indicus*	99
WT52	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	98
WT53	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	99
WT54	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	98
WT55	Actinobacteria	Actinomycetaceae	*Streptomyces bobili*	99
WT56	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas frederiksbergensis*	97
WT57	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas donghuensis*	99
WT58	Actinobacteria	Nocardiaceae	*Rhodococcus degradans*	99
WT59	Actinobacteria	Actinomycetaceae	*Streptomyces griseolus*	99
WT60	Firmicutes	Bacillaceae	*Bacillus thuringiensis*	99

Table S3.1 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
WA1	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	97
WA2	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	97
WA3	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	97
WA4	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella grimontii*	97
WA5	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter cancerogenus*	98
WA6	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella grimontii*	98
WA7	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	98
WA8	Gammaproteobacteria	Erwiniaceae	*Pantoea* sp.	98
WA9	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter* sp.	99
WA10	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella* sp.	99
WA11	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	97
WA12	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	99
WA13	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas donghuensis*	99
WA14	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* donghuensis	98
WA15	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas* sp.	96
WA16	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	94
WA17	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	98
WA18	Gammaproteobacteria	Enterobacteriaceae	*Raoultella terrigena*	98
WA19	Gammaproteobacteria	Enterobacteriaceae	*Citrobacter freundii*	98
WA20	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter cancerogenus*	98
WA21	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fluorescens*	99
WA22	Betaproteobacteria	Comamonadaceae	*Variovorax boronicumulans*	98
WA23	Actinobacteria	Pseudonocardiaceae	*Amycolatopsis azurea*	98
WA24	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas kilonensis*	99
WA25	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas brassicacearum*	99
WA28	Betaproteobacteria	Comamonadaceae	*Variovorax paradoxus*	99

[1] Isolate code refers to the environmental niche and isolation medium from which the isolate came. The first letter (W) indicates that the isolate was from willow rhizosphere. The second letter indicates the isolation media used to cultivates bacterial isolates (B= Bushnell-Haas medium amended with 1 % diesel, as the sole carbon and energy source, T= One-tenth-strength Trypticase Soy Agar (TSA) medium and A= DF-ACC agar. The Isolate numbers was randomly assigned.

Table S3.2 Taxonomic affiliations of rhizospheric bacteria isolated from *Eleocharis* rhizosphere on different media based on 16S rRNA gene.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
EB1	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas helmanticensis*	99
EB2	Firmicutes	Bacillaceae	*Bacillus indicus*	99
EB3	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas songnenensis*	98
EB4	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas geniculate*	98
EB5	Betaproteobacteria	Comamonadaceae	*Variovorax boronicumulans*	98
EB6	Betaproteobacteria	Burkholderiaceae	*Chitinimonas taiwanensis*	98
EB7	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas alcaligenes*	98
EB8	Actinobacteria	Microbacteriaceae	*Microbacterium pumilum*	99
EB9	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	97
EB10	Actinobacteria	Actinomycetaceae	*Streptomyces stelliscabiei*	99
EB11	Actinobacteria	Microbacteriaceae	*Microbacterium lacus*	98
EB12	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	97
EB13	Gammaproteobacteria	Moraxellaceae	*Acinetobacter junii*	99
EB14	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	96
EB15	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	99
EB16	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	99
EB17	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	98
EB18	Betaproteobacteria	Comamonadaceae	*Acidovorax facilis*	98
EB19	Gammaproteobacteria	Xanthomonadaceae	*Dyella ginsengisoli*	98
EB20	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas nitritireducens*	96
EB21	Gammaproteobacteria	Aeromonadaceae	*Aeromonas salmonicida*	99
EB22	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	97
EB23	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	98
EB24	Actinobacteria	Microbacteriaceae	*Microbacterium lacus*	97
EB25	Gammaproteobacteria	Aeromonadaceae	*Aeromonas salmonicida*	99
EB26	Gammaproteobacteria	Aeromonadaceae	*Aeromonas hydrophila*	98
EB27	Actinobacteria	Microbacteriaceae	*Microbacterium kitamiense*	99
EB28	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	99
EB29	Gammaproteobacteria	Aeromonadaceae	*Aeromonas sobria*	99
EB30	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fluorescens*	99
EB31	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas pavanii*	98
EB32	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	99
EB33	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	99
EB34	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fildesensis*	98
EB35	Betaproteobacteria	Comamonadaceae	*Comamonas odontotermitis*	99
EB36	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	98
EB37	Actinobacteria	Micrococcales	*Lysinimonas* sp.	99
EB38	Betaproteobacteria	Comamonadaceae	*Delftia lacustris*	99
EB39	Actinobacteria	Microbacteriaceae	*Microbacterium proteolyticum*	97
EB40	Actinobacteria	Microbacteriaceae	*Microbacterium saccharophilum*	98

Table S3.2 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
EB41	Unidentified	Unidentified	*Unidentified bacterium*	98
EB42	Alphaproteobacterial	Sphingomonadaceae	*Sphingopyxis soli*	98
EB43	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas entomophila*	99
EB44	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	98
EB45	Alphaproteobacteria	Xanthobacteraceae	*Azorhizobium* sp.	97
EB46	Alphaproteobacteria	Rhizobiaceae	*Rhizobium petrolearium*	99
EB47	Alphaproteobacteria	Bradyrhizobiaceae	*Bosea thiooxidans*	97
EB48	Betaproteobacteria	Alcaligenaceae	*Achromobacter spanius*	98
EB49	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	98
EB50	Unidentified	Unidentified	*Unidentified bacterium*	98
EB51	Alphaproteobacteria	Xanthobacteraceae	*Azorhizobium doebereinerae*	99
EB52	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas stutzeri*	98
EB53	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	99
EB54	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas stutzeri*	98
EB55	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	98
EB56	Betaproteobacteria	Comamonadaceae	*Delftia lacustris*	99
EB57	Actinobacteria	Microbacteriaceae	*Agromyces indicus*	98
EB58	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas japonensis*	99
EB59	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	98
EB60	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas silesiensis*	99
ET1	Betaproteobacteria	Burkholderiaceae	*Chitinimonas taiwanensis*	98
ET2	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas mexicana*	99
ET3	Unidentified	Unidentified	*Unidentified bacterium*	98
ET4	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	99
ET5	Actinobacteria	Microbacteriaceae	*Microbacterium testaceum*	98
ET6	Actinobacteria	Micrococcales	*Lysinimonas* sp.	99
ET7	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium candidae*	96
ET8	Alphaproteobacteria	Rhizobiaceae	*Rhizobium selenitireducens*	96
ET9	Alphaproteobacteria	Rhizobiaceae	*Rhizobium rosettiformans*	98
ET10	Alphaproteobacteria	Rhizobiaceae	*Rhizobium selenitireducens*	99
ET11	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ET12	Firmicutes	Bacillaceae	*Bacillus indicus*	96
ET13	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ET14	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	99
ET15	Alphaproteobacteria	Sphingomonadaceae	*Sphingopyxis soli*	98
ET16	Firmicutes	Bacillaceae	*Bacillus aquimaris*	98
ET17	Bacteroidetes	Flavobacteriaceae	*Flavihumibacter cheonanensis*	99
ET18	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium elymi*	97
ET19	Actinobacteria	Microbacteriaceae	*Microbacterium saccharophilum*	95
ET20	Unidentified	Unidentified	*Unidentified bacterium*	97

Table S3.2 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
ET21	Alphaproteobacteria	Sphingomonadaceae	*Sphingomonas dokdonensis*	96
ET22	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
ET23	Unidentified	Unidentified	*Unidentified bacterium*	98
ET24	Unidentified	Unidentified	*Unidentified bacterium*	98
ET25	Firmicutes	Bacillaceae	*Bacillus marisflavi*	99
ET26	Unidentified	Unidentified	*Unidentified bacterium*	98
ET27	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	99
ET28	Actinobacteria	Microbacteriaceae	*Microbacterium testaceum*	97
ET29	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
ET30	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fildesensis*	99
ET31	Alphaproteobacteria	Rhodospirillaceae	*Rhodospirillum* sp.	97
ET32	Betaproteobacteria	Comamonadaceae	*Variovorax paradoxus*	99
ET33	Betaproteobacteria	Comamonadaceae	*Delftia lacustris*	99
ET34	Actinobacteria	Microbacteriaceae	*Agromyces tropicus*	97
ET35	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	99
ET36	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ET37	Gammaproteobacteria	Chromatiaceae	*Pararheinheimera arenilitoris*	97
ET38	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	98
ET39	Alphaproteobacteria	Sphingomonadaceae	*Sphingopyxis soli*	96
ET40	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas chengduensis*	97
ET41	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	99
ET42	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mendocina*	99
ET43	Gammaproteobacteria	Yersiniaceae	*Serratia* sp.	97
ET44	Gammaproteobacteria	Yersiniaceae	*Serratia* sp.	98
ET45	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fluorescens*	99
ET46	Gammaproteobacteria	Yersiniaceae	*Serratia* sp.	97
ET47	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ET48	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	98
ET49	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter cancerogenus*	98
ET50	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	99
ET51	Gammaproteobacteria	Yersiniaceae	*Serratia* sp.	97
ET52	Gammaproteobacteria	Yersiniaceae	*Serratia* sp.	98
ET53	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	98
ET54	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	99
ET55	Alphaproteobacteria	Bradyrhizobiaceae	*Bosea thiooxidans*	98
ET56	Alphaproteobacteria	Xanthobacteraceae	*Brevundimonas denitrificans*	98
ET57	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas monteilii*	99
ET58	Firmicutes	Bacillaceae	*Exiguobacterium acetylicum*	99
ET59	Firmicutes	Bacillaceae	*Exiguobacterium undae*	98
ET60	Gammaproteobacteria	Pseudomonadaceae	*Azomonas macrocytogenes*	98

Table S3.2 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
EA1	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA2	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA3	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	99
EA4	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	99
EA5	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA6	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA7	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	99
EA8	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella michiganensis*	97
EA9	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter cancerogenus*	97
EA10	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA11	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
EA12	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	97
EA13	Bacteroidetes	Flavobacteriaceae	*Myroides odoratimimus*	97
EA14	Gammaproteobacteria	Moraxellaceae	*Acinetobacter pittii*	99
EA15	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	99
EA16	Bacteroidetes	Flavobacteriaceae	*Empedobacter tilapiae*	99
EA17	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
EA18	Bacteroidetes	Flavobacteriaceae	*Myroides odoratus*	98
EA19	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter* sp.	97
EA20	Bacteroidetes	Flavobacteriaceae	*Myroides odoratimimus*	97
EA21	Gammaproteobacteria	Enterobacteriaceae	*Pantoea agglomerans*	98
EA22	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas koreensis*	98
EA23	Actinobacteria	Mycobacteriaceae	*Mycobacterium aquiterrae*	99
EA24	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	99
EA25	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	99
EA27	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	98

[1] Isolate code refers to the environmental niche and isolation medium from which the isolate came. The first letter (E) indicates that the isolate where from *Eleocharis* rhizosphere. The second letter indicates the isolation media used to cultivates bacterial isolates (B= Bushnell-Haas medium amended with 1 % diesel, as the sole carbon and energy source, T= One-tenth-strength Trypticase Soy Agar (TSA) medium and A= DF-ACC agar. The Isolate numbers was randomly assigned.

