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Structure fonctionnelle du plasmidome rhizosphérique dans un contexte de contamination aux hydrocarbures

par Fanny Rohrbacher

Département des Sciences Biologiques
Faculté des Arts et des Sciences

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Résumé

La phytoremédiation, la technique de bioremédiation qui utilise les plantes, est considérée comme une technologie « verte » et efficace pour décontaminer des sols pollués aux hydrocarbures. Les plantes agissent essentiellement à travers la stimulation des microorganismes de la rhizosphère, où l'exsudation racinaire semble être le moteur majeur de l'activité microbienne qui s'y déroule. Beaucoup de gènes responsables de l'adaptation bactérienne, dans le sol contaminé ou dans la rhizosphère, semblent être portés par les plasmides conjugatifs et transférés entre les bactéries. Une meilleure compréhension de ce phénomène pourrait améliorer le développement de techniques de manipulation du microbiome rhizosphérique afin d'accélérer la bioremédiation.

Le but de cette recherche est d'étudier le plasmidome, soit le contenu total en plasmides, de la rhizosphère de saules provenant d'un sol contaminé aux hydrocarbures. Des analyses métagénomiques, de données obtenues à partir d'un séquençage Illumina, ont été utilisées pour étudier les fonctions portées par les plasmides. Nos résultats ont indiqué un fort effet de la contamination aux hydrocarbures sur la composition des plasmides. De plus, les plasmides contenaient des gènes impliqués dans de nombreuses voies métaboliques, telles que la biodégradation d'hydrocarbures, la production d'énergie, la transduction du signal, le chimiotactisme, et les métabolismes des sucres, acides aminés et métabolites secondaires. À ce jour, c'est la première étude de métagénomique comparative documentant la diversité des plasmides dans un contexte de phytoremédiation. Ces résultats fournissent de nouvelles connaissances sur le rôle du transfert latéral de gènes dans l'adaptation bactérienne dans la rhizosphère et dans le sol contaminé aux hydrocarbures.

Mots-clés : Métagénomique, rhizosphère, phytoremédiation, plasmide, transfert latéral de gènes, hydrocarbures, exsudats racinaires.

Abstract

Phytoremediation, a bioremediation technique that uses plants, is considered to be an effective and affordable “green technology” to clean up hydrocarbon contaminated soils. Plants essentially act indirectly through the stimulation of rhizosphere microorganisms. Root exudation is thought to be one of the predominant drivers of microbial communities in the rhizosphere and is therefore a potential key factor behind enhanced hydrocarbon biodegradation. Many of the genes responsible for bacterial adaptation in contaminated soil and the plant rhizosphere are thought to be carried by conjugative plasmids and transferred among bacteria. A better understanding of these phenomena could thus inform the development of techniques to manipulate the rhizospheric microbiome in ways that improve hydrocarbon bioremediation.

This research aims to study the plasmidome (the overall plasmid content) in the rhizosphere of willow growing in hydrocarbon contaminated and non-contaminated soils, as compared with unplanted soil. Metagenomic analyses based on Illumina sequencing were used to highlight functions carried by plasmids. Our results indicate a strong effect of hydrocarbon contamination on plasmid composition. Furthermore, plasmids harbored genes involved in several metabolic pathways, such as hydrocarbon biodegradation, energy production, signal transduction, chemotaxis, metabolisms of carbohydrates, amino acids and secondary metabolites. To date, this is the first comparative soil metagenomics documenting the plasmidome diversity in a phytoremediation system. The results provide new knowledge on the role of lateral gene transfer in the bacterial adaptation in rhizosphere and in hydrocarbon contaminated soil.

Keywords : Metagenomics, rhizosphere, phytoremediation, plasmid, lateral gene transfer, hydrocarbon, root exudates.

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ABC transporters : ATP-Binding Cassette transporters

ACLAME : A CLAssification of Mobile genetic Elements

ADN : Acide DésoxiriboNucléique

AHL : Acyl Homoserine Lactone

ALMT : ALuminum-activated Malate Transporter

ANOVA : ANalysis Of VAriance

ATP : Adenosine Tri-Phosphate

ATPase : Adenosine TriPhosphate synthase

BLAST : Basic Local Alignment Search Tool

BTEX : Benzène, Ethylbenzene, Toluene, Xylene

C : Contaminated

C2,3O : Catechol 2,3 diOxygenase

CC : Connected Components

CONJ : CONJugation

DDE : Dichloro Diphenyl dichloroEthylene

DNA : Desoxyribo Nucleic Acid

EDTA : Ethylene Diamine Tetra Acetic acid

EGN : Evolutionary Gene and genomes Network

FA : Firmicutes Actinobacteria

FATA : Firmicutes Actinobacteria, Tenericutes Archaea

FPM : FlavoProtein Monooxygenases

HAP : Hydrocarbures Aromatiques Polycycliques

HMM : Hidden Markov Model

HMWC : High Molecular Weight Compound

ICA : Integrative Conjugative Elements

IDBA-UD : Iterative De Bruijn graph *de novo* Assembler – Uneven sequencing Depth

IP : Information Processing

KEGG : Kyoto Encyclopedia of Genes and Genomes

LCO : LipoChitine Oligosaccharide

LMWC : Low Molecular Weight Compound

LMWOA : Low Molecular Weight Organic Acids

MATE : Multidrug And Toxic compound Extrusion

MCP : Methyl-accepting Chemotaxis Protein

MEGAN : MEtaGenome ANalyzer

MFS : Major Facilitator Superfamily

MOB : Mobility

MPF : Mating Pair Formation

NC : Non Contaminated

NDO : Naphthalene DiOxygenase

NGS : Next Generation Sequencing

ORF : Open Reading Frame

OTUs : Operational Taxonomic Units

PAH : Polycyclic Aromatic Hydrocarbon

PGPR : Plant Growth Promoting Rhizobacteria

R : Rhizosphere

RNA : RiboNucleic Acid

RNHO : Rieske Non-Heme iron Oxygenases

RR : Response Regulator

SDM : Soluble Di-iron multicomponent Monooxygenases

SK : Sensor Kinase

SOM : Soil Organic Matter

T4CP : Type IV Coupling Protein

T4SS : Type IV Secretion System

TCE : TriChloroEthylene

TLG : Transfert Latéral de Gènes

TPH : Total Petroleum Hydrocarbon

tRNA : Transfer RiboNucleic Acid

UPGMA : Unweighted Pair Group Method with Arithmetic mean

US : Unplanted Soil

YSL : Yellow Stripe-Like

Liste des abréviations

% : Pourcent

± : Plus ou moins

≤ : Plus petit que

≥ : Plus grand que

°C : Degré celsius

bp : Base pairs

Che : Chemotaxis

Cl : Chlore

CO_2 : Dioxide de carbone/carbon dioxide

CoA: Coenzyme A

Cu : Copper

e.g. : *exempli gratia* (par exemple)

et al. : *et alii* (et les autres)

Etc : *et caetera* (et les autres choses)

F : Fisher's value

Fe : Iron

g : Gramme

Gln : Glutamine

H : Hydrogène

i.e. : *id est* (c'est-à-dire)

Imp : Inner membrane protein

Inc. : Incorporation

K : Potassium

Kdp : Potassium

L : Litre

Lia : Lipid II Interacting Antibiotics

Meta: Meta cleavage patway

mg/kg *ou* $mg.kg^{-1}$: Milligrammes par kilogrammes

Mn : Manganese

n : Number

Na : Sodium

Nar : Respiratory nitrate reductase

Ntr : Nitrogen

O_2 : Dioxygène

Omp : Orotidine monophosphate

Ortho: Ortho cleavage pathway

P : P-value

pH : Potentiel hydrogène

Pho : Phosphate

Pil : Pilus

QC : Québec

Rpo : Ribonucleic acid polymerase

Subterm. ox.: Subterminal oxidation.

Term. ox.: Terminal oxidation

Trp : Tryptophan

µg : Microgramme

var. : *varietas* (variété)

Vir : Virulence

Zn : Zinc

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Introduction

Depuis le XIX^{ème} siècle, la production industrielle d'hydrocarbures - autant que leur utilisation inappropriée, leur élimination incorrecte et les pertes accidentelles - a provoqué la pollution de nombreux environnements. Au Canada seulement, plus de 22 000 sites ont été identifiés comme étant contaminés, dont plus de la moitié contenant des hydrocarbures (FCSAP 2013). Ces sites contaminés représentent principalement les friches industrielles et actuelles raffineries de pétrole, les stations essence, les industries agrochimiques, pétrochimiques et pharmaceutiques. En 2009, la société de technologies environnementales Ventix a réalisé une étude sur les friches industrielles à Montréal. Dans la métropole, l'étude a évalué à 135 km² la superficie des friches industrielles contaminées, soit plus du tiers de l'île de Montréal (Joncas 2013). Un recul de quelques décennies nous permet de constater alors que Montréal a déjà été le plus important centre de raffinage au Canada. Sur les quatre des six raffineries montréalaises, quatre ont cessé leurs activités dans les années 1980, devenant des friches industrielles contaminées. Dans ces sites, il est fréquent que des hydrocarbures, tels que les hydrocarbures aromatiques polycycliques (HAP) et les BTEX (benzène, éthylbenzène, toluène et xylène), y aient été oubliés ou cachés. Ces polluants, fréquemment présents en grandes concentrations (centaines de mg/kg), deviennent alors une source durable de pollution des sols. De plus, la plupart des composés aromatiques sont récalcitrants et persistent dans l'environnement pour de longues périodes de temps. En plus de nuire à la pérennité des écosystèmes, la présence d'hydrocarbures dans l'environnement constitue un danger pour la santé publique du fait de leur toxicité, leurs propriétés mutagènes et cancérigènes, et de leur habilité à s'accumuler dans la chaine alimentaire (Mrozik and Piotrowska-Seget 2010). La dépollution de ces environnements est donc devenue un enjeu écologique et sanitaire majeur.

Quatre catégories de techniques de dépollution des sols ont été développées : le confinement, les méthodes physico-chimiques, thermiques et biologiques. Les trois premières techniques, dites « traditionnelles », en plus de représenter un coût très élevé (plus d'un million de dollars par hectare), sont lourdes à mettre en œuvre et requièrent une haute demande énergétique. Elles nécessitent soit le transport de sol contaminé vers un autre lieu de décontamination, soit un traitement *in situ*, ces derniers ayant un impact néfaste sur la biodiversité du milieu du fait de la

consommation de réactifs chimiques (Mrozik and Piotrowska-Seget 2010). Or, les sites contaminés ne sont pas toujours accessibles aux traitements *in situ* ni à l'excavation. De ces faits, une grande proportion des sites contaminés sont soit laissés tels quels ou bien la réhabilitation est remise à plus tard. Quant aux techniques biologiques, dites de bioremédiation, elles tirent directement profit de la capacité de certains organismes (plantes, champignons et bactéries) à séquestrer, concentrer et/ou dégrader les contaminants (Das and Chandran 2011). Elles permettent également de dépolluer des sites inaccessibles à l'excavation, ne nécessitent pas de transport du sol contaminé et moins de main d'œuvre, et possèdent une empreinte carbone (quantité de CO_2 émis) basse, et la biomasse aérienne peut être convertie en bioénergie (Das and Chandran 2011). De plus, de nombreuses études ont démontré qu'implémenter la bioremédiation pouvait réduire les couts de réhabilitation de 50 à 80%, comparé aux techniques traditionnelles (Drake 1997, Glass 1998, EPA 2000, van Epps 2006). La bioremédiation est donc une méthode « verte » qui s'inscrit dans les perspectives de développement durable.