Table S3.3 Taxonomic affiliations of bacteria isolated from bulk soil on different media based on 16S rRNA gene.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
SB1	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB2	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	99
SB3	Firmicutes	Bacillaceae	*Bacillus toyonensis*	99
SB4	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	97
SB5	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	98
SB6	Actinobacteria	Bogoriellaceae	*Georgenia muralis*	95
SB7	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	99
SB8	Actinobacteria	Microbacteriaceae	*Microbacterium lacus*	95
SB9	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	99
SB10	Actinobacteria	Nocardiaceae	*Rhodococcus erythropolis*	98
SB11	Betaproteobacteria	Oxalobacteraceae	*Massilia oculi*	99
SB12	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* sp.	98
SB13	Unidentified	Unidentified	Unidentified bacterium	99
SB14	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	98
SB15	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
SB16	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	98
SB17	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	98
SB18	Actinobacteria	Microbacteriaceae	*Agromyces indicus*	98
SB19	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	98
SB20	Gammaproteobacteria	Moraxellaceae	*Acinetobacter johnsonii*	98
SB21	Unidentified	Unidentified	Unidentified bacterium	95
SB22	Alphaproteobacteria	Rhizobiaceae	*Rhizobium* sp.	99
SB23	Unidentified	Unidentified	Unidentified bacterium	97
SB24	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas chelatiphaga*	98
SB25	Firmicutes	Bacillaceae	*Bacillus siamensis*	99
SB26	Alphaproteobacteria	Paracoccus	*Paracoccus* sp.	99
SB27	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	98
SB28	Unidentified	Unidentified	Unidentified bacterium	99
SB29	Gammaproteobacteria	Pseudomonadaceae	Pseudomonas kunmingensis	98
SB30	Firmicutes	Paenibacillaceae	*Brevibacillus nitrificans*	97
SB31	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas tumulicola*	99
SB32	Actinobacteria	Microbacteriaceae	*Microbacterium hatanonis*	98
SB33	Gammaproteobacteria	Moraxellaceae	*Acinetobacter* sp.	98
SB34	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB35	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB36	Gammaproteobacteria	Moraxellaceae	*Acinetobacter pittii*	98
SB37	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fulva*	99
SB38	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas stutzeri*	98
SB39	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	98
SB40	Actinobacteria	Microbacteriaceae	*Microbacterium oxydans*	98

Table S3.3 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
SB41	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB42	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas stutzeri*	96
SB43	Alphaproteobacteria	Sphingomonadaceae	*Sphingomonas taxi*	99
SB44	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas hunanensis*	98
SB45	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	99
SB46	Actinobacteria	Microbacteriaceae	*Microbacterium lacus*	98
SB47	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
SB48	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* sp.	99
SB49	Betaproteobacteria	Oxalobacteraceae	*Massilia oculi*	98
SB50	Alphaproteobacteria	Sphingomonadaceae	*Sphingobium yanoikuyae*	98
SB51	Unidentified	Unidentified	Unidentified bacterium	98
SB52	Unidentified	Unidentified	Unidentified bacterium	99
SB53	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas monteilii*	98
SB54	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB55	Gammaproteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SB56	Firmicutes	Bacillaceae	*Bacillus indicus*	99
SB57	Betaproteobacteria	Oxalobacteraceae	*Massilia* sp.	99
SB58	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas stutzeri*	99
SB59	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas mosselii*	99
SB60	Gammaproteobacteria	Moraxellaceae	*Acinetobacter* sp.	98
ST1	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas nitritireducens*	99
ST2	Firmicutes	Bacillaceae	*Bacillus idriensis*	99
ST3	Actinobacteria	Microbacteriaceae	*Agromyces indicus*	95
ST4	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	97
ST5	Betaproteobacteria	Oxalobacteraceae	*Massilia* sp.	98
ST6	Gammaproteobacteria	Chromatiaceae	*Rheinheimera arenilitoris*	98
ST7	Actinobacteria	Microbacteriaceae	*Agromyces indicus*	96
ST8	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas* sp.	97
ST9	Alphaproteobacteria	Caulobacteraceae	*Brevundimonas nasdae*	97
ST10	Unidentified	Unidentified	Unidentified bacterium	98
ST11	Unidentified	Unidentified	Unidentified bacterium	97
ST12	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	97
ST13	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
ST14	Betaproteobacteria	Comamonadaceae	*Hydrogenophaga* sp.	98
ST15	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* sp.	97
ST16	Firmicutes	Bacillaceae	*Bacillus cibi*	99
ST17	Gammaproteobacteria	Rhodanobacteraceae	*Luteibacter jiangsuensis*	97
ST18	Gammaproteobacteria	Bacillaceae	*Bacillus aquimaris*	96
ST19	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	97
ST20	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium halperniae*	95

Table S3.3 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
ST21	Gammaproteobacteria	Rhodanobacteraceae	*Dyella ginsengisoli*	99
ST22	Unidentified	Unidentified	Unidentified	98
ST23	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	99
ST24	Gammaproteobacteria	Rhodanobacteraceae	*Dyella ginsengisoli*	98
ST25	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas nitritireducens*	98
ST26	Actinobacteria	Microbacteriaceae	*Agrococcus* sp.	99
ST27	Firmicutes	Bacillaceae	*Bacillus thuringiensis*	98
ST28	Unidentified	Unidentified	Unidentified bacterium	98
ST29	Unidentified	Unidentified	Unidentified bacterium	98
ST30	Firmicutes	Bacillaceae	*Bacillus idriensis*	99
ST31	Actinobacteria	Microbacteriaceae	*Microbacterium natoriense*	99
ST32	Unidentified	Unidentified	*Unidentified bacterium*	98
ST33	Firmicutes	Bacillaceae	*Bacillus aryabhattai*	99
ST34	Actinobacteria	Nocardiaceae	*Rhodococcus erythropolis*	99
ST35	Alphaproteobacteria	Caulobacteraceae	*Brevundimonas alba*	99
ST36	Unidentified	Unidentified	Unidentified bacterium	96
ST37	Actinobacteria	Microbacteriaceae	*Microbacterium* sp.	99
ST38	Firmicutes	Bacillaceae	*Bacillus aquimaris*	98
ST39	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas nitritireducens*	97
ST40	Firmicutes	Bacillaceae	*Exiguobacterium* sp.	98
ST41	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium elymi*	96
ST42	Firmicutes	Bacillaceae	*Bacillus megaterium*	99
ST43	Gammaproteobacteria	Chromatiaceae	*Rheinheimera arenilitoris*	96
ST44	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas* sp.	99
ST45	Actinobacteria	Gordoniaceae	*Gordonia amicalis*	99
ST46	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium elymi*	97
ST47	Gammaproteobacteria	Rhodanobacteraceae	*Dyella ginsengisoli*	99
ST48	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	99
ST49	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas alcaligenes*	97
ST50	Gammaproteobacteria	Pseudomonadaceae	*Pseudoxanthomonas spadix*	99
ST51	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ST52	Alphaproteobacteria	Sphingomonadaceae	*Sphingopyxis soli*	99
ST53	Firmicutes	Bacillaceae	*Bacillus indicus*	99
ST54	Firmicutes	Bacillaceae	*Bacillus cereus*	97
ST55	Unidentified	Unidentified	Unidentified bacterium	96
ST56	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	98
ST57	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas* sp.	95
ST58	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas spadix*	99
ST59	Actinobacteria	Nocardiaceae	*Rhodococcus* sp.	95
ST60	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas* sp.	98

Table S3.3 Continued.

Isolate code	Phyla	Family	Closest NCBI relative	SIM (%)
SA1	Gammaproteobacteria	Moraxellaceae	*Acinetobacter pittii*	98
SA2	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	97
SA3	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	97
SA4	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	98
SA5	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella* variicola	97
SA6	Gammaproteobacteria	Enterobacteriaceae	*Citrobacter freundii*	98
SA7	Gammaproteobacteria	Enterobacteriaceae	*Pantoea agglomerans*	96
SA8	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella oxytoca*	98
SA9	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas plecoglossicida*	98
SA10	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter* sp.	98
SA11	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	99
SA12	Gammaproteobacteria	Enterobacteriaceae	*Klebsiella variicola*	99
SA13	Bacteroidetes	Flavobacteriaceae	*Empedobacter tilapiae*	99
SA14	Alphaproteobacteria	Sphingomonadaceae	*Sphingobacterium* sp.	98
SA15	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas maltophilia*	97
SA16	Bacteroidetes	Flavobacteriaceae	*Myroides odoratus*	98
SA17	Bacteroidetes	Flavobacteriaceae	*Myroides odoratus*	98
SA18	Bacteroidetes	Flavobacteriaceae	*Empedobacter tilapiae*	99
SA19	Gammaproteobacteria	Enterobacteriaceae	*Citrobacter freundii*	98
SA20	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas maltophilia*	99
SA21	Firmicutes	Staphylococcaceae	*Staphylococcus capitis*	99
SA22	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	99
SA23	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	97
SA24	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	98
SA25	Actinobacteria	Nocardiaceae	*Rhodococcus ruber*	98
SA26	Actinobacteria	Mycobacteriaceae	*Mycolicibacterium vanbaalenii*	99

[1] Isolate code refers to the environmental niche and isolation medium from which the isolate came. The first letter (S) indicates that the isolate where from bulk soil. The second letter indicates the isolation media used to cultivates bacterial isolates (B= Bushnell-Haas medium amended with 1 % diesel, as the sole carbon and energy source, T= One-tenth-strength Trypticase Soy Agar (TSA) medium and A= DF-ACC agar. The Isolate numbers was randomly assigned.

Figure S3.1 Bars indicate the relative abundance of phyla among isolates that possesses different PGP-associated traits *in vitro*.

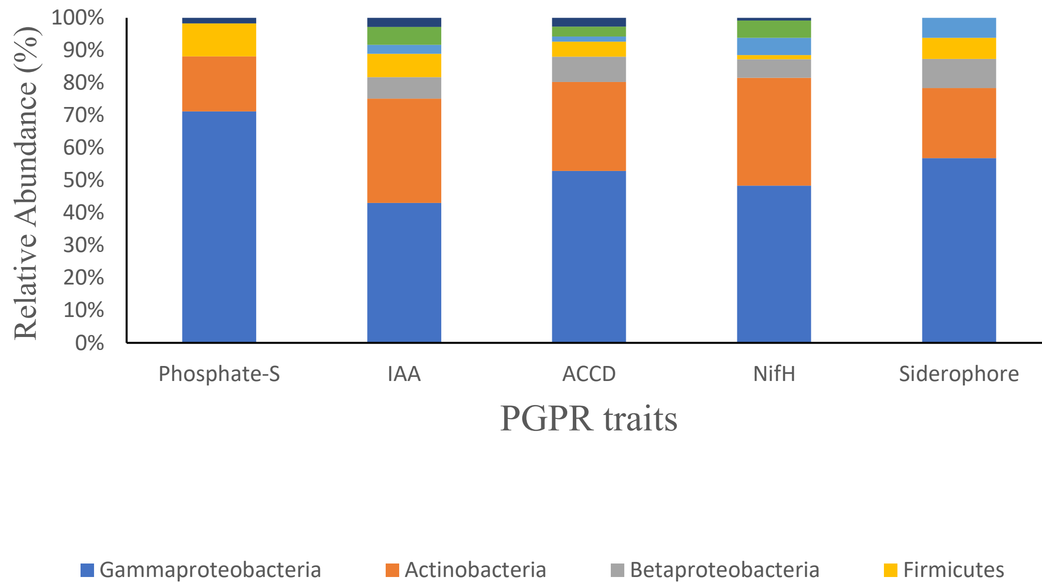


Figure S3.2 Qualitative representation of genera among isolates presenting different PGP-associated traits *in vitro*.

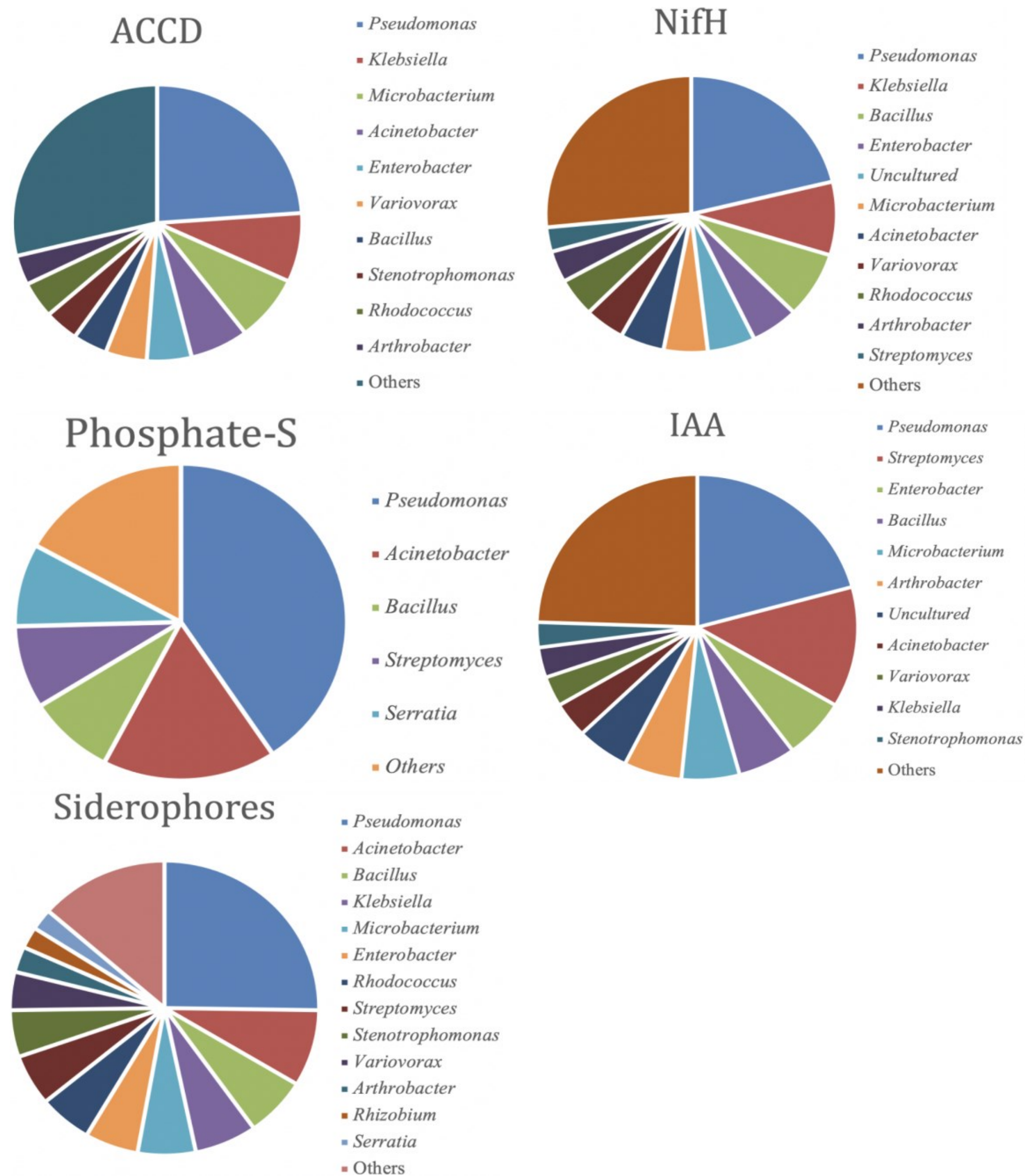


Figure S3.3 Venn diagram of comparison between isolates reported with all five PHC degradation and all five PGP traits.