Parmi les techniques de bioremédiation des hydrocarbures, la phytoremédiation est une technique prometteuse qui utilise la plante comme acteur principal de l'exportation d'un contaminant hors du milieu environnant. Les arbres à croissance rapide, tels que les saules et les peupliers, sont souvent privilégiés en phytoremédiation. La plante peut : (1) soit absorber le contaminant pour le métaboliser (phytodégradation/ phytotransformation), (2) soit empêcher sa libération dans d'autres compartiments de l'environnement en le stabilisant/séquestrant dans des racines (phytostabilisation) ou le concentrant dans ses parties aériennes (phytoextraction), ou (3) l'éliminer par évapotranspiration (phytovolatilisation) (Salt, Smith et al. 1998, Favas, Pratas et al. 2014, Rohrbacher and St-Arnaud 2016). Cependant, la phytoremédiation ne repose pas seulement sur l'action de la plante mais aussi et surtout des microorganismes qui lui sont associés.

À la périphérie des racines, la rhizosphère constitue le premier habitat de la plante peuplé de microorganismes. Cette fine couche de sol est profondément influencée par le métabolisme de la plante à travers le rejet d'oxygène et la sécrétion d'un éventail complexe d'exsudats racinaires, telles que des molécules riches en carbone (acides organiques, sucres, acides aminés). Grâce au chimiotactisme, les microorganismes sont attirés vers ces exsudats racinaires qui sont ensuite utilisés comme substrats pour leur métabolisme (Lioussanne, Jolicoeur et al. 2008,

Huang, Chaparro et al. 2014, Vandenkoornhuyse, Quaiser et al. 2015). Le microbiote racinaire est alors principalement recruté à partir du sol environnant et son développement est fortement influencé par les espèces microbiennes à proximité (Vandenkoornhuyse, Quaiser et al. 2015). De ce fait, la rhizosphère est jusqu'à 100 fois plus riche en biomasse microbienne : c'est l'effet rhizosphérique (Cheng and Coleman 1990, Lynch and Whipps 1990, Anderson, Guthrie et al. 1993, Marilley and Aragno 1999, Kowalchuk, Buma et al. 2002, Nie, Yang et al. 2010, Martin, George et al. 2014). Cela fait de la rhizosphère un environnement hautement dynamique où des communautés complexes de microorganismes se développent. Cependant, la diversité bactérienne de la rhizosphère, ou richesse spécifique, semble être moins importante que celle du sol environnant (Bulgarelli, Rott et al. 2012). Ceci peut s'expliquer par le fait que certains microorganismes adaptés à la plante peuvent avoir été sélectionnés par celle-ci car ils lui fournissent un avantage sélectif. En effet, du fait de leur mode de vie sessile, les plantes ont besoin de moyens pour s'ajuster à toutes sortes de contraintes. Les plantes et leurs microorganismes associés ont en fait co-évolué pour former une association symbiotique, où chacun en tire des bénéfices. Les fonctions écologiques portées par le microbiote sélectionné permettent à la plante non seulement d'améliorer sa croissance et sa nutrition, mais aussi de s'adapter aux stress biotiques (résistance aux antibiotiques et pathogènes) et abiotiques, telle que la contamination aux hydrocarbures (Berendsen, Pieterse et al. 2012, Bulgarelli, Schlaeppi et al. 2013, Yergeau, Sanschagrin et al. 2014, Vandenkoornhuyse, Quaiser et al. 2015).

Beaucoup de bactéries exposées à long terme à une contamination finissent par développer la capacité de biodégrader les hydrocarbures, constituant en retour une source de carbone et d'énergie pour leur respiration et leur croissance. Ces bactéries biodégradent les hydrocarbures des plus simples (alcanes linéaires et ramifiés) aux plus complexes (composés aromatiques), à l'exception des HAP qui ne peuvent être biodégradés en totalité (Atlas and Bragg 2009). La voie de biodégradation la plus rapide et la plus complète de la majorité des polluants organiques est réalisée sous des conditions aérobies (Das and Chandran 2011, Rohrbacher and St-Arnaud 2016). La plupart des bactéries n'étant pas équipées de toutes les enzymes nécessaires à la biodégradation totale d'un hydrocarbure, celle-ci est alors principalement réalisée par un consortium de microorganismes possédant des systèmes enzymatiques variés.

Si les bactéries sont capables de vivre et de s'adapter aux conditions extrêmes, telles que la contamination aux hydrocarbures, c'est parce qu'elles évoluent rapidement. Cette évolution accélérée repose sur la capacité des bactéries à acquérir des gènes directement de leurs consœurs, autrement appelée transfert latéral de gènes (TLG). Dagan, Artzy-Randrup et al. (2008) ont estimé que la quantité de gènes acquis latéralement dans un génome procaryote variait entre 66 et 96%. Le TLG est conduit par 3 mécanismes : la transformation (acquisition d'ADN étranger nu), la transduction (transfert de gènes médié par les bactériophages) et la conjugaison (plasmides et éléments conjugatifs intégratifs - ICEs) (Popa and Dagan 2011). Ces trois phénomènes permettent à des populations microbiennes aux propriétés physiologiques, aux structures cellulaires ou aux niches écologiques différentes d'acquérir de nouvelles fonctions pour améliorer leurs performances dans leur niche écologique actuelle ou d'en envahir une nouvelle (Gogarten, Doolittle et al. 2002, Cohan and Koeppel 2008). Par exemple, l'acquisition de gènes cataboliques, comme ceux de biodégradation des hydrocarbures, est un réel atout pour l'adaptation bactérienne dans un sol contaminé.

La rhizosphère est considérée comme un *hotspot* pour le transfert latéral. En effet, la grande quantité de nutriments (exsudats racinaires) et la haute densité cellulaire encouragent le contact entre les bactéries, et par extension la probabilité de transfert de gènes via la conjugaison (Heuer and Smalla 2012, Sentchilo, Mayer et al. 2013, Wang, Kou et al. 2014, Wei, Wang et al. 2014). Ce mécanisme, considéré majeur pour acquérir des gènes de dégradation aux hydrocarbures dans le sol, se base sur le transfert de plasmides (Halary, Leigh et al. 2010, Zhang, Pereira e Silva Mde et al. 2014). Ces molécules d'ADN extra-chromosomiques auto-réplicatives et majoritairement circulaires, sont transmises à la fois verticalement (de la cellule mère aux cellules filles lors de la division bactérienne) et horizontalement (d'une cellule donneuse à une cellule receveuse) (Mela, Fritsche et al. 2008). Les plasmides possèdent en général des spectres d'hôtes étroits, c'est-à-dire qu'ils sont capables de se répliquer et se maintenir que dans un nombre limité de taxa. Certains plasmides ont cependant des spectres d'hôtes très larges qui s'étendent à travers toutes les divisions bactériennes, et de ce fait jouent un rôle majeur dans l'adaptation bactérienne (Norman, Hansen et al. 2009, Heuer and Smalla 2012). Les plasmides sont qualifiés de génomes « mosaïques », combinant des gènes acquis de différentes sources par transfert latéral, et incluent deux régions distinctes (Thomas 2000). D'une part les gènes de

squelette - souvent conservés parmi les membres d'une même famille de plasmides - assurent la réplication, le maintien dans la cellule et le transfert (Li, Top et al. 2015). D'autre part les gènes accessoires codent des protéines aux fonctions diverses qui confèrent souvent des bénéfices importants à l'hôte et varient entre les plasmides : dégradation des hydrocarbures (Ono, Miyazaki et al. 2007, Obayori and Salam 2010), virulence (Schluter, Krause et al. 2008), résistance aux antibiotiques (Rhodes, Parkhill et al. 2004) et aux éléments traces métalliques (Schneiker, Keller et al. 2001), utilisation d'exsudats racinaires, etc...

Malgré l'importance du rôle des plasmides dans l'adaptation bactérienne à la contamination aux hydrocarbures, les études portant sur ce sujet restent récentes. Avant les années 2000, l'étude de la diversité des microorganismes et de leur physiologie était entravée par l'incapacité de cultiver en laboratoire 99% des microorganismes (Suenaga 2012). En effet, les conditions de culture de la plupart des microorganismes n'étant pas connues et les conditions environnementales adéquates étant difficilement reproductibles en laboratoire, la culture en laboratoire a donc limité notre vision de la diversité microbienne (Rappe and Giovannoni 2003). Dès le début des années 2000, le développement de techniques indépendantes de la culture et l'arrivée du NGS (Next Generation Sequencing) ont fondamentalement changé les études menées en microbiologie environnementale (Lombard, Prestat et al. 2011, Bell, Joly et al. 2014, Kumar, Krishnani et al. 2015). La métagénomique, basée sur l'extraction d'ADN microbien directement à partir d'un échantillon environnemental, permet d'avoir accès à tous les génomes microbiens (métagénome) dudit échantillon (Suenaga 2012). D'une part, des projets récents de métagénomique et plasmidomique (étude des plasmides) ont révélé que les fonctions essentielles à l'adaptation bactérienne dans un milieu contaminé étaient encodées sur des plasmides (Ono, Miyazaki et al. 2007, Yergeau, Sanschagrin et al. 2012, Fondi, Rizzi et al. 2013, Jutkina 2013, Yakimov, Crisafi et al. 2016). D'autre part, l'utilisation combinée de plantes et de bactéries modifiées - contenant des gènes de dégradation d'hydrocarbures sur leurs plasmides - a été exploitée et appliquée avec succès en phytoremédiation (Taghavi, Barac et al. 2005, Gerhardt, Huang et al. 2009, Glick 2010, Weyens, Schellingen et al. 2013). Cependant, la diversité fonctionnelle des plasmides reste largement inexplorée dans un contexte de phytoremédiation, et pourtant la connaissance de cette diversité est nécessaire à l'optimisation de cette technique (Thijs, Sillen et al. 2016).

Mon mémoire de maitrise présente deux chapitres. Le premier chapitre est une revue de littérature sur l'impact des exsudats racinaires sur le microbiome rhizosphérique, qui a été publiée dans le journal Agronomy MDPI (co-auteur : Marc St-Arnaud). Le second chapitre est un article qui étudie le plasmidome, soit l'ensemble des plasmides d'un métagénome, dans le sol et la rhizosphère de saule, et ce en présence d'une contamination aux hydrocarbures (co-auteurs : Sébastien Halary, Yves Terrat, Marc St-Arnaud). Cette étude permet non seulement de mettre en lumière la diversité fonctionnelle des plasmides, mais aussi d'améliorer nos connaissances et notre compréhension de l'adaptation bactérienne dans un contexte de phytoremédiation. J'ai également écrit un article de vulgarisation scientifique pour le journal DIRE de l'Université de Montréal, présent en annexe, sur le rôle du transfert latéral de gènes dans la bioremédiation de sites contaminés aux hydrocarbures.

Objectifs et hypothèses

Root exudation : the ecological driver of hydrocarbon rhizo-remediation

Dans cette revue de littérature, je m'intéresse à l'effet de l'exsudation racinaire sur les bactéries dans un sol contaminé aux hydrocarbures, ainsi que sur le transfert latéral de gènes *via* les plasmides.

Les objectifs étaient (1) de décrire les acteurs de la phytoremédiation, en ciblant tout particulièrement les bactéries de la rhizosphère, (2) d'expliquer le processus de rhizoremédiation des hydrocarbures, c'est-à-dire la biodégradation des hydrocarbures par les bactéries de la rhizosphère, (3) de détailler les mécanismes de l'exsudation racinaire, (4) d'expliquer pourquoi l'exsudation racinaire est considérée comme le moteur des communautés microbiennes rhizosphériques et (5) quels sont ses effets sur la biodégradation des hydrocarbures et (6) sur le transfert de plasmides.

Je m'attendais à ce que l'exsudation racinaire impacte la diversité microbienne et que la plante « sélectionne » par ce mécanisme les bactéries qui lui sont bénéfiques, telles que les rhizobactéries. Je m'attendais également à ce que les plantes utilisent les exsudats racinaires, notamment des antibiotiques, pour lutter contre les pathogènes. De plus, je m'attendais à ce que les exsudats racinaires améliorent la biodégradation des hydrocarbures, en fournissant par exemple une source de nutriments. Enfin, je m'attendais aussi à ce que le phénomène de transfert de gènes *via* les plasmides soit amplifié dans la rhizosphère.

Functional structure of rhizosphere plasmidome in a context of hydrocarbon contamination

Dans cet article, je m'intéresse à l'effet de la contamination aux hydrocarbures et de la présence de plantes sur la diversité des fonctions métaboliques encodées sur des plasmides dans le métagénome microbien du sol.

Les objectifs étaient (1) de comparer la diversité des gènes portés par les plasmides dans un sol contaminé aux hydrocarbures et non contaminé, ainsi que (2) dans la rhizosphère de saule et le sol non planté, (3) d'étudier la diversité des gènes impliqués dans la dégradation des hydrocarbures, (4) dans l'utilisation des exsudats racinaires et la relation plante-bactéries et (5) dans le processus de conjugaison, et (6) d'annoter taxonomiquement les gènes.

Je m'attendais à ce que la diversité des gènes de dégradation des hydrocarbures portés par les plasmides soit plus grande dans le milieu contaminé aux hydrocarbures. Je m'attendais également à la présence de gènes impliqués dans les échanges avec la plante dans la rhizosphère, comme l'utilisation des exsudats racinaires. Aussi, je m'attendais à ce qu'il y ait plus de gènes impliqués dans le processus de conjugaison dans la rhizosphère. De plus, je m'attendais à une plus petite diversité de taxons dans la rhizosphère.

Root exudation: the ecological driver of hydrocarbon rhizoremediation

Fanny Rohrbacher, Marc St-Arnaud

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Contribution des auteurs

Je suis responsable des recherches bibliographiques, de la rédaction et de la réalisation des figures. Marc St-Arnaud a fourni une révision critique et l'approbation finale.

Root exudation: the ecological driver of hydrocarbon rhizoremediation

Introduction

Since the nineteenth century, the industrial production of chemicals (including their improper use, incorrect elimination and accidental losses) has caused pollution of many environments. The loss of crude oil was estimated at 600 000 tonnes (± 200 000) per year worldwide (Committee on Oil in the Sea 2003, Kvenvolden and Cooper 2003). Anthropogenic substances that cause serious ecotoxicological problems include halogenated aromatic compounds, polycyclic aromatic hydrocarbons (PAHs) and BTEX (benzene, ethylbenzene, toluene and xylene) (Mrozik and Piotrowska-Seget 2010). PAHs are frequently found in the soil environment at relatively large concentrations (hundreds of mg.kg−1) (Nadal, Schuhmacher et al. 2004, Wehrer and Totsche 2008, An, Huang et al. 2011). The main sources of these toxic substances are oil refineries, gas stations, agrochemical, petrochemical and pharmaceutical industries. In addition to harming the sustainability of various ecosystems, the presence of these pollutants in the environment has posed a danger to public health due to their toxicity, their mutagenic and carcinogenic properties, and their ability to accumulate in the food chain (Mrozik and Piotrowska-Seget 2010, Ling, Sun et al. 2015). Most aromatic compounds are recalcitrant and persist in the environment for long periods of time. Cleaning up these environments has become a major environmental and health issue.

In Canada alone, 30,000 contaminated sites have been identified (De Sousa 2006) and their rehabilitation could cost billions of dollars using standard approaches (these consist of soil excavation and transportation to a landfill, commonly referred as the "dig-and-dump" strategy). Due to the prohibitive cost of standard strategies, a high proportion of contaminated sites are either left as is or the rehabilitation is postponed. Thus, others alternatives are clearly required. Biological techniques of remediation, called bioremediation, directly benefit from the ability of some organisms (plants, fungi and bacteria) to sequester, concentrate and/or degrade contaminants (Das and Chandran 2011). Bioremediation is considered to be a "green" approach

that is one of the prospects for sustainable development. Bioremediation helps to clean up the sites that are inaccessible to the excavation or that do not allow for the high cost required for standard strategies, does not require transportation of contaminated soil and requires less labor, is less expensive and has a lower carbon footprint (amount of CO_2 emitted) than traditional techniques of remediation (Das and Chandran 2011). Current rehabilitation costs can total over $1 million per hectare and some studies have indicated that implementing phytoremediation may result in a cost savings of 50 to 80 percent over traditional technologies (Drake 1997, Glass 1998, EPA 2000, van Epps 2006).

Phytoremediation is the direct use of living plants for *in situ* bioremediation of contaminated environments, such as soils (Pilon-Smits 2005, Hassan, St-Arnaud et al. 2010). This technique can be used to clean up sites with shallow, low to moderate levels of various contaminants, such as hydrocarbons (Miller 1996, Wenzel, Adriano et al. 1999, Vidali 2001, Khan 2005, Pilon-Smits 2005). It is a promising technique which saves biological activity of micro and macroorganisms, and improves soil structure and fertility (Salt, Smith et al. 1998). However, phytoremediation is a relatively slow process and may take several years to reduce the contaminant content in the soil to a safe and acceptable level due to small size and slow growth of most plants used for that purpose (Khan 2005). One avenue to circumvent this problem and accelerate decontamination is the use of fast-growing trees, such as willows and poplars (Tesar, Reichenauer et al. 2002, Labrecque and Teodorescu 2005, Bissonnette, St-Arnaud et al. 2010, Guidi, Kadri et al. 2012, Desjardins, Pitre et al. 2015). Through their extensive and widespread root systems, which can measure up to several meters long, willows improve the structure and texture of soil (Abrahamson, Robison et al. 1998) and reduce erosion (Kenney, Sennerby-Forsse et al. 1990). Furthermore, willows have demonstrated an ability to adapt to varying soil textures (sandy to clay) and humidity, due to their high transpiration rate and their roots ability to tolerate seasonal floods. In addition to improving the landscape and providing habitat for wildlife, willows play a positive role in the restoration and accelerated revegetation of degraded areas (lands disturbed by industrial activities or left by agriculture) in temperate and boreal regions of the world due to their ability to remove contaminants (Kuzovkina and Quigley 2005). After their harvest, they provide wood, fiber, biofuel and other forest products, due to their high biomass production. Some fast growing grasses and forbs are also used extensively in phytoremediation

for similar purposes (Olson and Fletcher 2000, Olson, Flechter et al. 2001, Merkl, Schultze-Kraft et al. 2005).

Phytoremediation is considered more effective for native microbial communities than human induced bioaugmentation. There are two types of factors that influence the effectiveness and survival of microbial strains introduced into the soil: (i) biotic factors (quick growth, easy to cultivate, tolerance to high concentrations of contaminants, competition between indigenous and exogenous microorganisms for carbon sources) and (ii) abiotic factors (type and amount of contaminants, temperature, humidity, pH, organic matter, aeration, soil type, chemical composition of the root exudates, etc)(Atlas and Cerniglia 1995, Cho, Rhee et al. 2000, Bento, Camargo et al. 2005, Pilon-Smits 2005, Wolski, Murialdo et al. 2007, Karamalidis, Evangelou et al. 2010, Tyagi, da Fonseca et al. 2011). Selection of microorganisms can be done either from sites with similar types of contaminants or by pre-adaptation in the laboratory. Indeed, bacteria can be isolated from contaminated soils, grown under certain conditions to obtain pure strains and then returned to the same soil: this is considered a re-inoculation with indigenous microorganisms (Dong, Hong et al. 2008, Cordova-Rosa, Dams et al. 2009, Mrozik and Piotrowska-Seget 2010). In many cases, inoculation of a consortium of hydrocarbon-degrading bacteria is more effective than single strains, because the intermediate of a catabolic pathway for a strain can be degraded by other strains that have the suitable catabolic pathway (Goux, Shapir et al. 2003, Ghazali, Rahman et al. 2004, Heinaru, Merimaa et al. 2005, Mrozik and Piotrowska-Seget 2010).