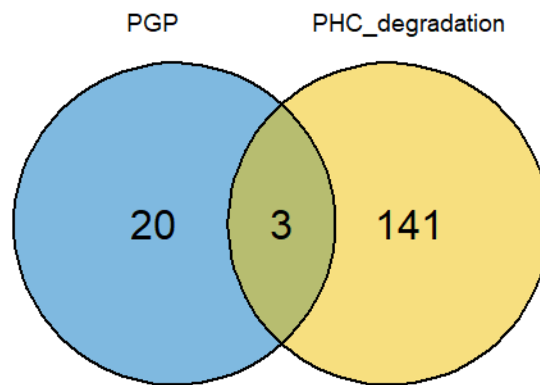


Figure S3.4 Example of production of siderophore by *Pseudomonas putida* strain ET27 on CAS agar plate.

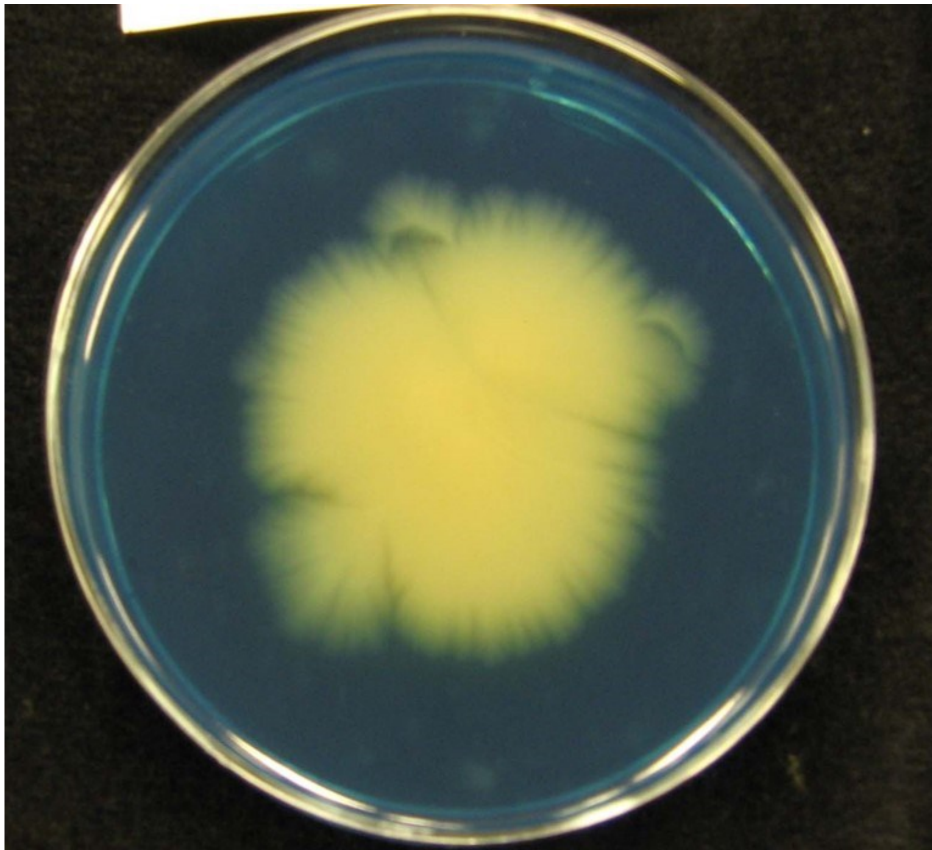
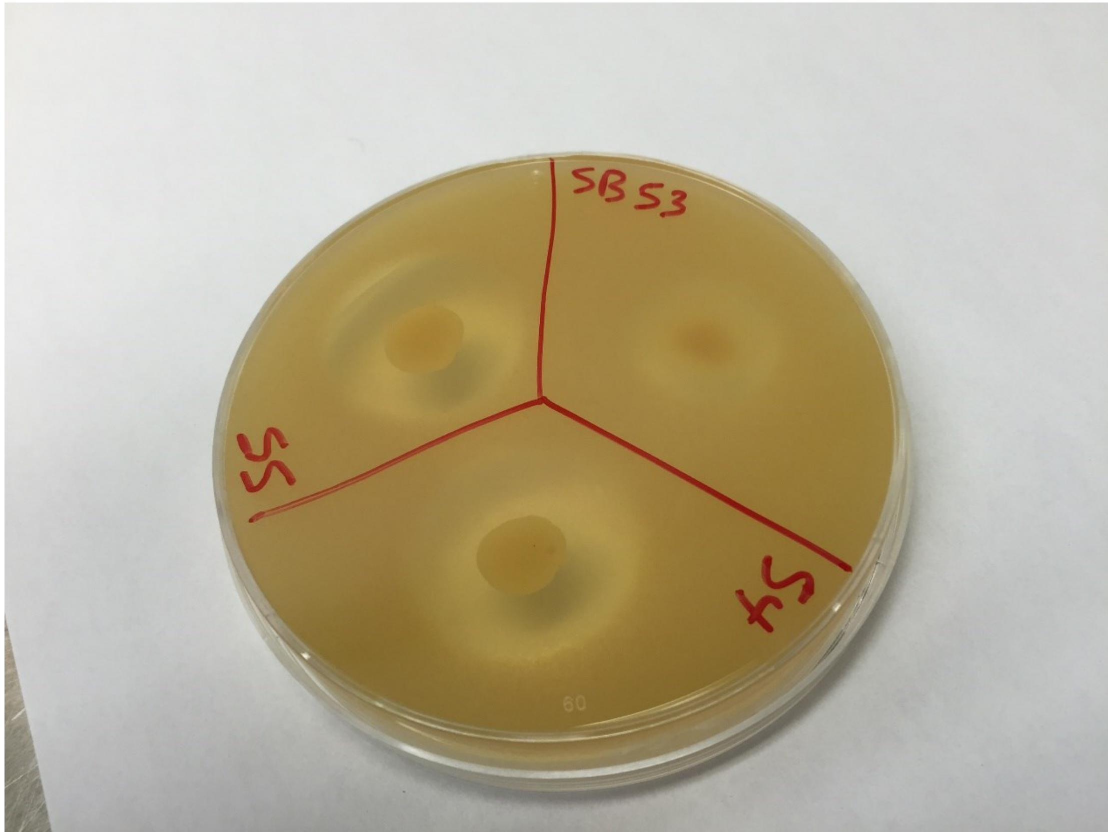


Figure S3.5 Example of phosphate solubilization by bacterial isolates *Pseudomonas monteilii* strain SB53, *Acinetobacter calcoaceticus* strain SB54 and *Bacillus indicus* strain SB55 as indicated by clear zone on the PDYA-CaP medium.



APPENDIX C: Supporting information (Chapter 4)

Table S1 PCR conditions used to amplify hydrocarbon degradation genes and plant growth promotion-related genes present in bacterial genomes.

Target gene	Primers	PCR Conditions	Reference
Alkane monooxygenase (*alkB*)	AlkB-F AlkB-R	Initial denaturation step of 4 min at 94 °C; 32 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C and a final elongation step of 10 min at 72 °C.	Kloos et al., 2006
Cytochrome P450 hydroxylase (*CYP153*)	P450-F P450-R	Initial denaturation step of 4 min at 94 °C; 32 cycles of 30 s at 94 °C, 30 s at 52 °C, and 1 min at 72 °C and a final extension step of 10 min at 72 °C.	Wang et al., 2010
Naphthalene dioxygenase (*nah*)	nah-F nah-F	Initial denaturation step of 5 min at 95 °C; 30 cycles of 1 min at 95 °C, 1 min at 47 °C, and 2 min at 72 °C and a final elongation for 10 min at 72 °C.	Baldwin et al., 2003
ACCD enzyme (*acdS*)	F1936 F1938	Initial denaturation step of 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C and a final elongation step of 7 min at 72 °C.	Blaha et al., 2006
Nitrogen fixation (*nifH*)	nifH-F nifH-R	Initial denaturation step of 5 min at 95 °C; 1 cycle of 20 s at 96 °C, 30 s at 65 °C, and 30 s at 72 °C; 2 cycles of 20 s at 96 °C, 30 s at 62 °C, and 35 s at 72 °C; 3 cycles of 20 s at 96 °C, 30 s at 59 °C, and 40 s at 72 °C; 4 cycles of 20 s at 96 °C, 30 s at 56 °C, and 45 s at 72 °C; 5 cycles of 20 s at 96 °C, 30 s at 53 °C, and 50 s at 72 °C; 25 cycles of 20 s at 94 °C, 45 s at 50 °C, and 60 s at 72 °C; and an extension step of 10 min at 72 °C.	Rösch et al., 2002

APPEDIX D: Hydrocarbons degradations potentials of bacterial isolates

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
WB1	*Variovorax paradoxus*	+	+	-	+	+
WB2	*Arthrobacter sulfonivorans*	+++	++	++	++	++
WB3	*Arthrobacter nicotinovorans*	+++	+++	+++	++	++
WB4	*Variovorax paradoxus*	+	+	+	+	+
WB5	*Streptomyces ederensis*	+	++	+	++	++
WB6	*Streptomyces ederensis*	+	++	++	+	+
WB7	*Nocardia asteroides*	+++	+++	+++	+	+++
WB8	*Variovorax paradoxus*	++	+	+	+	+
WB9	*Variovorax ureilyticus*	+	+	+	-	+
WB10	*Variovorax ureilyticus*	+	++	++	+	+
WB11	*Streptomyces* sp.	++	++	+++	-	+
WB12	*Variovorax paradoxus*	-	+	-	+	++
WB13	*Arthrobacter* sp.	+++	+++	+++	+++	+++
WB14	*Arthrobacter* sp.	+++	+++	+++	++	++
WB15	*Variovorax boronicumulans*	+	+	-	+	+
WB16	*Variovorax paradoxus*	+	-	-	-	+
WB17	*Arthrobacter* sp.	+++	++	+++	+++	+++
WB18	*Variovorax paradoxus*	+	+	++	+	+
WB19	*Streptomyces* sp.	+	++	++	++	++
WB20	*Nocardioides albus*	-	-	-	++	-
WB21	*Amycolatopsis speibonae*	++	+++	+++	+++	+++
WB22	*Arthrobacter pascens*	+	+	+	+	+
WB23	*Variovorax paradoxus*	++	++	++	++	+++
WB24	*Streptomyces canus*	+++	+++	-	++	++
WB25	*Sphingomonas sanxanigenens*	+	++	++	-	+
WB26	*Streptomyces umbrinus*	+	++	+	++	++
WB27	*Streptomyces phaeochromogenes*	-	-	+	+	+
WB28	*Nocardioides albus*	-	-	-	+	-
WB29	*Streptomyces chartreusis*	++	++	+	+	++
WB30	*Nocardioides albus*	++	++	++	+++	+++
WB31	*Pseudomonas frederiksbergensis*	-	+	+	++	++
WB32	*Kribbella aluminosa*	++	+	+	+	++
WB33	*Paenarthrobacter nitroguajacolicus*	+++	+++	+++	+++	+++
WB34	*Microbacterium oxydans*	+	+	+	++	+
WB35	*Kribbella sindirgiensis*	+	+	-	+	+
WB36	*Pseudarthrobacter oxydans*	+	+	+	+	++
WB37	*Kribbella koreensis*	-	+	+++	+	+
WB38	*Nocardioides* sp.	+	+	++	+	+
WB39	*Streptomyces umbrinus*	+	++	++	++	++
WB40	*Pseudarthrobacter siccitolerans*	+++	+++	+++	++	+++
WB41	*Streptomyces phaeochromogenes*	+	++	++	++	++
WB42	*Staphylococcus warneri*	++	+++	++	+++	++
WB43	*Phycicoccus aerophilus*	++	++	+	+	+
WB44	*Streptomyces umbrinus*	+	++	++	++	++
WB45	*Streptomyces umbrinus*	+	+	+	+	+
WB46	*Nocardia asteroides*	+++	++	+++	++++	++
WB47	*Streptomyces umbrinus*	+	++	+	++	++
WB48	*Nocardia* sp.	++++	++++	+++	++++	+++
WB49	*Nocardioides alpinus*	++++	++++	+++	++++	+++
WB50	*Arthrobacter humicola*	++	++	+	+	++

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
WB51	*Gordonia* sp.	++	++++	+++	+++	+++
WB52	*Nocardioides albus*	+++	-	+	+++	+++
WB53	*Streptomyces canus*	-	+	+	+	++
WB54	*Phycicoccus bigeumensis*	+	+	-	-	+
WB55	*Streptomyces* sp.	++	++	+	++	++
WB56	*Pseudarthrobacter oxydans*	+	+	-	++	+++
WB57	*Mycolicibacterium vanbaalenii*	+++	++++	+++	+++	+++
WB58	*Micromonospora palomenae*	+++	++	++	++	++
WB59	*Pseudarthrobacter sulfonivorans*	+++	++	++	+++	++
WB60	*Nocardioides albus*	++	+	+	++	++
WT1	*Bacillus cereus*	+	-	-	+	-
WT2	*Pseudomonas putida*	-	+	-	++	++
WT3	*Paenibacillus polysaccharolyticus*	-	+	-	-	-
WT4	*Pseudomonas mandelii*	++	-	-	++	+
WT5	*Bacillus cereus*	-	-	-	+	+
WT6	*Bacillus indicus*	-	-	-	+	++
WT7	*Streptomyces griseolus*	-	+	+	+	+
WT8	*Streptomyces atriruber*	++	++	++	+	+
WT9	*Streptomyces umbrinus*	+	++	++	+++	+++
WT10	*Bacillus megaterium*	+	++	++	+++	++
WT11	*Streptomyces bobili*	+++	+	+	+	+
WT12	*Gordonia amicalis*	+	+	+	++++	++++
WT13	*Streptomyces pseudovenezuelae*	-	+	+	+	+
WT14	*Streptomyces bobili*	-	+	+	+	+
WT15	*Bacillus aryabhattai*	-	++	++	+++	-
WT16	*Micromonospora halotolerans*	++	++	++	++	+++
WT17	*Pseudomonas kilonensis*	-	-	-	-	+
WT18	*Nocardioides albus*	+	+	+	+	+++
WT19	*Pseudarthrobacter siccitolerans*	+	+	++	+++	++
WT20	*Bacillus indicus*	+	-	+	-	-
WT21	*Mesorhizobium norvegicum*	-	-	+	-	-
WT22	*Pseudomonas frederiksbergensis*	-	-	-	+	+
WT23	*Pseudarthrobacter siccitolerans*	-	+	+	++	+++
WT24	*Pseudarthrobacter defluvii*	++	++	+	++	+++
WT25	*Bacillus simplex*	+	+	+	-	-
WT26	*Streptomyces griseolus*	-	-	-	+	+
WT27	*Streptomyces umbrinus*	-	+	-	++	++
WT28	*Bacillus aryabhattai*	-	-	-	++	+++
WT29	*Bacillus cereus*	-	+	-	++	-
WT30	*Bacillus indicus*	+	+	-	-	-
WT31	*Lysinibacillus xylanilyticus*	++	++	++	-	++
WT32	*Bacillus megaterium*	++	++	+	+++	+++
WT33	*Mycolicibacterium vanbaalenii*	+	+	+	+++	+++
WT34	*Pseudarthrobacter oxydans*	+++	+++	+++	+++	+++
WT35	*Bacillus thuringiensis*	-	-	-	+++	+++
WT36	*Bacillus indicus*	-	++	++	++++	++
WT37	*Arthrobacter* sp.	++	++	+	+++	+++
WT38	*Arthrobacter* sp.	+++	+++	+++	+++	+++
WT39	*Streptomyces atratus*	++	-	-	++++	++++
WT40	*Paenarthrobacter nitroguajacolicus*	+	+	+	+	++

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
WT41	*Massilia suwonensis*	-	+	+	-	-
WT42	*Streptomyces pseudovenezuelae*	+	+	+	-	-
WT43	*Streptomyces pseudovenezuelae*	+	+	+	-	-
WT44	*Bacillus simplex*	-	-	-	+	+
WT45	*Caulobacter rhizosphaerae*	+	+	+	+	++
WT46	*Rhodococcus degradans*	+	+	-	+++	+++
WT47	*Bacillus indicus*	-	++	+	++	++
WT48	*Arthrobacter* sp.	+++	+	-	++++	++++
WT49	*Gordonia amicalis*	+	+	-	+	+
WT50	*Pseudomonas frederiksbergensis*	+	-	-	+	+
WT51	*Bacillus indicus*	+	+	+	+	+
WT52	*Streptomyces bobili*	+	+	+	+	+
WT53	*Streptomyces bobili*	+	+	+	++	++
WT54	*Streptomyces bobili*	+	++	++	++	-
WT55	*Streptomyces bobili*	-	-	-	-	-
WT56	*Pseudomonas frederiksbergensis*	+	+	++	-	-
WT57	*Pseudomonas donghuensis*	+	+	+	-	-
WT58	*Rhodococcus degradans*	+	+	+	-	-
WT59	*Streptomyces griseolus*	+	+	+	-	-
WT60	*Bacillus thuringiensis*	-	-	-	-	-
WA1	*Raoultella terrigena*	++	+++	++	++	++
WA2	*Raoultella terrigena*	+++	+++	+++	+++	+++
WA3	*Raoultella terrigena*	+++	+++	-	+++	+++
WA4	*Klebsiella grimontii*	++	++	++	-	-
WA5	*Enterobacter cancerogenus*	+	++	++	+++	++
WA6	*Klebsiella grimontii*	+	+	+++	-	+
WA7	*Raoultella terrigena*	++	+++	+++	++	+++
WA8	*Pantoea* sp.	+	+	+	++	+++
WA9	*Enterobacter* sp.	++	+++	++	+++	+++
WA10	*Klebsiella* sp.	+++	++	+	++	+++
WA11	*Raoultella terrigena*	+	++	+	+	+++
WA12	*Klebsiella oxytoca*	-	-	+	-	+
WA13	*Pseudomonas donghuensis*	+	++	-	++	++
WA14	*Pseudomonas* donghuensis	++	++	++	++	+++
WA15	*Stenotrophomonas* sp.	++	++	+++	-	+++
WA16	*Pseudomonas mosselii*	++	+++	+++	++	+++
WA17	*Pseudomonas plecoglossicida*	++	-	+	++	++
WA18	*Raoultella terrigena*	-	-	-	+++	+++
WA19	*Citrobacter freundii*	+	++	+	++	+++
WA20	*Enterobacter cancerogenus*	++	-	+	++	++
WA21	*Pseudomonas fluorescens*	-	-	-	+	+
WA22	*Variovorax boronicumulans*	-	-	-	-	+
WA23	*Amycolatopsis azurea*	-	-	-	++	++
WA24	*Pseudomonas kilonensis*	-	-	++	-	+
WA25	*Pseudomonas brassicacearum*	-	-	+	-	+++
WA28	*Variovorax paradoxus*	+	-	-	+	+

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
EB1	*Pseudomonas helmanticensis*	+	-	-	-	+
EB2	*Bacillus indicus*	-	-	-	-	-
EB3	*Pseudomonas songnenensis*	++	+	+	++	++
EB4	*Pseudomonas geniculate*	++	++	+	+++	+++
EB5	*Variovorax boronicumulans*	+	+	-	+	+
EB6	*Chitinimonas taiwanensis*	-	++	+	-	+
EB7	*Pseudomonas alcaligenes*	+	+	-	++	-
EB8	*Microbacterium pumilum*	+	+	+	-	-
EB9	*Pseudomonas mosselii*	-	-	-	-	-
EB10	*Streptomyces stelliscabiei*					
EB11	*Microbacterium lacus*	+	+	+	-	+
EB12	*Pseudomonas mosselii*	+	+	+	++	++
EB13	*Acinetobacter junii*	++	++	-	+++	+++
EB14	*Pseudomonas mosselii*	-	-	-	+	+
EB15	*Pseudomonas plecoglossicida*	+	+	-	++	+
EB16	*Pseudomonas plecoglossicida*	++	-	-	++	+
EB17	*Microbacterium oxydans*	+	-	-	+	+
EB18	*Acidovorax facilis*	-	-	-	-	-
EB19	*Dyella ginsengisoli*	+	+	+	++	++
EB20	*Stenotrophomonas nitritireducens*	+	+	+	++	+
EB21	*Aeromonas salmonicida*	+	+	+	+	-
EB22	*Pseudomonas mosselii*	+	-	-	+	+
EB23	*Pseudomonas putida*	+	+	+	++	+
EB24	*Microbacterium lacus*	++	++	+	-	-
EB25	*Aeromonas salmonicida*	+	+	+	+	-
EB26	*Aeromonas hydrophila*	+	+	++	-	-
EB27	*Microbacterium kitamiense*	+	+	+	+	+
EB28	*Pseudomonas plecoglossicida*	++	++	+++	+++	+++
EB29	*Aeromonas sobria*	+	+	+	-	-
EB30	*Pseudomonas fluorescens*	+	+	+	+	+
EB31	*Stenotrophomonas pavanii*	-	++	-	++	++
EB32	*Pseudomonas putida*	+	+	-	++	+
EB33	*Pseudomonas plecoglossicida*	++	+	+	++	++
EB34	*Pseudomonas fildesensis*	+	+	-	++	+
EB35	*Comamonas odontotermitis*	++	++	++	+	-
EB36	*Pseudomonas plecoglossicida*	++	++	++	+++	+++
EB37	*Lysinimonas* sp.	++	+	++	++++	++++
EB38	*Delftia lacustris*	+	-	-	-	-
EB39	*Microbacterium proteolyticum*	+	+	+	+	+
EB40	*Microbacterium saccharophilum*	++	++	+	++	++
EB41	Unidentified bacterium	+	+	+	+++	++++
EB42	*Sphingopyxis soli*	+	+	+	+	+
EB43	*Pseudomonas entomophila*	+	+++	++	+	++++
EB44	*Pseudomonas mosselii*	++	++	++	+++	+++
EB45	*Azorhizobium* sp.	+	-	-	-	+
EB46	*Rhizobium petrolearium*	-	+	+	-	+
EB47	*Bosea thiooxidans*	+	+	+	-	+
EB48	*Achromobacter spanius*	-	-	-	-	-
EB49	*Rhodococcus ruber*	++	++	+	++	+++
EB50	Unidentified bacterium	+	+	-	+	+

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
EB51	*Azorhizobium doebereinerae*	-	-	-	-	+
EB52	*Pseudomonas stutzeri*	+	+	-	-	+
EB53	*Microbacterium oxydans*	-	-	-	-	-
EB54	*Pseudomonas stutzeri*	+	+	-	-	-
EB55	*Pseudomonas putida*	+	+	+	++	++
EB56	*Delftia lacustris*	+	+	-	+	+
EB57	*Agromyces indicus*	-	-	-	-	-
EB58	*Pseudoxanthomonas japonensis*	+	+	+	+	+
EB59	*Pseudomonas mosselii*	++	+	+	+	+++
EB60	*Pseudomonas silesiensis*	+	+	+	++	+
ET1	*Chitinimonas taiwanensis*	-	-	-	-	-
ET2	*Pseudoxanthomonas mexicana*	+	+	+	+	+
ET3	Unidentified bacterium	-	-	-	-	-
ET4	*Pseudoxanthomonas spadix*	+	+	+	+	-
ET5	*Microbacterium testaceum*	-	+	+	++	++
ET6	*Lysinimonas* sp.	-	-	-	-	-
ET7	*Chryseobacterium candidae*	-	-	-	+	++
ET8	*Rhizobium selenitireducens*	+	+	+	+	+
ET9	*Rhizobium rosettiformans*	+	+	+	+	+
ET10	*Rhizobium selenitireducens*	+	++	++	+	+
ET11	*Bacillus indicus*	-	++	-	-	-
ET12	*Bacillus indicus*	+	++	-	-	-
ET13	*Bacillus indicus*	+	-	-	-	-
ET14	*Pseudoxanthomonas spadix*	-	-	-	+	-
ET15	*Sphingopyxis soli*	+	+	+	+	+
ET16	*Bacillus aquimaris*	-	-	-	-	-
ET17	*Flavihumibacter cheonanensis*	-	-	-	-	-
ET18	*Chryseobacterium elymi*	-	-	-	-	-
ET19	*Microbacterium saccharophilum*	+	-	-	+	+
ET20	Unidentified bacterium	-	-	-	+	-
ET21	*Sphingomonas dokdonensis*	++	++	++	-	+
ET22	*Pseudoxanthomonas spadix*	-	-	-	+	-
ET23	Unidentified bacterium	-	-	-	-	-
ET24	Unidentified bacterium	-	-	-	+	-
ET25	*Bacillus marisflavi*	+	+	+	-	-
ET26	Unidentified bacterium	-	-	-	+	-
ET27	*Pseudomonas plecoglossicida*	+	-	+	++++	++++
ET28	*Microbacterium testaceum*	+	-	-	-	+
ET29	*Pseudoxanthomonas spadix*	-	-	-	+	-
ET30	*Pseudomonas fildesensis*	+++	+	-	++	++
ET31	*Rhodospirillum* sp.	-	-	-	-	-
ET32	*Variovorax paradoxus*	+++	+	-	-	-
ET33	*Delftia lacustris*	-	++	+	+	+
ET34	*Agromyces tropicus*	-	-	-	-	-
ET35	*Microbacterium oxydans*	-	-	-	-	-
ET36	*Bacillus indicus*	-	-	-	-	-
ET37	*Pararheinheimera arenilitoris*	+	-	-	-	-
ET38	*Rhodococcus ruber*	++	++	++	++++	++++
ET39	*Sphingopyxis soli*	-	-	+	+	+
ET40	*Pseudomonas chengduensis*	+	-	-	-	-