In addition to providing mechanical support, water and nutrients, roots perform more specialised roles in the rhizosphere including the ability to synthesise and secrete a multitude of metabolites (Bertin, Yang et al. 2003, Walker, Bais et al. 2003, Weston, Ryan et al. 2012). Plant roots release between 6 and 21% of photosynthetically fixed carbon (Bowen and Rovira 1999, Bertin, Yang et al. 2003, Uren 2007, Huang, Chaparro et al. 2014) in organic, amino and fatty acids, carbohydrates, vitamins, nucleotides, phenolic compounds, polysaccharides and proteins (Badri and Vivanco 2009, Badri, Chaparro et al. 2013, Badri, Zolla et al. 2013). Microorganisms are attracted through chemotaxis (Lioussanne, Jolicoeur et al. 2008, Huang, Chaparro et al. 2014) to these substrates and can use them for their metabolism. As a result, the rhizosphere is up to 100 times richer in microorganism biomass but poorer in diversity than bulk soil (Lynch and

Whipps 1990, Marilley and Aragno 1999, Kowalchuk, Buma et al. 2002, Martin, George et al. 2014): this is called "the rhizosphere effect" (Cheng and Coleman 1990, Anderson, Guthrie et al. 1993, Nie, Yang et al. 2010). In addition to exudation of nutrients, vitamins, and minerals, the plant and its associated microbiome also produce and/or exude various secondary metabolites, many of which act as signals (Walker, Bais et al. 2003) or as compounds inhibitory to the growth and functioning of microorganisms or roots called allelochemicals (Bertin, Yang et al. 2003, Field, Jordán et al. 2006, Dayan and Duke 2009, Mathesius and Watt 2010). For example, specific specific secondary products can initiate communication between microorganisms and conduct specialized interactions such as nodulation, pathogenesis, antibiotic production and DNA transfer between bacteria (conjugation) (Ben 2015). Many genes responsible for bacterial adaptation in soil and rhizosphere (e.g. degradation of organic compounds) are carried by conjugative plasmids (Heuer and Smalla 2012). In return, the ecological functions carried by the microbiota allow the plant to adapt to many types of environmental conditions and environmental changes (Bulgarelli, Schlaeppi et al. 2013).

Actors of phytoremediation: the holobiont

In phytoremediation, plants can absorb, concentrate, sequester, transform and eliminate contaminants (**Figure 1.1**)(Vidali 2001, Khan 2005, Favas, Pratas et al. 2014). Even if plants can also produce several enzymes to degrade organic compounds (such as peroxidases and phenol oxidases), they are generally considered as a minor contributor to the dissipation of organic contaminants in soil (Gao, Yang et al. 2011). Phytoremediation of hydrocarbons depends primarily on rhizoremediation which involves the breakdown of contaminants in soil as a result of microbial activity at the root surface (Yang, Ratte et al. 2001, Corgié, Joner et al. 2003, Favas, Pratas et al. 2014). Microorganisms can colonize three distinct areas of the root zone of a plant (Huang, Chaparro et al. 2014, Ben 2015): (1) the endosphere, i.e. all the cells inside the roots (Compant, Clément et al. 2010); (2) the rhizoplane which is the root surface (van Loon and Bakker 2006, Ben 2015), usually as biofilm (i.e. multiple layers of mature microcolonies covered by mucus (Ben 2015)); and (3) the rhizosphere, i.e. the soil immediately adjacent to roots (a few millimeters thick) and influenced by plant roots (Morgan, Bending et al. 2005, Hartmann, Rothballer et al. 2008). Rhizosphere is a complex ecosystem characterized

by a large amount of microniches - spatially close, but chemically heterogeneous - containing a high diversity of microorganisms (Nihorimbere, Ongena et al. 2011). The structure of the rhizosphere microbial community depends on soil type (Marschner, Yang et al. 2001, Fierer and Jackson 2006, Lauber, Strickland et al. 2008, Berg and Smalla 2009), temporal changes (seasons) and environmental factors (Mahaffee and Kloepper 1997, Kuzyakov 2002), plant species and genotype (Broeckling, Broz et al. 2008, Berg and Smalla 2009, Micallef, Channer et al. 2009, Micallef, Shiaris et al. 2009, Bulgarelli, Rott et al. 2012, Lundberg, Lebeis et al. 2012), plant development stage (Micallef, Channer et al. 2009, Inceoglu, Abu Al-Soud et al. 2011, Chaparro, Badri et al. 2014) and signaling of plant hormones (Carvalhais, Dennis et al. 2013).

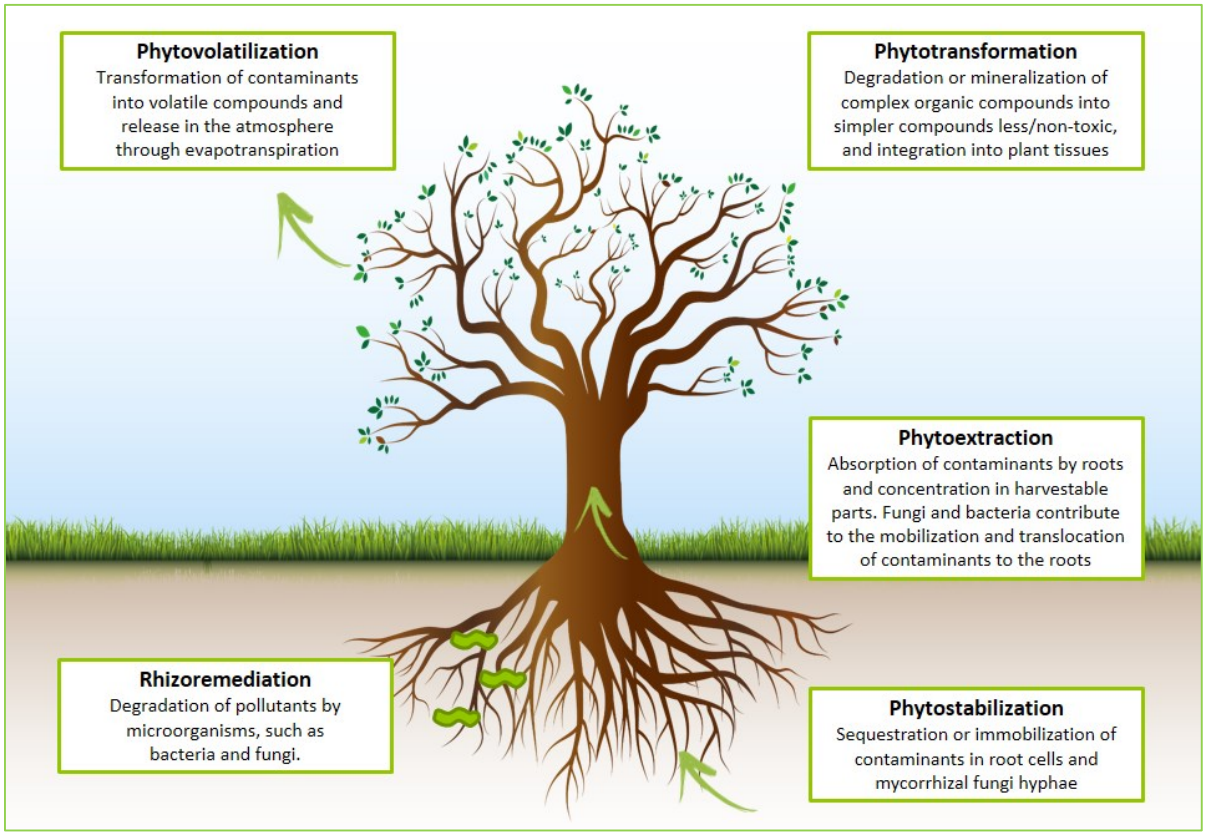

Figure 1.1 Mecanisms involved in phytoremediation (Vidali 2001, Khan 2005, Favas, Pratas et al. 2014).

Plants and microorganisms have co-evolved to each take advantage of their association. Because of their sessile lifestyle, plants need to adjust to biotic and abiotic stresses present in their immediate environment (Field, Jordán et al. 2006). Thus, a plant may promote the presence of

certain microorganisms if they provide a selective advantage, in particular to act as a defense against certain pathogens (Berendsen, Pieterse et al. 2012) or enhance its growth and nutrition (Vandenkoornhuyse, Quaiser et al. 2015). This symbiotic system is called “a holobiont”. This notion extended the concept of an organism to all internal or surface microbial communities associated with it, i.e. the plant with its microbiota (Zilber-Rosenberg and Rosenberg 2008). The microbial portion of the plant holobiont can be seen as a "facilitator component" that provides additional genes to the host and allows it to adjust to environmental conditions (Bordenstein and Theis 2015, Vandenkoornhuyse, Quaiser et al. 2015). It is therefore considered that the unit of selection processes and adaptation is not the isolated individual but the whole "meta-organism", i.e. the species community ; and the metabolites it produces is referred to as the metabolome (Zilber-Rosenberg and Rosenberg 2008, Weston, Skoneczny et al. 2015). This meta-organism results from the interaction of the host genome (plant) and its associated microbiome (including metabolites produced by both the plant and its associated microbiome), making a single dynamic entity: the hologenome, a target of natural selection (Guerrero, Margulis et al. 2013, Rosenberg and Zilber-Rosenberg 2013). Co-evolution shows that microorganisms present in a plant, whether parasite or symbiont, may have a considerable influence on their host. On one hand, the survival of the plant may be affected through changes to many physiological functions, such as reproduction, development or immunity (Smith, Handelsman et al. 1999, Stougaard 2000, Singh, Millard et al. 2004, Somers, Vanderleyden et al. 2004, Kiers and Heijden 2006, Jones, Kobayashi et al. 2007, Egamberdieva, Kamilova et al. 2008, Zilber-Rosenberg and Rosenberg 2008). Disruptions of this association can then overturn acclimation capacity and thus the fitness of the species assemblage (Zilber-Rosenberg and Rosenberg 2008). On the other hand, the interaction between heterogeneous communities with rapid (microbiota) and slow (macro-organisms) adaptability can bring out new selective traits and influence the species adaptability (Zilber-Rosenberg and Rosenberg 2008). Indeed, under stressful environmental conditions, the symbiotic community can change quickly and this plasticity may play an important role both in the acclimatization and evolution of the plant (Vandenkoornhuyse, Quaiser et al. 2015). In fact, phytoremediation results in a symbiotic interaction in the rhizosphere where plants and microorganisms act jointly in the degradation of organic contaminants (Yang, Ratte et al. 2001, Corgié, Joner et al. 2003, Ma, He et al. 2010, Favas, Pratas et al. 2014).

Hydrocarbon rhizoremediation

Organic contaminants can provide microorganisms with a carbon source and electrons required in respiration (Germida, Frick et al. 2002). Microorganisms are able to degrade simple hydrocarbons (linear and branched alkanes) to complex (aromatic compounds), with the exception of complex polyaromatic hydrocarbons (PAHs) that are not, in general, fully degraded (Atlas and Bragg 2009). Soil conditions were shown to be important for hydrocarbon degradation by microorganisms, and some authors proposed the following levels as optima: soil moisture at 30% of water holding capacity, soil pH between 6.5 and 8, oxygen content between 10 and 40%, and low clay or silt content for soil type (Vidali 2001, Das and Chandran 2011). Even if hydrocarbon biodegradation has been reported in psychrophilic environments in temperate regions (Delille, Coulon et al. 2004, Pelletier, Delille et al. 2004), the rate of biodegradation generally decreases with a decrease in temperature because it affects hydrocarbon solubility (Foght, Westlake et al. 1996) and microbial activity (Venosa and Zhu 2003). Generally, the optimum temperature for hydrocarbon degradation in a soil environment is between 20 and 40°C (Zhou and Crawford 1995, Margesin and Schinner 2001, Martin, George et al. 2014). Nutrients (nitrogen, phosphorus) have also been reported to be very important for hydrocarbon biodegradation (Zhou and Crawford 1995, Chaineau, Rougeux et al. 2005, Coulon, Pelletier et al. 2005). The optimum nutrient content has been found to follow a C:N:P ratio of 100:10:1 (Vidali 2001, Tibbett, George et al. 2011). However, excessive nutrient concentrations can also have negative effects, especially on aromatics, and inhibit hydrocarbon biodegradation (Carmichael and Pfaender 1997, Oudot, Merlin et al. 1998, Chaineau, Rougeux et al. 2005, Chaillan, Chaineau et al. 2006).