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
ET41	*Pseudomonas mosselii*	+	-	-	++	++
ET42	*Pseudomonas mendocina*	+	-	-	+	++
ET43	*Serratia* sp.	++	++	-	+	++
ET44	*Serratia* sp.	+	+	-	++	++
ET45	*Pseudomonas fluorescens*	+	+	-	+	++
ET46	*Serratia* sp.	-	+	++	+	++
ET47	*Bacillus indicus*	-	-	-	-	-
ET48	*Pseudomonas mosselii*	+	+	-	++	++
ET49	*Enterobacter cancerogenus*	+	+	+	+++	+++
ET50	*Pseudomonas putida*	+	-	-	+	++
ET51	*Serratia* sp.	+	+	+	+	+
ET52	*Serratia* sp.	+	+	-	+	++
ET53	*Pseudomonas mosselii*	+	+	-	+	++
ET54	*Pseudomonas mosselii*	-	-	-	+	+
ET55	*Bosea thiooxidans*	-	-	-	-	-
ET56	*Brevundimonas denitrificans*	-	-	-	-	-
ET57	*Pseudomonas monteilii*	++	+	-	+++	+++
ET58	*Exiguobacterium acetylicum*	-	-	-	+	-
ET59	*Exiguobacterium undae*	-	-	-	-	-
ET60	*Azomonas macrocytogenes*	++	+	-	++	++
EA1	*Klebsiella oxytoca*	+	-	-	++	++
EA2	*Klebsiella oxytoca*	+	-	-	+	+
EA3	*Klebsiella oxytoca*	-	+	-	+	+
EA4	*Klebsiella oxytoca*	-	-	-	-	-
EA5	*Klebsiella oxytoca*	+	-	-	+	-
EA6	*Klebsiella oxytoca*	-	-	+	+	+
EA7	*Klebsiella variicola*	-	-	-	+	+
EA8	*Klebsiella michiganensis*	-	-	-	-	-
EA9	*Enterobacter cancerogenus*	+	+	+	+++	+++
EA10	*Klebsiella oxytoca*	+	-	-	++	++
EA11	*Klebsiella oxytoca*	+++	+++	+++	++	++
EA12	*Klebsiella oxytoca*	+++	++	+++	++	++
EA13	*Myroides odoratimimus*	+	++	++	-	-
EA14	*Acinetobacter pittii*	+++	++	++	+++	+++
EA15	*Acinetobacter johnsonii*	+	-	-	+++	+++
EA16	*Empedobacter tilapiae*	-	-	-	-	-
EA17	*Acinetobacter calcoaceticus*	+	++	+++	+++	+++
EA18	*Myroides odoratus*	-	+	+	-	-
EA19	*Enterobacter* sp.	+	++	++	++	+++
EA20	*Myroides odoratimimus*	-	-	-	+	-
EA21	*Pantoea agglomerans*	+	+	+	-	-
EA22	*Pseudomonas koreensis*	-	-	-	+	-
EA23	*Mycobacterium aquiterrae*	++	++	++	+++	++
EA24	*Microbacterium oxydans*	-	-	-	+	-
EA25	*Mycolicibacterium vanbaalenii*	-	-	-	+++	+++
EA26	*Mycolicibacterium vanbaalenii*	+++	+++	+++	+++	+++

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
SB1	*Acinetobacter calcoaceticus*	++	+	++	+	+
SB2	*Acinetobacter johnsonii*	-	+	+	+	++
SB3	*Bacillus toyonensis*	+++	+++	+	++	++
SB4	*Microbacterium oxydans*	-	+	+	++	++
SB5	*Acinetobacter johnsonii*	++	++	+++	+	+
SB6	*Georgenia muralis*	+++	+	+	+++	+++
SB7	*Acinetobacter johnsonii*	+++	+++	++	+	++
SB8	*Microbacterium lacus*	-	-	-	++	+++
SB9	*Acinetobacter johnsonii*	++	+	-	+	+
SB10	*Rhodococcus erythropolis*	++	++	++	++++	++++
SB11	*Massilia oculi*	++	+	-	++	++
SB12	*Pseudomonas* sp.	+	+++	+	+	+
SB13	Unidentified bacterium	++	-	-	++	+++
SB14	*Microbacterium oxydans*	++	++	+	+++	++++
SB15	*Pseudoxanthomonas spadix*					
SB16	*Pseudomonas putida*	+	+	+	+++	+++
SB17	*Acinetobacter calcoaceticus*	+++	+	++	++	++
SB18	*Agromyces indicus*	+	-	+	+	+
SB19	*Pseudomonas putida*	+++	+++	-	++	++
SB20	*Acinetobacter johnsonii*	+++	-	-	++	++
SB21	Unidentified bacterium	-	-	-	-	-
SB22	*Rhizobium* sp.	-	-	-	-	-
SB23	Unidentified bacterium	+++	+	+	+++	+++
SB24	*Stenotrophomonas chelatiphaga*	-	-	-	+	-
SB25	*Bacillus siamensis*	+	-	+	+	+
SB26	*Paracoccus* sp.	+	+	+	++++	++++
SB27	*Rhodococcus ruber*	+++	-	-	+++	+++
SB28	Unidentified bacterium	++	+	+	+++	+++
SB29	Pseudomonas kunmingensis	+	+	-	+	+
SB30	*Brevibacillus nitrificans*	-	+	+	+++	+++
SB31	*Stenotrophomonas tumulicola*	++	++	++	++	++
SB32	*Microbacterium hatanonis*	++	++	++	++++	++++
SB33	*Acinetobacter* sp.	+++	+++	+++	++	++
SB34	*Acinetobacter calcoaceticus*	+	+	+	+	++
SB35	*Acinetobacter calcoaceticus*	++	+	+	+	++
SB36	*Acinetobacter pittii*	++	+	++	+++	+++
SB37	*Pseudomonas fulva*	+++	++	++	++	+++
SB38	*Pseudomonas stutzeri*	+++	++	+	++++	++++
SB39	*Microbacterium oxydans*	-	-	-	++	++
SB40	*Microbacterium oxydans*	-	-	-	++	++
SB41	*Acinetobacter calcoaceticus*	+	+	+	+	+
SB42	*Pseudomonas stutzeri*	-	++	-	+	-
SB43	*Sphingomonas taxi*	++	++	++	-	-
SB44	*Pseudomonas hunanensis*	+	+	+	+	+
SB45	*Pseudomonas mosselii*	+++	+++	+++	++	++
SB46	*Microbacterium lacus*	+++	+++	+++	+++	++++
SB47	*Pseudoxanthomonas spadix*	+	+	+	+++	++++
SB48	*Pseudomonas* sp.	++	++	++	++++	++++
SB49	*Massilia oculi*	+	-	+++	+++	++++
SB50	*Sphingobium yanoikuyae*	++	+++	+++	++++	++

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
SB51	Unidentified bacterium	+++	++	++	++	+++
SB52	Unidentified bacterium	-	+	-	+	++
SB53	*Pseudomonas monteilii*	-	+	-	++	++
SB54	*Acinetobacter calcoaceticus*	++	+	+	++	++
SB55	*Acinetobacter calcoaceticus*	++	-	-	++	++
SB56	*Bacillus indicus*	+	+	+	++	++
SB57	*Massilia* sp.	+	+	+	+++	+++
SB58	*Pseudomonas stutzeri*	-	-	-	-	-
SB59	*Pseudomonas mosselii*	+++	+++	+	++	++
SB60	*Acinetobacter* sp.	-	-	-	+	++
ST1	*Stenotrophomonas nitritireducens*	++	++	+	+++	+++
ST2	*Bacillus idriensis*	-	-	-	-	-
ST3	*Agromyces indicus*	-	-	-	-	-
ST4	*Rhodococcus ruber*	++	+	++	++++	++++
ST5	*Massilia* sp.	++	+	-	+	-
ST6	*Rheinheimera arenilitoris*	-	-	-	-	-
ST7	*Agromyces indicus*	+	-	-	+	+
ST8	*Pseudoxanthomonas* sp.	+	-	-	++	++
ST9	*Brevundimonas nasdae*	+	-	-	-	-
ST10	Unidentified bacterium	-	-	-	-	-
ST11	Unidentified bacterium	-	-	-	-	-
ST12	*Pseudoxanthomonas spadix*	-	-	-	+	-
ST13	*Pseudoxanthomonas spadix*	-	-	-	+	-
ST14	*Hydrogenophaga* sp.	-	-	-	+	-
ST15	*Pseudomonas* sp.	+	+	+	++	+++
ST16	*Bacillus cibi*	-	-	-	-	-
ST17	*Luteibacter jiangsuensis*	-	-	-	-	-
ST18	*Bacillus aquimaris*	-	-	-	-	-
ST19	*Pseudoxanthomonas spadix*	-	-	-	-	-
ST20	*Chryseobacterium halperniae*	-	-	-	-	-
ST21	*Dyella ginsengisoli*	+	-	-	+	-
ST22	Unidentified bacterium	-	-	-	-	-
ST23	*Rhodococcus ruber*	-	-	-	-	-
ST24	*Dyella ginsengisoli*	+	-	-	-	+
ST25	*Stenotrophomonas nitritireducens*	+	+	+	+++	+++
ST26	*Agrococcus* sp.	-	-	-	-	-
ST27	*Bacillus thuringiensis*	-	-	-	-	-
ST28	Unidentified bacterium	-	-	-	-	-
ST29	Unidentified bacterium	-	-	-	-	-
ST30	*Bacillus idriensis*	-	-	-	-	-
ST31	*Microbacterium natoriense*	+	+	-	+	-
ST32	Unidentified bacterium	-	-	-	-	-
ST33	*Bacillus aryabhattai*	-	-	-	-	-
ST34	*Rhodococcus erythropolis*	+	+	+	-	-
ST35	*Brevundimonas alba*	-	-	-	-	-
ST36	Unidentified bacterium	-	+	-	-	-
ST37	*Microbacterium* sp.	+	+	+	++	+++
ST38	*Bacillus aquimaris*	-	-	-	-	-
ST39	*Stenotrophomonas nitritireducens*	++	+	+	+++	+++
ST40	*Exiguobacterium* sp.	-	-	-	-	-

Degradation potentials of bacterial isolates

Isolate	I.D	Naphthalene	Phenanthrene	Pyrene	Dodecane	Hexadecane
ST41	*Chryseobacterium elymi*	-	-	-	+	+
ST42	*Bacillus megaterium*	-	-	-	-	-
ST43	*Rheinheimera arenilitoris*	-	-	-	-	-
ST44	*Stenotrophomonas* sp.	++	+	+	+++	+++
ST45	*Gordonia amicalis*	+++	+++	+++	+++	++++
ST46	*Chryseobacterium elymi*	+	+	-	+++	++
ST47	*Dyella ginsengisoli*	-	-	-	-	-
ST48	*Pseudoxanthomonas spadix*	-	-	-	-	+
ST49	*Pseudomonas alcaligenes*	+	+	-	++	+
ST50	*Pseudoxanthomonas spadix*	-	+	-	+	+
ST51	*Bacillus indicus*	-	-	-	-	-
ST52	*Sphingopyxis soli*	+	+	+	+	-
ST53	*Bacillus indicus*	-	-	-	-	-
ST54	*Bacillus cereus*	-	-	-	-	-
ST55	Unidentified bacterium	-	-	-	-	-
ST56	*Pseudoxanthomonas spadix*	-	+	-	+	+
ST57	*Pseudoxanthomonas* sp.	-	-	-	-	-
ST58	*Pseudoxanthomonas spadix*	-	-	-	+	-
ST59	*Rhodococcus* sp.	+	+	+	-	-
ST60	*Pseudoxanthomonas* sp.	-	+	-	++	++
SA1	*Acinetobacter pittii*	+	+	-	+++	+++
SA2	*Klebsiella variicola*	-	-	-	++	++
SA3	*Klebsiella variicola*	-	-	-	+	+
SA4	*Klebsiella variicola*	-	-	-	+	++
SA5	*Klebsiella variicola*	-	-	-	+	++
SA6	*Citrobacter freundii*	-	-	-	+	+
SA7	*Pantoea agglomerans*	++	++	+	++	++
SA8	*Klebsiella oxytoca*	-	-	-	+	+
SA9	*Pseudomonas plecoglossicida*	++	+	+	+++	+++
SA10	*Enterobacter* sp.	+	+	+	++	++
SA11	*Klebsiella variicola*	-	-	-	+	+
SA12	*Klebsiella variicola*	-	-	-	+	+
SA13	*Empedobacter tilapiae*	-	-	-	-	-
SA14	*Sphingobacterium* sp.	++	++	++	+	-
SA15	*Stenotrophomonas maltophilia*	++	++	+	+++	+++
SA16	*Myroides odoratus*	-	-	-	-	-
SA17	*Myroides odoratus*	+	-	-	+	-
SA18	*Empedobacter tilapiae*	-	+	+	+	-
SA19	*Citrobacter freundii*	-	-	-	++	+
SA20	*Stenotrophomonas maltophilia*	+	+	+	+++	+++
SA21	*Staphylococcus capitis*	+	++	++	++	+++
SA22	*Mycolicibacterium vanbaalenii*	-	-	-	+++	+++
SA23	*Rhodococcus ruber*	+	+	+	++	+++
SA24	*Mycolicibacterium vanbaalenii*	-	-	-	++	++
SA25	*Rhodococcus ruber*	+	+	+	+++	+++
SA27	*Mycolicibacterium vanbaalenii*	+++	+++	+++	+	+++