Most individual species are not equipped with all the appropriate enzymes, so degradation is mainly achieved via a consortium of microorganisms with various enzyme systems (Macek, Mackova et al. 2000, Chaudhry, Blom-Zandstra et al. 2005, Yateem, Al-Sharrah et al. 2008). Hydrocarbon-degrading microorganisms can be subdivided into two groups (Vidali 2001): aerobic (in presence of oxygen) and anaerobic (in absence of oxygen). The fastest and most complete degradation of most organic pollutants is performed under aerobic conditions, due to the metabolic advantage of having the availability of O_2 as an electron acceptor (**Figure 1.2**) (Das and Chandran 2011, Peixoto, Vermelho et al. 2011).

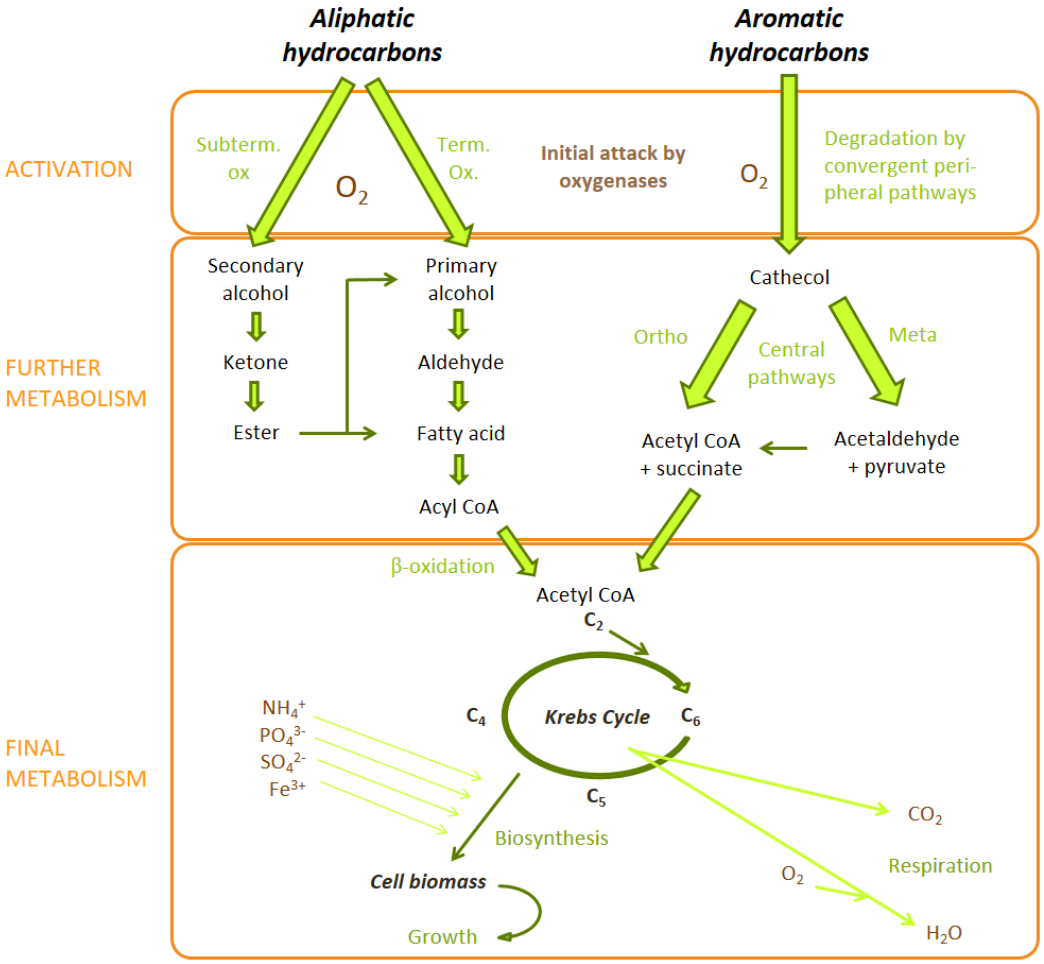

Figure 1.2 Degradation of aliphatic and aromatic hydrocarbons by aerobic bacteria. Subterm. ox.: subterminal oxidation. Term. ox.: terminal oxidation. Ortho: ortho cleavage pathway. Meta: meta cleavage patway. CoA: coenzyme A. (Hopwood and Chater 1988, Das and Chandran 2011, Peixoto, Vermelho et al. 2011, Guzik, Hupert-Kocurek et al. 2013, Kothari, Panchal et al. 2013, Segura and Ramos 2013, Sierra-Garcia and Oliveira 2013)

Aerobic bacteria such as *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium* have often been reported to degrade hydrocarbons, both alkanes and polyaromatic compounds (Vidali 2001). Many of these bacteria use hydrocarbons as the only source of carbon and energy. The key step of aerobic degradation is the activation, i.e. the addition of one or two oxygen atoms to the hydrocarbon molecule (monooxygenases introduce one oxygen atom to a substrate while dioxygenases introduce two). For aliphatic hydrocarbons, the activation is catalyzed either by substrate-specific terminal oxygenases (monooxygenases, non-heme iron monooxygenases, dioxygenases) or subterminal oxidation (cytochrome P450)

(Das and Chandran 2011, Peixoto, Vermelho et al. 2011, Hassanshahian and Cappello 2013). In aromatic hydrocarbons, four types of enzymes are involved : the Rieske non-heme iron oxygenases (RNHO), the flavoprotein monooxygenases (FPM), the soluble di-iron multicomponent monooxygenases (SDM) and the CoA ligases (Sierra-Garcia and Oliveira 2013). This activation makes the hydrocarbon more soluble in water, marks a reactive site, and introduces a reactive site for the next reactions (Das and Chandran 2011, Peixoto, Vermelho et al. 2011). In the case of aliphatic hydrocarbons, the activated molecule is then converted to an alkanol, then oxidized to the corresponding aldehyde, and converted into a fatty acid. Fatty acid is conjugated to CoA - which form an acyl CoA - and processed by β-oxidation to generate acetyl-CoA (Sierra-Garcia and Oliveira 2013). So, the final product of the oxidation of aliphatic hydrocarbons is acetyl-CoA, which is catabolised in the Krebs cycle, and fully oxidised to CO_2 (Peixoto, Vermelho et al. 2011). In the case of aromatic hydrocarbons, the activated molecule is not converted in alkanol, but in phenol intermediates (catechol or a structurally related compound) via peripheral pathways (Peixoto, Vermelho et al. 2011). Phenol intermediates are further degraded by central pathways (intradiol or extradiol dioxygenases), resulting in di- or trihydroxylated aromatic compounds that can be introduced into the Krebs cycle, and fully degraded to CO_2 (Peixoto, Vermelho et al. 2011, Sierra-Garcia and Oliveira 2013). Biosynthesis of cell biomass occurs from the central precursor metabolites, for example acetyl-CoA, succinate and pyruvate. Methylotrophs (methanogens) bacteria are also aerobic bacteria able to degrade hydrocarbons, using methane as source of carbon and energy (Orphan, Goffredi et al. 2003, Nazina, Shestakova et al. 2006). In methanogenesis, the terminal process of biomass degradation, acetate and hydrogen are the most important immediate precursors. They are respectively converted into methane by acetoclastic and hydrogenotrophic methanogens (Garcia, Patel et al. 2000). Acetate can also be a precursor for methanogenesis through syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis, which is mediated by syntrophic bacteria and methanogenic archaea (Zinder and Koch 1984, Garcia, Patel et al. 2000, Hattori, Kamagata et al. 2000, Balk, Weijma et al. 2002).

Anaerobic bacteria are able to use different terminal electron acceptors to degrade hydrocarbons, such as sulfate (sulphate-reducing bacteria), nitrate, iron, manganese and, more recently found, chlorate (van Hamme, Singh et al. 2003). These bacteria use a complete different pathway, based

in reductive reactions to attack the aromatic ring: alkane activation at the subterminal carbon by the addition of fumarate, or carboxylation (Das and Chandran 2011, Peixoto, Vermelho et al. 2011, Sierra-Garcia and Oliveira 2013). Anaerobic degradation has also been coupled to methanogenesis, fermentation and phototrophic metabolism but growth of these microorganisms and as a result, biodegradation rates are significantly lower compared to aerobic degraders**.** Despite not often being considered as aerobic bacteria, there is an increasing interest in the use of anaerobic bacteria for bioremediation (Vidali 2001, Zhang and Bennett 2005, Foght 2008, Weelink, van Eekert et al. 2010, Jaekel, Zedelius et al. 2015).

Mechanisms of root exudation

Rhizosphere microorganisms generally live under conditions of "nutrient starvation" and are thus constantly looking for nutrients. The most important nutrient sources excreted by roots are organic acids (citric, malic, succinic, oxalic and pyruvic), carbohydrates (glucose, xylose, fructose, maltose, sucrose, ribose), amino acids, fatty acids, proteins, enzymes, nucleotides and vitamins (Bertin, Yang et al. 2003, Narasimhan, Basheer et al. 2003, Lioussanne, Jolicoeur et al. 2008, Badri and Vivanco 2009, Ben 2015). Microorganisms have developed sensory systems (chemotaxis) that guide them to these roots-secreted components in order to provide the necessary nutrition and energy for their survival and reproduction (Joner and Leyval 2003, Yan-Zheng and Li-Zhong 2005).

Root exudates can be grouped into two specific categories: low molecular weight compounds (LMWCs: amino and organic acids, sugars, phenolic compounds and other secondary metabolites) and high molecular weight compounds (HMWCs: polysaccharides and proteins)(Ryan, Delhaize et al. 2001, Bertin, Yang et al. 2003, Bais, Weir et al. 2006, Badri and Vivanco 2009). The quantity and quality of root exudates are determined by the cultivar, plant species, developmental stage, various environmental factors (soil type, pH, temperature, nutrient availability) and the presence of microorganisms (Gransee and Wittenmayer 2000, Hutsch, Augustin et al. 2002, Leigh, Fletcher et al. 2002, Neumann 2007, Badri and Vivanco 2009, Shukla, Sharma et al. 2011, Xue, Wu et al. 2013). The greatest concentration of exudates occurs mostly at the root tips and at sites of lateral branching, decreasing with increasing distance from the root surface (Neumann 2007, Gao, Yang et al. 2011, Marschner, Crowley et al. 2011, Martin,

George et al. 2014). Gao, Yang et al. (2011) proposed that decreases in concentrations of root exudates depend mainly on two aspects : diffusion and degradation due to chemical (sorption or desorption)(Gao, He et al. 2003, van Hees, Jones et al. 2005) or biological process (e.g., microbial consumption). Microbial consumption contributes to the dissipation of root exudates because it provides the nutrition and energy necessary for survival and reproduction of rhizosphere microorganisms (Joner and Leyval 2003, Yan-Zheng and Li-Zhong 2005). Diversity in root exudates generates different microbial communities that are specific to each plant species (Huang, Chaparro et al. 2014).