APPEDIX E: Plant growth promoting abilities of bacterial isolates

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
WB1	*Variovorax paradoxus*	+	-	+++	++	-
WB2	*Arthrobacter sulfonivorans*	-	-	-	-	-
WB3	*Arthrobacter nicotinovorans*	-	-	+	-	-
WB4	*Variovorax paradoxus*	+	-	+++	++	+
WB5	*Streptomyces ederensis*	+	+	-	+	-
WB6	*Streptomyces ederensis*	+++	-	-	-	-
WB7	*Nocardia asteroides*	-	-	-	++	-
WB8	*Variovorax paradoxus*	-	-	++	++	+
WB9	*Variovorax ureilyticus*	+	-	-	-	-
WB10	*Variovorax ureilyticus*	++	-	+	-	+
WB11	*Streptomyces* sp.	+	-	-	-	-
WB12	*Variovorax paradoxus*	+	-	+++	++	+
WB13	*Arthrobacter* sp.	++	-	-	-	-
WB14	*Arthrobacter* sp.	-	-	++	+	+
WB15	*Variovorax boronicumulans*	-	-	+++	+	-
WB16	*Variovorax paradoxus*	+	-	+++	++	-
WB17	*Arthrobacter* sp.	-	-	+++	++	+
WB18	*Variovorax paradoxus*	-	-	++	++	+
WB19	*Streptomyces* sp.	++	-	-	-	+
WB20	*Nocardioides albus*	+++	-	-	+	-
WB21	*Amycolatopsis speibonae*	-	-	++	+++	-
WB22	*Arthrobacter pascens*	+++	-	-	-	-
WB23	*Variovorax paradoxus*	-	-	+++	+++	-
WB24	*Streptomyces canus*	++	+	-	+	-
WB25	*Sphingomonas sanxanigenens*	+	-	+++	-	+
WB26	*Streptomyces umbrinus*	+++	-	-	-	-
WB27	*Streptomyces phaeochromogenes*	++	-	-	-	-
WB28	*Nocardioides albus*	-	-	-	-	-
WB29	*Streptomyces chartreusis*	++	-	-	-	-
WB30	*Nocardioides albus*	-	-	-	-	-
WB31	*Pseudomonas frederiksbergensis*	++	++	+++	+++	++
WB32	*Kribbella aluminosa*	-	-	-	-	-
WB33	*Paenarthrobacter nitroguajacolicus*	-	-	+	++	+
WB34	*Microbacterium oxydans*	+	-	+	-	-
WB35	*Kribbella sindirgiensis*	-	-	-	-	-
WB36	*Pseudarthrobacter oxydans*	+	-	+	+	+
WB37	*Kribbella koreensis*	-	-	-	-	-
WB38	*Nocardioides* sp.	-	-	-	-	-
WB39	*Streptomyces umbrinus*	-	-	+	-	-
WB40	*Pseudarthrobacter siccitolerans*	+	-	-	-	-
WB41	*Streptomyces phaeochromogenes*	+++	-	-	-	-
WB42	*Staphylococcus warneri*	-	-	-	-	-
WB43	*Phycicoccus aerophilus*	++	-	-	-	-
WB44	*Streptomyces umbrinus*	++	-	-	-	-
WB45	*Streptomyces umbrinus*	+	-	-	-	+
WB46	*Nocardia asteroides*	++	-	+	-	+
WB47	*Streptomyces umbrinus*	++	-	-	-	-
WB48	*Nocardia* sp.	-	-	+++	+++	-
WB49	*Nocardioides alpinus*	+	-	-	-	-
WB50	*Arthrobacter humicola*	-	+	-	+	+

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
WB51	*Gordonia* sp.	-	-	++	++	-
WB52	*Nocardioides albus*	-	-	-	-	-
WB53	*Streptomyces canus*	++	++	-	-	-
WB54	*Phycicoccus bigeumensis*	++	++	+	-	-
WB55	*Streptomyces* sp.	++	-	-	-	+
WB56	*Pseudarthrobacter oxydans*	-	-	-	++	-
WB57	*Mycolicibacterium vanbaalenii*	+++	-	+	-	-
WB58	*Micromonospora palomenae*	-	-	-	+	+
WB59	*Pseudarthrobacter sulfonivorans*	-	-	+	+	-
WB60	*Nocardioides albus*	-	-	-	-	-
WT1	*Bacillus cereus*	-	-	-	-	-
WT2	*Pseudomonas putida*	-	-	+++	+++	-
WT3	*Paenibacillus polysaccharolyticus*	-	-	-	-	-
WT4	*Pseudomonas mandelii*	+	+++	++	++	+
WT5	*Bacillus cereus*	+	-	-	-	-
WT6	*Bacillus indicus*	-	-	-	-	-
WT7	*Streptomyces griseolus*	-	-	++	++	+
WT8	*Streptomyces atriruber*	+	++	+	+	+
WT9	*Streptomyces umbrinus*	+	-	+	+	-
WT10	*Bacillus megaterium*	-	++	-	-	+
WT11	*Streptomyces bobili*	-	-	+	-	+
WT12	*Gordonia amicalis*	-	+	+++	+++	+
WT13	*Streptomyces pseudovenezuelae*	-	-	+	-	-
WT14	*Streptomyces bobili*	+++	-	+	+	-
WT15	*Bacillus aryabhattai*	++	-	-	-	-
WT16	*Micromonospora halotolerans*	+++	-	-	-	-
WT17	*Pseudomonas kilonensis*	++	+++	+++	++	++
WT18	*Nocardioides albus*	-	-	-	-	-
WT19	*Pseudarthrobacter siccitolerans*	+++	-	++	+	-
WT20	*Bacillus indicus*	-	-	-	-	+
WT21	*Mesorhizobium norvegicum*	-	-	-	-	-
WT22	*Pseudomonas frederiksbergensis*	+	+++	+	++	++
WT23	*Pseudarthrobacter siccitolerans*	+	-	-	-	-
WT24	*Pseudarthrobacter defluvii*	-	-	-	-	-
WT25	*Bacillus simplex*	++	-	-	-	-
WT26	*Streptomyces griseolus*	+	-	++	++	+
WT27	*Streptomyces umbrinus*	-	-	-	-	-
WT28	*Bacillus aryabhattai*	-	-	-	-	-
WT29	*Bacillus cereus*	+	++	-	-	+
WT30	*Bacillus indicus*	+	-	-	-	-
WT31	*Lysinibacillus xylanilyticus*	+	-	-	-	-
WT32	*Bacillus megaterium*	+	+	+	+	+
WT33	*Mycolicibacterium vanbaalenii*	+	-	-	-	-
WT34	*Pseudarthrobacter oxydans*	+++	-	-	-	-
WT35	*Bacillus thuringiensis*	-	-	-	-	-
WT36	*Bacillus indicus*	-	-	-	-	-
WT37	*Arthrobacter* sp.	++	-	-	-	-
WT38	*Arthrobacter* sp.	-	-	-	-	-
WT39	*Streptomyces atratus*	-	-	-	-	+
WT40	*Paenarthrobacter nitroguajacolicus*	+	-	+	-	-

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
WT41	*Massilia suwonensis*	++	-	-	-	+
WT42	*Streptomyces pseudovenezuelae*	-	-	-	-	-
WT43	*Streptomyces pseudovenezuelae*	+	-	-	-	-
WT44	*Bacillus simplex*	+	-	-	-	-
WT45	*Caulobacter rhizosphaerae*	+	-	-	-	-
WT46	*Rhodococcus degradans*	-	-	+++	+++	+++
WT47	*Bacillus indicus*	-	-	-	-	-
WT48	*Arthrobacter* sp.	++	-	+	+	-
WT49	*Gordonia amicalis*	-	-	++	++	-
WT50	*Pseudomonas frederiksbergensis*	+	+++	++	++	++
WT51	*Bacillus indicus*	+	-	-	-	-
WT52	*Streptomyces bobili*	+++	-	-	-	+
WT53	*Streptomyces bobili*	+++	-	+	+	-
WT54	*Streptomyces bobili*	++	-	-	-	+
WT55	*Streptomyces bobili*	++	-	-	-	-
WT56	*Pseudomonas frederiksbergensis*	+	+++	+++	+	+++
WT57	*Pseudomonas donghuensis*	-	++	+	++	+
WT58	*Rhodococcus degradans*	-	-	+	-	++
WT59	*Streptomyces griseolus*	+	-	-	-	+
WT60	*Bacillus thuringiensis*	-	-	-	-	-
WA1	*Raoultella terrigena*	++	-	+++	++	+
WA2	*Raoultella terrigena*	++	-	+++	++	-
WA3	*Raoultella terrigena*	++	-	+++	++	+
WA4	*Klebsiella grimontii*	-	-	+++	+++	++
WA5	*Enterobacter cancerogenus*	+	-	+++	++	+
WA6	*Klebsiella grimontii*	-	-	+++	++	+
WA7	*Raoultella terrigena*	+	-	+++	++	+
WA8	*Pantoea* sp.	++	-	+++	++	-
WA9	*Enterobacter* sp.	+	-	+++	++	++
WA10	*Klebsiella* sp.	+	-	+++	+++	++
WA11	*Raoultella terrigena*	+	-	+++	++	+
WA12	*Klebsiella oxytoca*	-	-	+++	+++	++
WA13	*Pseudomonas donghuensis*	-	-	+++	+++	+
WA14	*Pseudomonas* donghuensis	-	-	+++	++	-
WA15	*Stenotrophomonas* sp.	-	-	+++	-	++
WA16	*Pseudomonas mosselii*	-	-	+++	+	+
WA17	*Pseudomonas plecoglossicida*	-	-	+++	++	-
WA18	*Raoultella terrigena*	+	-	+++	+	-
WA19	*Citrobacter freundii*	-	++	+++	+	+
WA20	*Enterobacter cancerogenus*	+	-	+++	++	+
WA21	*Pseudomonas fluorescens*	-	+++	+++	+++	++
WA22	*Variovorax boronicumulans*	-	-	+++	+++	+
WA23	*Amycolatopsis azurea*	-	-	+++	+++	-
WA24	*Pseudomonas kilonensis*	+	-	+++	++	+
WA25	*Pseudomonas brassicacearum*	-	+++	+++	+++	+++
WA28	*Variovorax paradoxus*	-	-	+++	++	+

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
EB1	*Pseudomonas helmanticensis*	+++	-	+	-	-
EB2	*Bacillus indicus*	-	++	++	++	-
EB3	*Pseudomonas songnenensis*	+	+	++	++	++
EB4	*Pseudomonas geniculate*	++	-	++	+	++
EB5	*Variovorax boronicumulans*	-	-	++	-	+
EB6	*Chitinimonas taiwanensis*	-	-	++	-	+
EB7	*Pseudomonas alcaligenes*	++	-	++	-	+++
EB8	*Microbacterium pumilum*	-	-	++	-	++
EB9	*Pseudomonas mosselii*	+	-	+++	+++	++
EB10	*Streptomyces stelliscabiei*	++	-	-	-	++
EB11	*Microbacterium lacus*	-	-	+	-	++
EB12	*Pseudomonas mosselii*	-	-	+	-	++
EB13	*Acinetobacter junii*	+++	-	+	-	++
EB14	*Pseudomonas mosselii*	-	-	+	-	++
EB15	*Pseudomonas plecoglossicida*	++	-	++	++	++
EB16	*Pseudomonas plecoglossicida*	++	-	++	++	+++
EB17	*Microbacterium oxydans*	+	-	++	++	++
EB18	*Acidovorax facilis*	-	-	-	-	+
EB19	*Dyella ginsengisoli*	-	-	+	+	++
EB20	*Stenotrophomonas nitritireducens*	-	-	+	-	++
EB21	*Aeromonas salmonicida*	+	-	-	-	+
EB22	*Pseudomonas mosselii*	-	-	+	-	+
EB23	*Pseudomonas putida*	-	-	+	+	-
EB24	*Microbacterium lacus*	-	-	+	+	++
EB25	*Aeromonas salmonicida*	+	-	-	-	+
EB26	*Aeromonas hydrophila*	+	-	+	-	++
EB27	*Microbacterium kitamiense*	-	-	++	++	-
EB28	*Pseudomonas plecoglossicida*	++	-	++	++	++
EB29	*Aeromonas sobria*	-	-	+	-	++
EB30	*Pseudomonas fluorescens*	-	+	++	++	++
EB31	*Stenotrophomonas pavanii*	+	++	++	+	++
EB32	*Pseudomonas putida*	+	+	++	++	-
EB33	*Pseudomonas plecoglossicida*	+	-	++	+	++
EB34	*Pseudomonas fildesensis*	-	-	+	+	-
EB35	*Comamonas odontotermitis*	-	-	-	-	-
EB36	*Pseudomonas plecoglossicida*	-	-	+	+	+
EB37	*Lysinimonas* sp.	-	-	+	-	-
EB38	*Delftia lacustris*	-	-	+	-	+
EB39	*Microbacterium proteolyticum*	++	-	++	++	-
EB40	*Microbacterium saccharophilum*	-	-	-	-	-
EB41	Unidentified bacterium	+++	-	+	+	-
EB42	*Sphingopyxis soli*	-	-	-	-	+
EB43	*Pseudomonas entomophila*	++	-	++	++	+
EB44	*Pseudomonas mosselii*	-	-	+	+	+
EB45	*Azorhizobium* sp.	-	-	-	++	-
EB46	*Rhizobium petrolearium*	-	-	-	+	+
EB47	*Bosea thiooxidans*	+	-	-	+	+
EB48	*Achromobacter spanius*	-	-	-	++	-
EB49	*Rhodococcus ruber*	-	-	+	++	++
EB50	Unidentified bacterium	+	-	-	-	-