Plants use several mechanisms to export and secrete compounds in the rhizosphere (Badri and Vivanco 2009, Weston, Ryan et al. 2012). Generally, root exudates are released either by passive (diffusion, ion channels and transport vesicles) or active mechanisms (secretion) (Bertin, Yang et al. 2003, Weston, Ryan et al. 2012, Huang, Chaparro et al. 2014). The majority of organic low-molecular weight compounds (LMWC) are released through a passive transport (i.e., not requiring energy) which permits passage of an ion or a molecule across a membrane without energy intake (Ryan, Delhaize et al. 2001, Huang, Chaparro et al. 2014). Passive transport is opposed to active transport that requires energy.

Passive transport can be achieved through two means (Cooper 2000). The first is a concentration gradient, spread by osmosis. Solutes diffuse through the cell membrane to reach an equilibrium concentration between the exterior and the interior of the cell (Cooper 2000). Non-polar molecules can pass through the membrane without making use of channels or transfer proteins due to their hydrophobic characteristics, this is simple diffusion (Cooper 2000). Polar-molecules and ions can diffuse through the membrane due to a phenomenon called facilitated diffusion, using channels or permeases. The channels allow the passage of water and specific small ions such as H^+, Na^+, K^+, and Cl^-, which establish a pH gradient and membrane potential, maintain osmotic balance and stabilize cell volume (Cooper 2000, Campbell 2014). Permeases can change their conformation upon binding with the molecule and upon its release. These proteins oscillate between two conformations, allowing them to spread the solute through the membrane. Small polar and uncharged molecules are transported by direct passive diffusion (Sanders and Bethke 2000), a process that depends on membrane permeability (Guern, Renaudin et al. 1987), polarity of excreted compound and cytosolic pH (Badri and Vivanco 2009). Passive transport

can also be mediated by an electrochemical gradient for charged molecules or ions. This gradient is influenced both by the concentration between intracellular and extracellular spaces and the electrical gradient of the membrane. Other compounds such as sugars, amino acids and caboxylates anions are transported across the membranes with the help of proteins, and their direction of movement is dependent on the electrochemical gradient that allows them to pass from the cytoplasm of root cells to the soil. There are specific transporters of sugars, amino acids and metals involved in the secretion of specific compounds by root cells (Williams, Pittman et al. 2000, Hussain, Haydon et al. 2004, Colangelo and Guerinot 2006, Hoekenga, Maron et al. 2006, Lee, Foster et al. 2007, Svennerstam, Ganeteg et al. 2007). Plants have metal homeostasis mechanisms to avoid excessive concentrations of free metal ions (e.g., Fe, Zn, Mn and Cu). These mechanisms involve coordination of transport for assimilation, translocation and compartmentalization (Haydon and Cobbett 2007). For example, grasses secrete mugineic acid, a ligand for metal secreted by the roots, and the complex Fe(III)-AM reduce Fe toxicity and get it into root cells through a specific YSL (Yellow Stripe-like) transporter identified in maize (Curie, Panaviene et al. 2001, Curie and Briat 2003). However, passive transport with an electrochemical gradient cannot be done against the concentration gradient: it requires active transport and an energy intake.

Active transport refers to the passage of an ion or molecule through a membrane against its concentration gradient, and involves the hydrolysis of ATP. Plants have a sessile lifestyle and require many adaptive strategies to interact with the environment, suggesting that the number of compounds produced by plants may require a large number of transporters (Dixon 2001). The excretion of compounds of high molecular weight by the roots usually involves transport vesicles (Battey and Blackbourn 1993, Weston, Ryan et al. 2012). Plant defence responses are accompanied by traffic of antimicrobial compounds at the site of infection by a pathogen. For example, pigmented-antimicrobial naphthoquinones are secreted in the apoplast of Boraginaceae roots (*Lithospermum erythrorhizon*) by a mechanism mediated by a vesicle in response to a fungal elicitation (induction and enhancement of secondary metabolites to stimulate plant natural defenses) (Brigham, Woo et al. 1998, Yazaki, Matsuoka et al. 2001, Yazaki 2005, Field, Jordán et al. 2006). Although other unrelated studies have demonstrated transport via vesicles in plant leaves, there is no evidence that this mechanism exists in the roots,

except for mucilage polysaccharides transported by Golgi through the root cap (Neumann and Romheld 2000). Root cells secrete secondary metabolites, polysaccharides and proteins, with the help of membrane transport proteins (Field, Jordán et al. 2006, Weston, Ryan et al. 2012). These transporters include ABC (ATP-binding cassette transporters) transporters (Loyola-Vargas, Broeckling et al. 2007, Sugiyama, Shitan et al. 2008, Badri, Quintana et al. 2009), MATEs (multidrug and toxic compound extrusion) (Yazaki 2005), MFS (major facilitator superfamily) (Reddy, Shlykov et al. 2012), and ALMT (aluminum-activated malate transporter) (Weston, Ryan et al. 2012). Badri, Quintana *et al.* (2009) found that 25 genes of ABC transporters were significantly overexpressed in roots of *Arabidopsis thaliana* and played important roles in the process of secretion. In bacteria, ABC transporters function as importers and exporters of compounds. In addition to the ABC transporters, MATEs are active transporters who export a wide range of substrates across membranes by using the electrochemical gradient of other ions (Weston, Ryan et al. 2012). Many MATE genes playing a role in the export of different compounds (such as alkaloids, antibiotic, anions citrate, phenolic compounds) have been identified and characterised in *Arabidopsis* (Diener, Gaxiola et al. 2001, Li, He et al. 2002, Liu, Magalhaes et al. 2009), sorghum (Magalhaes, Liu et al. 2007), barley (Furukawa, Yamaji et al. 2007), and rice (Ishimaru, Kakei et al. 2011). ABC transporters and MATEs are involved in the transport of flavonoids to the vacuole (Yazaki 2005).

Root exudation, the ecological driver of microbial communities in the rhizosphere

While plants produce and receive specific rhizosphere signals, they can also interfere with rhizosphere signals (Mathesius and Watt 2010). Root exudates are involved in determining the composition and diversity of the microbial community in the rhizosphere and can also play a significant role in the formation of the rhizosphere (Yang and Crowley 2000, Bertin, Yang et al. 2003, Jones, Hodge et al. 2004, Johnson, Anderson et al. 2005, Rentz, Alvarez et al. 2005, Singh, Munro et al. 2007, Badri, Quintana et al. 2009, Bonanomi, Vinale et al. 2009, Mathesius and Watt 2010, Badri, Chaparro et al. 2013).

First, root exudates can alterate the microbial diversity. Micallef, Shiaris et al. (2009) showed that each of eight different ecotypes of *Arabidopsis* released a unique suite of compounds into

its rhizosphere, and that these exudation differences targeted different bacterial communities. Furthermore, a positive correlation has been demonstrated between phenolic compounds and a large number of unique OTUs (operational taxonomic units), compared to other exudates, such as sugars and amino acids (Badri, Chaparro et al. 2013). For example, an *Arabidopsis*-mutant plant (containing ABC transporters that secrete more phenolic compounds to sugars compared to the wild type) caused significant changes in the microbial community (Badri, Quintana et al. 2009). Application of p-coumaric acid and vanillic acid, components of cucumber root exudates, increased the abundance and changed the composition of the rhizosphere bacterial community (Zhou and Wu 2012, Zhou and Wu 2013). The abundances of Firmicutes, Betaproteobacteria and Gammaproteobacteria increased, indicating that these taxa may be involved in the degradation of p-coumaric acid.

Secondly, roots can also alter the communication among bacteria, secreting compounds that mimic bacterial signals of quorum sensing (Gao, Teplitski et al. 2003). Quorum sensing is described as the coordination of bacterial behaviors by regulation of gene expression in response to population densities of bacteria (Mathesius and Watt 2010). It is a form of communication among bacteria, mediated by autoinducers (small signal molecules) which are generally N-acyl homoserine lactones (AHLs) in Gram-negative and peptides in Gram-positive bacteria (Walker, Bais et al. 2003, Mathesius and Watt 2010). After reaching a high population density, an autoinducer activates transcription proteins that induce specific genes. Thus, intercellular signals allow a bacterial population to control its expression of genes in response to cell density (Walker, Bais et al. 2003). In plants, quorum sensing plays an important role in establishment of symbiotic, pathogenic or beneficial plant-microbe associations, and for regulating bacterial behaviors important for host infection (including motility, biofilm formation, plasmid transfer, nitrogen fixation, and synthesis of virulence factors, exopolysaccharides, and degradative enzymes) (Mathesius and Watt 2010). For example, pea (*Pisum sativum*) root exudates contain bioactive compounds that mimic AHLs signals that stimulate AHL-regulated behaviors in certain strains of bacteria and inhibit in others (Teplitski, Robinson et al. 2000, Knee, Gong et al. 2001). Similarly, compounds mimicking the activity of AHLs and affecting bacterial behaviors regulated by quorum sensing are present in *Coronilla varia* (crownvetch), *Medicago truncatula* (barrel medic, at least 15 compounds)(Gao, Teplitski et al. 2003), *Oryza sativa* (rice),

Glycine max (soybean) and *Lycopersicon lycopersicum* (tomato)(Teplitski, Robinson et al. 2000, Daniels, De Vos et al. 2002, Teplitski, Chen et al. 2004). Moreover, strigolactones produced by *Physcomitrella patens* (moss) act as signaling factors that control the developmental process and the production of pseudo-quorum sensing signals (Proust, Hoffmann et al. 2011).

Thirdly, root exudates can mediate rhizospheric interactions (plant–microbe and microbe–microbe) by recruiting beneficial specific microorganisms such as PGPR (plant growth promoting rhizobacteria), mycorrhizal fungi or nitrogen-fixing bacteria (Benizri, Dedourge et al. 2002, Baudoin, Benizri et al. 2003, Butler, Williams et al. 2003, Broeckling, Broz et al. 2008, Hartmann, Schmid et al. 2009, Mathesius and Watt 2010, Bakker, Manter et al. 2012, Berendsen, Pieterse et al. 2012, Chaparro, Sheflin et al. 2012, Huang, Chaparro et al. 2014). The release of exudates, such as sugars and amino acids, was shown to attract PGPR (Somers, Vanderleyden et al. 2004, Huang, Chaparro et al. 2014). *Pseudomonas* possesses chemotactic proteins for malic acid, citric acid, and amino acids (especially leucine) that help colonization of tomato roots (de Weert, Vermeiren et al. 2002, Oku, Komatsu et al. 2012). Likewise, Rudrappa, Czymmek et al. (2008) demonstrated that released-malic acid allows the recruitment of *Bacillus subtilis* (PGPR). Some cell surface glycoproteins called arabino galactan proteins are secreted by the root cap of *Arabidopsis*, and also play an important role in the recognition and attachment of rhizobia on the root surface (Vicre, Santaella et al. 2005). Regarding the phenolic compounds, they can act as specific substrates and signaling molecules, while at the same time playing multifunctional roles in rhizospheric plant-microbe interactions such as legume-rhizobia symbioses (Blum, Staman et al. 2000, Martens 2002, Mandal, Chakraborty et al. 2010, Badri, Chaparro et al. 2013, Fang, Zhuang et al. 2013, Michalet, Rohr et al. 2013). Indeed, legume plants can secrete phenolic compounds to attract and induce the chemotaxis of *Rhizobium* species, which have the ability to use phenolic acids as a carbon source (van Rossum, Schuurmans et al. 1995, Irisarri, Milnitsky et al. 1996), and can also initiate symbiosis between them (Mandal, Mandal et al. 2009). Weston and Mathesius (2013), Weston and Mathesius (2014) described production, transport and roles in the rhizosphere of two phenolic compounds: (1) flavonoids (low molecular weight compounds), generally described as non-essential for plant survival, unlike primary metabolites ; and (2) sorgoleones (long chain hydroquinones,

phenolic lipids), released by passive exudation from *Sorghum* spp. (Weston, Alsaadawi et al. 2013). Some of the more well-known biological roles of flavonoids in the rhizosphere include (Mathesius and Watt 2010, Weston and Mathesius 2013, Weston and Mathesius 2014): (1) chemo-attraction that lead rhizobia to the root surface (Neal, Ahmad et al. 2012) (2) induction of the expression of *nod* genes, which encode enzymes for the synthesis of Nod factors or lipochitine oligosaccharides (LCOs) (Blum, Staman et al. 2000, Mandal, Chakraborty et al. 2010) (3) activation of nodule formation in which bacteria fix atmospheric nitrogen for plants (Abdel-Lateif, Bogusz et al. 2012, Ben 2015) (4) increasing of the efficiency of indol-3-acetic acid production by *Rhizobium* species and regulate nodule morphogenesis (Mandal, Mandal et al. 2009). The auxin phytohormone indol-3-acetic acid can also be produced by bacteria and is suspected to act as a signaling molecule that can affect their gene expression (Spaepen and Vanderleyden 2011). Similarly, phenolic acids involved nodule morphogenesis and in rhizobial defense have been detected in roots and root nodules of *Arachis hypogaea* (Chakraborty and Mandal 2008).

Plants can also use exudates to defend themselves against pathogens (Neal, Ahmad et al. 2012). Proteins secreted by roots are important for recognition of pathogenic and non-pathogenic bacteria (Wen, VanEtten et al. 2007, De-La-Pena, Lei et al. 2008). For example, lectins function as defense factors and recognition in symbiotic interactions (De Hoff, Brill et al. 2009). During the flowering period of *Arabidopsis thaliana*, roots excrete more protein involved in defense, such as chitinases, glucanases and myrosinases (De-la-Pena, Badri et al. 2010). Furthermore, protein patterns released as root exudates depend on the identity of microorganisms exposed to the roots of *A. thaliana* (De-La-Pena, Lei et al. 2008). *Pseudomonas syringae*, an *A. thaliana* pathogen, highly induced secretion (from *A. thaliana*) of defense proteins, such as peroxidases, glycosyl hydrolase family 17, chitinase, and glycosyl hydrolase family 18 (De-La-Pena, Lei et al. 2008)

In return, the quality and quantity of exudates is highly influenced by the presence of microorganisms. For example, Matilla*, et al.* [138] found that *A. thaliana* produce distinct patterns of root exudation when it grows with and without *Pseudomonas putida* KT2440. Also, when the biocontrol strain *Pseudomonas* WCS365 is added in sufficient quantity for the biocontrol to operate, organic acids (citric acid especially) is strongly increased, while the

amount of succinic acid drastically decreased (Kamilova, Kravchenko et al. 2006). Similarly, mycorrhizal colonization was shown to modify the exudates of tomato roots (Lioussanne, Jolicoeur et al. 2008), and the stimulatory effect of root exudates from many plant species to the germination of conidia of *Fusarium oxysporum* f. sp. *lycopersici*, a tomato root pathogen (Scheffknecht, St-Arnaud et al. 2007).

Impact of root exudates on hydrocarbon degradation

Root exudation is now considered to be the most important factor in the mediation of hydrocarbon biodegradation in the rhizosphere (Miya and Firestone 2001, Yoshitomi and Shann 2001, Germida, Frick et al. 2002, Joner, Corgie et al. 2002, Joner and Leyval 2003, Da Silva, Kamath et al. 2006, Dzantor 2007, Gao, Yang et al. 2011, Phillips, Greer et al. 2012, Martin, George et al. 2014). Root exudates serve as a carbon source and energy for microorganisms, and also improve the hydrocarbon degradation in the rhizosphere by stimulating hydrocarbon-degrader populations (Gao, Yang et al. 2011).

In contaminated soil, the contaminant distribution gradient is negatively correlated with the gradient of root exudates, with the lowest hydrocarbon concentration and the highest exudate concentration close to the roots (Gao, Yang et al. 2011, Ling, Dang et al. 2013). Corgié, Joner et al. (2003) reported that phenanthrene biodegradation reached 86% in the first 3 mm from the roots, 48% between 3 and 6 mm, and 36% between 6 and 9 mm. They observed a parallel bacterial gradient, where high numbers of heterotrophs and PAH-degrading bacteria were close to the roots. Similarly, in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) growing in a petroleum hydrocarbon contaminated soil, the highest rates of hydrocarbon degradation and the microbial degraders were mainly found within 3 mm of the root surface (Corgie, Beguiristain et al. 2004). In a phenanthrene-contaminated soil, Cébron, Louvel et al. (2011) observed that major phenanthrene degraders were *Pseudoxanthomonas* spp. (Gammaproteobacteria) and *Microbacterium* spp. (Actinobacteria). But when root exudates of ryegrass were added, the population of phenanthrene degraders shifted towards mostly the Actinobacterium *Arthrobacter* spp., the Gammaproteobacteria *Pseudomonas stutzeri* and *Pseudoxanthomonas mexicana*. Interestingly, the Firmicutes *Bacillus* spp., *Paenibacillus* spp. and *Pseudomonas* spp. were also found to be able to use both root exudates and phenanthrene as carbon source (Rentz, Alvarez

et al. 2005). *Arthrobacter* spp. was shown to degrade hydrocarbons and more specifically phenanthrene (Radwan, Al-Awadhi et al. 1998, Seo, Keum et al. 2006). Kozdrój and van Elsas (2000) also found *Pseudomonas* and *Arthrobacter* as dominant active phenanthrene degraders either in the presence of artificial root exudates or with phenanthrene alone.

Plant roots may enhance microbial biodegradation of petroleum hydrocarbons via physical processes such as nutrient and pollutant transport, microbial attachment sites and soil aeration (**Figure 1.3**) (Martin, George et al. 2014). The first and most significant role of root exudates in the improvement of petroleum hydrocarbon degradation is that they provide microorganisms with a source of energy and nutrients, which supports their growth and activity (Walton, Anderson et al. 1995, Kuiper, Lagendijk et al. 2004) (**Figure 1.3, A**). The forms of carbon and nitrogen exuded by roots, i.e. high molecular weight organic polymers, are complex and mostly insoluble (Martin, George et al. 2014). Therefore, they may have relatively long biodegradation times (Kuzyakov 2002, Kalbitz, Schmerwitz et al. 2003). Increasing the microbial biomass and activity by exudation of labile C and N can be a solution. For example, the addition of root debris to phenanthrene contaminated soils only improve phenanthrene degradation after 20 days, in contrast to more labile water soluble exudates, which reflects the low biodegradability of the root debris (Miya and Firestone 2001). On the contrary, the high solubility of low molecular weight exudates allows them a higher mobility in soil and a rapid uptake by microbial cells (Martin, George et al. 2014). Carbohydrates, amino acids and organic acid represent the largest proportion of low molecular weight root exudates, and are fundamental to the provision of easily degradable energy and nutrient sources to the rhizosphere (van Hees, Jones et al. 2005). For example, the addition of low molecular weight root exudates can generate a rapid response (within 1 h) in the microbial respiration. However, several days may be required to observe changes in gene expression and microbial biomass (Darrah 1991, Jones and Murphy 2007). Furthermore, root secreted compounds involved in plant nutrient acquisition, such as enzymes (e.g. acid phosphatases), protons and chelating agents (e.g. organic acid anions and other phytosiderophores), also provide a source of nutrients to rhizosphere microorganisms (Dakora and Phillips 2002, Rengel and Marschner 2005, Marschner 2007, Marschner, Crowley et al. 2011).

Secondly, plants may directly improve degradation via the root exudation of enzymes, such as laccases, phenol oxidases and peroxidases which catalyse the oxidation of various hydrocarbons and degrade them into intermediate products (**Figure 1.3, B**) (Gao, Yang et al. 2011, Martin, George et al. 2014). However, microbially derived enzymatic breakdown is considered to be the primary pathway for petroleum hydrocarbons degradation (Martin, George et al. 2014).

Thirdly, many secondary plant metabolites exuded by roots, such as flavonoids, are structurally similar to aromatic hydrocarbons (**Figure 1.3, C**) (Singer 2006, Bais, Broeckling et al. 2008). Therefore, structural analogy improve hydrocarbon degradation by stimulating co-metabolic processes, which involves the oxidation /mineralisation of petroleum hydrocarbons molecules that do not supporting growth (for example benzo-*a*-pyrene (Kanaly and Bartha 1999)) in the presence of other growth supporting root exudates (Fletcher and Hegde 1995, Yergeau, Sanschagrin et al. 2014). Co-metabolism seems to be the primary process underlying degradation of recalcitrant hydrocarbons (Cunningham and Berti 1993, Kuiper, Lagendijk et al. 2004).