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
EB51	*Azorhizobium doebereinerae*	+++	-	-	+	-
EB52	*Pseudomonas stutzeri*	+	-	++	+	++
EB53	*Microbacterium oxydans*	++	-	++	+	++
EB54	*Pseudomonas stutzeri*	++	-	+	+	-
EB55	*Pseudomonas putida*	+	-	+	++	-
EB56	*Delftia lacustris*	+	-	+	-	+
EB57	*Agromyces indicus*	-	-	++	++	-
EB58	*Pseudoxanthomonas japonensis*	-	-	-	+	-
EB59	*Pseudomonas mosselii*	-	-	+	-	+
EB60	*Pseudomonas silesiensis*	-	-	+	++	+
ET1	*Chitinimonas taiwanensis*	-	-	-	+++	-
ET2	*Pseudoxanthomonas mexicana*	-	-	-	+	-
ET3	Unidentified bacterium	+	-	-	-	-
ET4	*Pseudoxanthomonas spadix*	+	+++	-	-	-
ET5	*Microbacterium testaceum*	+++	-	+	++	+
ET6	*Lysinimonas* sp.	+	-	+	+	-
ET7	*Chryseobacterium candidae*	-	-	-	+	-
ET8	*Rhizobium selenitireducens*	-	-	-	+++	+
ET9	*Rhizobium rosettiformans*	+++	-	-	+++	+
ET10	*Rhizobium selenitireducens*	+++	-	-	+++	+
ET11	*Bacillus indicus*	-	-	+	++	+
ET12	*Bacillus indicus*	-	-	+	+	-
ET13	*Bacillus indicus*	-	-	-	++	-
ET14	*Pseudoxanthomonas spadix*	-	-	-	-	-
ET15	*Sphingopyxis soli*	+	-	-	-	+
ET16	*Bacillus aquimaris*	-	-	-	+	+
ET17	*Flavihumibacter cheonanensis*	-	-	-	-	-
ET18	*Chryseobacterium elymi*	+	-	-	-	-
ET19	*Microbacterium saccharophilum*	-	-	++	++	+
ET20	Unidentified bacterium	+	-	-	-	-
ET21	*Sphingomonas dokdonensis*	++	-	-	+	+
ET22	*Pseudoxanthomonas spadix*	-	-	-	-	+
ET23	Unidentified bacterium	-	-	-	+	-
ET24	Unidentified bacterium	-	-	-	-	-
ET25	*Bacillus marisflavi*	-	-	+++	++	+
ET26	Unidentified bacterium	-	-	-	-	-
ET27	*Pseudomonas plecoglossicida*	++	+++	+++	+++	+++
ET28	*Microbacterium testaceum*	+	-	++	++	++
ET29	*Pseudoxanthomonas spadix*	+	-	-	-	-
ET30	*Pseudomonas fildesensis*	-	++	++	++	++
ET31	*Rhodospirillum* sp.	-	-	-	-	-
ET32	*Variovorax paradoxus*	-	-	-	++	+
ET33	*Delftia lacustris*	+++	-	+++	+	+
ET34	*Agromyces tropicus*	+	+	++	+	-
ET35	*Microbacterium oxydans*	-	-	-	-	+
ET36	*Bacillus indicus*	-	-	-	+	+
ET37	*Pararheinheimera arenilitoris*	-	-	-	-	-
ET38	*Rhodococcus ruber*	-	-	+++	+++	-
ET39	*Sphingopyxis soli*	+	-	-	-	+
ET40	*Pseudomonas chengduensis*	+++	-	++	+	++

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
ET41	*Pseudomonas mosselii*	-	++	+++	+	+++
ET42	*Pseudomonas mendocina*	++	-	++	++	++
ET43	*Serratia* sp.	-	++	++	+	++
ET44	*Serratia* sp.	-	++	+++	-	+++
ET45	*Pseudomonas fluorescens*	+	++	++	++	+++
ET46	*Serratia* sp.	+	++	++	+	++
ET47	*Bacillus indicus*	-	-	-	+	-
ET48	*Pseudomonas mosselii*	++	++	++	++	-
ET49	*Enterobacter cancerogenus*	+++	-	++	++	++
ET50	*Pseudomonas putida*	+	+++	++	+	++
ET51	*Serratia* sp.	-	++	++	++	++
ET52	*Serratia* sp.	+	+	++	+	++
ET53	*Pseudomonas mosselii*	-	++	+++	++	-
ET54	*Pseudomonas mosselii*	-	++	++	++	++
ET55	*Bosea thiooxidans*	-	-	-	-	+
ET56	*Brevundimonas denitrificans*	-	-	-	-	-
ET57	*Pseudomonas monteilii*	+	+++	+++	+++	+
ET58	*Exiguobacterium acetylicum*	-	+	-	++	-
ET59	*Exiguobacterium undae*	-	-	-	+	-
ET60	*Azomonas macrocytogenes*	++	++	+++	+++	++
EA1	*Klebsiella oxytoca*	+	-	+++	+++	+
EA2	*Klebsiella oxytoca*	+	-	+++	+++	+
EA3	*Klebsiella oxytoca*	+	-	+++	+++	+
EA4	*Klebsiella oxytoca*	-	-	+++	+++	-
EA5	*Klebsiella oxytoca*	++	-	+++	+++	+
EA6	*Klebsiella oxytoca*	++	-	+++	+++	+
EA7	*Klebsiella variicola*	-	-	+++	+++	-
EA8	*Klebsiella michiganensis*	-	-	+++	+++	-
EA9	*Enterobacter cancerogenus*	++	-	+++	++	+++
EA10	*Klebsiella oxytoca*	-	-	+++	+++	+
EA11	*Klebsiella oxytoca*	-	-	+++	+++	-
EA12	*Klebsiella oxytoca*	-	-	+++	+++	+
EA13	*Myroides odoratimimus*	+	-	+++	-	-
EA14	*Acinetobacter pittii*	+	-	+++	+	++
EA15	*Acinetobacter johnsonii*	+	-	+++	+	-
EA16	*Empedobacter tilapiae*	-	-	+++	-	-
EA17	*Acinetobacter calcoaceticus*	-	-	+++	+	+
EA18	*Myroides odoratus*	-	-	+++	-	-
EA19	*Enterobacter* sp.	++	-	+++	++	++
EA20	*Myroides odoratimimus*	-	-	+++	-	-
EA21	*Pantoea agglomerans*	+++	+	+++	+++	+
EA22	*Pseudomonas koreensis*	-	++	+++	+++	++
EA23	*Mycobacterium aquiterrae*	-	-	+++	++	++
EA24	*Microbacterium oxydans*	-	-	+++	-	-
EA25	*Mycolicibacterium vanbaalenii*	-	-	+++	+	-
EA26	*Mycolicibacterium vanbaalenii*	-	-	+++	+++	-

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
SB1	*Acinetobacter calcoaceticus*	-	+	+++	+	+
SB2	*Acinetobacter johnsonii*	-	-	+++	++	+
SB3	*Bacillus toyonensis*	-	-	+++	+	-
SB4	*Microbacterium oxydans*	+	-	+++	-	-
SB5	*Acinetobacter johnsonii*	+	-	+	-	+
SB6	*Georgenia muralis*	-	-	++	++	-
SB7	*Acinetobacter johnsonii*	+	-	+	-	+
SB8	*Microbacterium lacus*	-	-	-	-	+
SB9	*Acinetobacter johnsonii*	-	-	+	-	+
SB10	*Rhodococcus erythropolis*	-	-	+++	+++	++
SB11	*Massilia oculi*	-	-	+	+	-
SB12	*Pseudomonas* sp.	-	-	++	-	+
SB13	Unidentified bacterium	-	-	-	-	+
SB14	*Microbacterium oxydans*	+	-	++	++	-
SB15	*Pseudoxanthomonas spadix*	+	-	++	-	-
SB16	*Pseudomonas putida*	-	+	++	++	++
SB17	*Acinetobacter calcoaceticus*	-	+	+++	+++	++
SB18	*Agromyces indicus*	-	-	+++	-	-
SB19	*Pseudomonas putida*	+	-	+++	++	+
SB20	*Acinetobacter johnsonii*	+	-	-	+	-
SB21	Unidentified bacterium	-	-	-	-	-
SB22	*Rhizobium* sp.	-	-	+	+	+
SB23	Unidentified bacterium	-	-	+	+	-
SB24	*Stenotrophomonas chelatiphaga*	-	-	-	-	+
SB25	*Bacillus siamensis*	-	-	+	-	-
SB26	*Paracoccus* sp.	-	-	-	+	-
SB27	*Rhodococcus ruber*	-	-	++	++	++
SB28	Unidentified bacterium	-	+++	+	-	+
SB29	Pseudomonas kunmingensis	+++	-	+++	+++	-
SB30	*Brevibacillus nitrificans*	++	-	-	+	-
SB31	*Stenotrophomonas tumulicola*	-	-	+	-	+
SB32	*Microbacterium hatanonis*	-	-	++	+++	-
SB33	*Acinetobacter* sp.	+	-	-	-	+
SB34	*Acinetobacter calcoaceticus*	-	+++	+++	+	+
SB35	*Acinetobacter calcoaceticus*	-	++	++	++	+
SB36	*Acinetobacter pittii*	-	+++	+++	+	++
SB37	*Pseudomonas fulva*	+	-	+++	+++	++
SB38	*Pseudomonas stutzeri*	+++	-	+	-	++
SB39	*Microbacterium oxydans*	+	-	+++	+	-
SB40	*Microbacterium oxydans*	++	-	+++	-	+
SB41	*Acinetobacter calcoaceticus*	+	+++	+++	++	++
SB42	*Pseudomonas stutzeri*	+++	-	++	+	-
SB43	*Sphingomonas taxi*	-	-	-	-	+
SB44	*Pseudomonas hunanensis*	+	-	+++	+++	++
SB45	*Pseudomonas mosselii*	+++	-	+++	+++	+
SB46	*Microbacterium lacus*	+	-	++	+	-
SB47	*Pseudoxanthomonas spadix*	-	-	-	+	-
SB48	*Pseudomonas* sp.	++	-	++	+	+
SB49	*Massilia oculi*	++	-	+	-	+
SB50	*Sphingobium yanoikuyae*	+++	-	++	++	-

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
SB51	Unidentified bacterium	-	-	+	-	-
SB52	Unidentified bacterium	+	-	-	-	+
SB53	*Pseudomonas monteilii*	+++	-	+++	+	++
SB54	*Acinetobacter calcoaceticus*	-	+	++	-	+
SB55	*Acinetobacter calcoaceticus*	+	+	++	+	+
SB56	*Bacillus indicus*	-	-	-	+	-
SB57	*Massilia* sp.	+++	-	-	-	-
SB58	*Pseudomonas stutzeri*	++	-	++	-	+
SB59	*Pseudomonas mosselii*	-	-	-	-	-
SB60	*Acinetobacter* sp.	+	+++	++	+	++
ST1	*Stenotrophomonas nitritireducens*	+	-	++	-	+
ST2	*Bacillus idriensis*	-	-	-	+	-
ST3	*Agromyces indicus*	-	-	-	+	-
ST4	*Rhodococcus ruber*	+	-	++	+	++
ST5	*Massilia* sp.	++	-	+	-	+
ST6	*Rheinheimera arenilitoris*	+	-	+	+	-
ST7	*Agromyces indicus*	-	-	-	+	-
ST8	*Pseudoxanthomonas* sp.	-	-	-	-	-
ST9	*Brevundimonas nasdae*	-	-	-	++	-
ST10	Unidentified bacterium	-	-	-	+	-
ST11	Unidentified bacterium	-	-	+++	++	-
ST12	*Pseudoxanthomonas spadix*	-	-	-	+	-
ST13	*Pseudoxanthomonas spadix*	-	-	-	-	-
ST14	*Hydrogenophaga* sp.	-	-	-	-	-
ST15	*Pseudomonas* sp.	++	-	++	+	++
ST16	*Bacillus cibi*	-	-	-	+	+
ST17	*Luteibacter jiangsuensis*	-	-	-	-	-
ST18	*Bacillus aquimaris*	-	-	-	-	-
ST19	*Pseudoxanthomonas spadix*	-	-	-	+	-
ST20	*Chryseobacterium halperniae*	-	-	-	-	-
ST21	*Dyella ginsengisoli*	-	-	-	-	-
ST22	Unidentified bacterium	-	-	-	-	-
ST23	*Rhodococcus ruber*	-	-	-	+++	++
ST24	*Dyella ginsengisoli*	-	-	-	-	-
ST25	*Stenotrophomonas nitritireducens*	+	-	+++	-	+
ST26	*Agrococcus* sp.	-	-	+	-	-
ST27	*Bacillus thuringiensis*	-	-	++	++	-
ST28	Unidentified bacterium	+	-	-	+	-
ST29	Unidentified bacterium	+	-	-	-	-
ST30	*Bacillus idriensis*	-	-	-	+_	-
ST31	*Microbacterium natoriense*	-	-	+	++	+
ST32	Unidentified bacterium	-	-	+	+	-
ST33	*Bacillus aryabhattai*	+	++	++	++	-
ST34	*Rhodococcus erythropolis*	-	-	+++	+++	++
ST35	*Brevundimonas alba*	-	-	-	-	-
ST36	Unidentified bacterium	-	-	-	-	-
ST37	*Microbacterium* sp.	-	-	+	-	+
ST38	*Bacillus aquimaris*	+	-	-	+	-
ST39	*Stenotrophomonas nitritireducens*	-	-	++	-	+
ST40	*Exiguobacterium* sp.	-	-	-	-	-

PGP potentials of bacterial isolates

Isolate	I.D	IAA	P-solubilization	ACCD	N-fixation	Siderophore
ST41	*Chryseobacterium elymi*	+	-	-	-	-
ST42	*Bacillus megaterium*	+	-	+	+	-
ST43	*Rheinheimera arenilitoris*	-	-	-	-	-
ST44	*Stenotrophomonas* sp.	+	-	++	-	+
ST45	*Gordonia amicalis*	-	-	++	+++	+
ST46	*Chryseobacterium elymi*	+	-	-	-	-
ST47	*Dyella ginsengisoli*	-	-	-	-	-
ST48	*Pseudoxanthomonas spadix*	-	-	-	-	-
ST49	*Pseudomonas alcaligenes*	-	-	+	+	+
ST50	*Pseudoxanthomonas spadix*	-	-	-	-	+
ST51	*Bacillus indicus*	-	-	-	-	+
ST52	*Sphingopyxis soli*	-	-	++	++	+
ST53	*Bacillus indicus*	-	-	-	-	-
ST54	*Bacillus cereus*	-	-	-	++	+
ST55	Unidentified bacterium	-	-	-	-	-
ST56	*Pseudoxanthomonas spadix*	-	-	-	-	-
ST57	*Pseudoxanthomonas* sp.	-	-	-	-	+
ST58	*Pseudoxanthomonas spadix*	+		-	-	-
ST59	*Rhodococcus* sp.	-		++	++	++
ST60	*Pseudoxanthomonas* sp.	-		-	-	-
SA1	*Acinetobacter pittii*	-	-	+++	++	-
SA2	*Klebsiella variicola*	-	-	+++	+++	++
SA3	*Klebsiella variicola*	-	-	+++	+++	+
SA4	*Klebsiella variicola*	-	-	+++	+++	++
SA5	*Klebsiella variicola*	-	-	+++	+++	-
SA6	*Citrobacter freundii*	-	-	+++	+	-
SA7	*Pantoea agglomerans*	+++	-	+++	++	++
SA8	*Klebsiella oxytoca*	-	-	+++	+++	+
SA9	*Pseudomonas plecoglossicida*	+	-	+++	++	+++
SA10	*Enterobacter* sp.	++	-	+++	++	++
SA11	*Klebsiella variicola*	-	-	+++	+++	-
SA12	*Klebsiella variicola*	-	-	+++	+++	-
SA13	*Empedobacter tilapiae*	++	-	+++	-	-
SA14	*Sphingobacterium* sp.	-	-	+++	+	-
SA15	*Stenotrophomonas maltophilia*	+	-	+++	+	+++
SA16	*Myroides odoratus*		-	+++	+	-
SA17	*Myroides odoratus*	-	-	+++	-	-
SA18	*Empedobacter tilapiae*	-	+	+++	-	-
SA19	*Citrobacter freundii*	-	-	+++	+	+
SA20	*Stenotrophomonas maltophilia*	-	-	+++	+	+++
SA21	*Staphylococcus capitis*	-	-	+++	+++	-
SA22	*Mycolicibacterium vanbaalenii*	-	-	+++	++	-
SA23	*Rhodococcus ruber*	-	-	+++	++	+
SA24	*Mycolicibacterium vanbaalenii*	-	-	+++	+	-
SA25	*Rhodococcus ruber*	-	-	+++	++	+
SA27	*Mycolicibacterium vanbaalenii*	-	-	+++	+++	-

Appendix F: Scientific Communications

Scientific Communications

Alotaibi, **F**, Mohamed Hijri and St-Arnaud. M. 2019. Potential for Plant Growth Promotion of Rhizobacteria Associated with *Salix* and *Eleocharis* Plants Growing in Petrochemical Contaminated Soils. Rhizosphere 5, 7-11 July 2019, Saskatoon, Saskatchewan, Canada.

Alotaibi, **F**, Chih-Ying Lay, Mohamed Hijri and St-Arnaud. M. **2017**. Potential for Plant Growth Promotion of Rhizobacteria Associated with *Salix* and *Eleocharis* Plants Growing in Petrochemical Contaminated Soils. 14th International Phytotechnologies Conference (IPC2017) September 25-29, 2017. Montréal, Québec, Canada.

Alotaibi, **F**, Chih-Ying Lay, Mohamed Hijri and St-Arnaud. M. **2017**. Selecting Plant Growth-Promoting Rhizobacteria for phytoremediation of petroleum-hydrocarbons contaminated soils. 67th annual Canadian Society of Microbiologists Conference. June 20-23th, 2013. Waterloo, Ontario, Canada (poster).

Alotaibi, **F**, Chih-Ying Lay, Mohamed Hijri and St-Arnaud. M. **2016**. Selecting Plant Growth-Promoting Rhizobacteria for phytoremediation of petroleum-hydrocarbons contaminated soils. 16th International Symposium on Microbial Ecology (ISME 16) August 21-26, 2016. Montréal, Québec, Canada

Bell, TH, B. Cloutier-Hurteau, **F. Alotaibi**, C. Turmel, E. Yergeau, F. Courchesne, M. St-Arnaud. **2015**. Sphaerosporella brunnea and Inocybe sp. naturally dominated the microbiome composition and determined growth and Zn uptake of willow introduced to a former landfill. 8th International Conference on Mycorrhiza (ICOM8), Northern Arizona University, August 3-7th, 2015, Flagstaff, Arizona, USA.

Alotaibi F, Bell TH, Hassan SE, Yergeau E, Hijri M and St--Arnaud M. **2015**. Shifts in soil bacterial functional gene composition in response to willow planting and contamination level. Soil Interfaces for Sustainable Development (ISMOM 2015) conference, held from July 5th to July 10th, 2015 in Montréal, Québec. (poster)

Bell, TH, B. Cloutier-Hurteau, **F. Alotaibi**, C. Turmel, E. Yergeau, F. Courchesne, M. St-Arnaud. 2015. The relationship between dominant ectomycorrhizal fungi, willow growth, and accumulation of contaminant metals. Mycorrhiza 2015 symposium, 8-9 May, Ottawa, Ontario, Canada.

Bell, TH, B. Cloutier-Hurteau, **F. Alotaibi**, C. Turmel, E. Yergeau, F. Courchesne, M. St-Arnaud. **2014**. Willow growth and bioaccumulation of contaminant metals: links with dominant ectomycorrhizal fungi. Complex Soil Systems Conference, September 3-5th, 2014, Berkely, California, USA.

Alotaibi F, Bell TH, Hassan SE, Yergeau E, Hijri M and St--Arnaud M. **2014**. Functional diversity of the rhizosphere microbiome in petroleum-hydrocarbon contaminated soils. The International Union of Microbiological Societies Congress, held from July 27th, to August 1st, 2014 in Montreal, Québec. (poster)

Bell, TH, Hassan, SE, Lauron-Moreau, A, **Alotaibi, F**, Hijri, M, Yergeau, E, and St-Arnaud, M. **2013**. Bacterial and fungal rhizosphere communities diverge in

hydrocarbon-contaminated soils according to phylogeny of introduced willows. Gordon Research Conference on Applied and Environmental Microbiology. July 7-12th, 2013 South Hadley. MA. (poster).

Alotaibi F, Bell, TH, Chanda, D, St-Arnaud, M, and Hijri, M, **2013**. Isolation and characterization of petroleum-hydrocarbons degrading bacteria from contaminated sediment environment. Canadian Society of Microbiologists Conference. June 17-20th, 2013. Ottawa. ON. (poster)

Bell, TH, S. El-Din Hassan, Y. Terrat, F. Rohrbacher, S. Tardiff, **F. Alotaibi**, CW. Greer, E. Yergeau, M. St-Arnaud. 2013. Contaminants and introduced willowa structure soil bacterial and fungal communities. Colloque GenoRem, 31st Janvier, Centre de recherche sur la biodiversité, Montréal.

St-Arnaud, M, S. El-Din Hassan, Y. Terrat, S. Halary, E. Yergeau, **F. Alotaibi**, I. de la Providencia, CW. Greer, M. Hijri. 2013. Biodiversity and bioremediation functions of arbuscular mycorrhizal fungi in soils contaminated from industrial activities. 7th International Conference on Mycorrhiza (ICOM7), 6-11th January, New Delhi, India.

Terrat, Y, S. Halary, E. Yergeau, S. El-Din Hassan, **F. Alotaibi**, CW. Greer, M. Hijri, M. St-Arnaud. 2012. La métagénomique des sols au service de la phytoremediation. Dans le Panel: Apport de la génomique pour comprendre la boidiversité. Colloque 2012 du CSBQ, 12-14 décembre 2012, Coeur des Sciences de l'UQAM, Montréal.

Halary, S, S. El-Din Hassan,**F. Alotaibi**, I. de la Providencia, Y. Terrat, E. Yergeau, CW. Greer, M. Hijri, M. St-Arnaud. 2012. Améliorer la bioremediation par la génomique environnementale: structure et function des communautés microbiennes dans la depollution des sols par une association saules-microorganismes. P. 24 Dans Programme du colloque Mycorhizes 2012, 5 octobre, Centre de recherche sur la biodiversité, Montréal.

Terrat, Y, S. Halary, E. Yergeau, S. El-Din Hassan, **F. Alotaibi**, CW. Greer, M. Hijri, M. St-Arnaud. 2012. A phytoremediation study: how soil metagenomics reveals key functions for degradation of pollutants. 4th Annual Agronne Soil Metagenomics Workshop, 3-5th October, Chicago, IL, USA.

APPENDIX G: List of publications of the author resulting from the thesis or indirectly with the thesis

LIST Of PUBLICATIONS

Fahad Alotaibi, Marc St-Arnaud and Mohamed Hijri. **2022**. In-Depth Characterization of Plant Growth Promotion Potentials of Selected Alkanes-Degrading Plant Growth-Promoting Bacterial Isolates. Frontiers in Microbiology. 13:863702.

Fahad Alotaibi, Soon-Jae Lee, Marc St-Arnaud and Mohamed Hijri. **2021**. *Salix purpurea* and *Eleocharis obtusa* Rhizospheres Harbor a Diverse Rhizospheric Bacterial Community Characterized by Hydrocarbons Degradation Potentials and Plant Growth-Promoting Properties. Plants. 10, 1987-2008.

Fahad Alotaibi, Mohamed Hijri and Marc St-Arnaud. **2021**. Overview of Approaches to Improve Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils. Appl. Microbiol. 1, 329–351.

Terrence H. Bell, Benoît Cloutier-Hurteau, **Fahad Alotaibi**, Marie-Claude Turmel, Etienne Yergeau, François Courchesne and Marc St-Arnaud, M. **2015**. Early rhizosphere microbiome composition is related to the growth and Zn uptake of willows introduced to a former landfill. Environmental Microbiology: 17(8), 3015-3038.

Bell, TH, Hassan, SE, Lauron-Moreau, A, **Alotaibi, F**, Hijri, M, Yergeau, E, and St-Arnaud, M. **2014**. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. ISME J 8: 331-343.