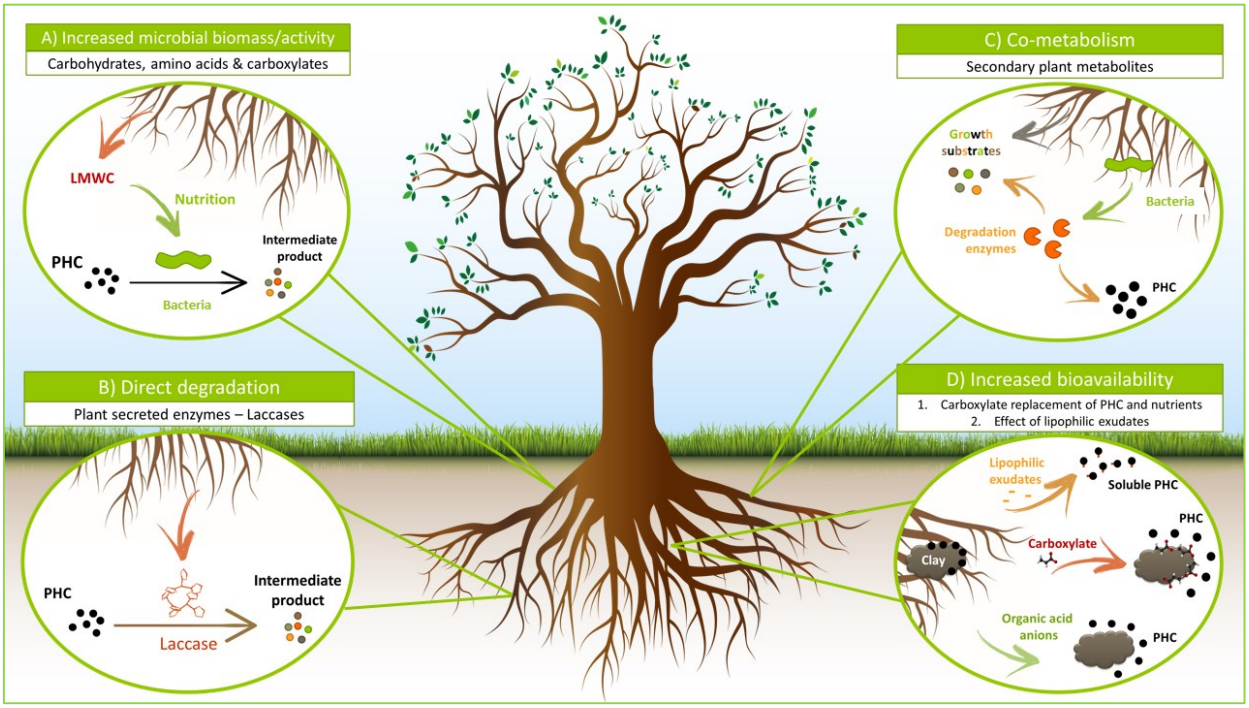

Figure 1.3 Improvement of petroleum hydrocarbon degradation by root exudates. PHC : Petroleum hydrocarbon, LMWC : Low molecular weight compounds. Adapted from Martin, George et al. (2014).

Finally, bioavailability is often a limiting factor for hydrocarbon degradation beacause polycyclic aromatic hydrocarbons are easily adsorbed by soil solids, such as soil organic matter, after entering the soil (**Figure 1.3, D**) (Gao, Yang et al. 2011). Root exudates can increase the solubility of hydrocarbons and alter their bioavailability (Ouvrard, Lapole et al. 2006, Gao, Ren et al. 2010, Martin, George et al. 2014), making them more available for a future microbial attacks (Read, Bengough et al. 2003, Siciliano, Germida et al. 2003). Root exudates collected from plant roots (Miya and Firestone 2001, Yoshitomi and Shann 2001, Xie, Liao et al. 2012) or artificial root exudate mixtures (Joner, Corgie et al. 2002) can be amended to contaminated soils to enhance desorption and promote the success of rhizoremediation. For example, Gao, Ren et al. (2010) used artificial root exudates as amendments, and observed an considerable increase of desorption of phenanthrene and pyrene from soils. Similarly, LeFevre, Hozalski et al. (2013) showed that root exudates, both artificial and harvested from plants, enhanced naphthalene desorption from soils, providing an abiotic contribution to the "rhizosphere effect" for degradation of naphthalene. As exudate amendments, low-molecular-weight organic acids (LMWOAs) can influence desorption of hydrophobic organic contaminants from soil and hence alter their bioavailability (Drever and Stillings 1997, Jones 1998, Jones and Brassington 1998, Ling, Sun et al. 2015). White, Mattina et al. (2003) showed that bioavailability of dichlorodiphenyldichloroethylene (DDE) in soils is increased in the presence of LMWOAs, such as succinic, tartaric, malic, malonic, oxalic, citric and ethylenediaminetetraacetic acid (EDTA). Similarly, Zhao, Wang et al. (2006) observed an enhanced release of hexachlorocyclohexane from soils in the presence of oxalic, tartaric and citric acid. Yang, Ratte et al. (2001), Ling, Ren et al. (2009), Ling, Sun et al. (2015) found that LMWOAs (such as citric and oxalic acids) significantly enhanced the desorption availability of hydrocarbons from a contaminated soil, and continued to desorb more PAHs from soil during multiple desorption cycles. In fact, the mechanism by which LMWOAs promote desorption of PAHs from soil was proposed to begin by a disruption of soil organic matter (SOM)–metal cation–mineral linkages in soils, thus releasing SOM and simultaneously increasing dissolved organic carbon in the surrounding solution which causes the enhanced PAH desorption from soil (Ling, Sun et al. 2015). All these results suggest that the amendment of contaminated soils with LMWOAs can promote PAH desorption from soils, which might be used as a new approach to enhance bioavailability – and therefore bioremediation – of hydrophobic organic compounds in

soils (Ling, Sun et al. 2015). Organic acid anions exuded by roots may also enhance desorption of hydrocarbons and/or compete for soil adsorption sites from the soil matrix, such as clay surfaces (An, Huang et al. 2010, Gao, Ren et al. 2010, An, Huang et al. 2011). Moreover, some microorganisms are able to release biosurfactants, such as rhamnolipids, that can increase the solubility of certain organic contaminants and improve the ability of microbial cells to attach to oil droplets (Clifford, Ioannidis et al. 2007, Wang, Fang et al. 2007, Cao, Wang et al. 2012).

Lateral gene transfer in rhizosphere and hydrocarbon degradation

The evolution of bacteria is accelerated by their ability to acquire genes directly from other organisms (Cohan and Koeppel 2008). The acquired amount of genes in a prokaryotic genome has been found to vary between 66 and 96% (Dagan, Artzy-Randrup et al. 2008). Horizontal acquisition of catabolic genes allows microbial populations that have different physiological properties, cellular structures or ecological niches, to acquire new capabilities to improve their performance in their current ecological niche (such as using root exudates for growth) or to invade a new niche (Gogarten, Doolittle et al. 2002, Cohan and Koeppel 2008). Lateral gene transfer is a major driver of bacterial evolution and adaptability (de la Cruz and Davies 2000), and is driven by three mechanisms: the transformation (acquisition of foreign naked DNA), transduction (bacteriophage-mediated gene transfer) and conjugation (plasmids and integrative conjugative elements - ICEs) (Popa and Dagan 2011). Of the three mechanisms of lateral gene transfer, conjugation is one of the most important for exchanging genes (Halary, Leigh et al. 2010, Zhang, Pereira e Silva Mde et al. 2014). Plasmids are autoreplicatives extrachromosomal molecules of DNA, mostly circular but otherwise linear (Mela, Fritsche et al. 2008), and are transmitted both vertically (from mother cell to daughter during bacterial division) and horizontally (from a donor cell to a recipient cell). Plasmids are mosaic genomes that include two distinct regions (Thomas 2000): (1) skeleton genes, often conserved among members of the same family of plasmids, ensure replication and maintenance of the plasmid in the cell and transfer (Li, Top et al. 2015); and (2) accessory genes encoding functions that are frequently beneficial to the host cell. These functions benefit the host cell in many different ways, e.g. degrading environmental pollutants and using them as a carbon or nitrogen source,

or providing virulence (Schluter, Krause et al. 2008), resistance to an antibiotic (Rhodes, Parkhill et al. 2004) or a metal or metalloid trace element, and other catabolic functions (Ono, Miyazaki et al. 2007).

Root exudation has an effect on conjugation. A large number of environmental 'hot spots' where lateral gene transfer is high were identified (Molbak, Molin et al. 2007), including the rhizosphere of plants (Droge, Puhler et al. 1999). Lateral gene transfer is known to occur with high frequency in the rhizosphere (van Elsas, Trevors et al. 1988, van Elsas, Turner et al. 2003, Jussila, Zhao et al. 2007), and to be stimulated in part by an increased root exudation (Molbak, Molin et al. 2007). The high rates of lateral gene transfer in the rhizosphere, compared to bulk soil, were attributed to the high cell density, distribution, and accessibility to root exudates that stimulate bacterial activity, exudation and root growth (Kroer, Barkay et al. 1998, van Elsas, Turner et al. 2003).

Molbak, Molin et al. (2007) studied lateral gene transfer in the rhizospheres of pea and barley. They discovered that there was 17 times more lateral gene transfer in pea rhizosphere than in barley ($3.5 \pm 0.7 \times 10^{-3}$ trans-conjugants per donor for peas and $2 \pm 0.5 \times 10^{-4}$ for barley). They proposed that in the pea rhizosphere, dense and uniform colonization of donor cells along the whole length of the root resulted in a higher encounter frequency between donor and recipient cells, and therefore a larger transfer rate. The spread into pea roots deeper in the soil is suspected to be due to passive transport or motility. In barley rhizosphere, populations are present mainly in the spermosphere and in the root portion closer to the surface of the soil, so few encounters between donor and recipient cells take place. Schwaner and Kroer (2001) observed similar results using different bacterial strains, plasmids and inoculation techniques which confirmed species-specific differences in the frequency of rhizospheric lateral gene transfer. Differences in bacterial activity have also been suggested as an important determinant of the transfer (Schwaner and Kroer 2001).

Many bacterial genes involved in PAH degradation are carried on conjugative plasmids (Nojiri, Shintani et al. 2004). Phillips, Greer et al. (2012) studied two catabolic genes involved in hydrocarbon degradation commonly carried by conjugative plasmids: C2,3O (catechol 2,3 dioxygenase) and *nahAc* (naphthalene dioxygenase (NDO) iron sulfur protein). On one hand, C2,3O is an enzyme encoding an archetypal pathway for the catabolism of monocyclic

aromatics to aliphatic metabolites (Ishida, Kita et al. 2002). It is more specifically involved in the degradation of xylene, catalyzing the oxidative cleavage of catechols (i.e. incorporate dioxygen into the substrate catechol) to form a 2-hydroxymuconate semialdehyde (Ishida, Kita et al. 2002). C2,3O is carried by the TOL plasmid of *Pseudomonas putida* mt-2 (ATCC 23973) and the bacterium degrades *m*-xylene to CO_2, acetate, acetaldehyde, and pyruvate. On the other hand, biodegradation pathways of aromatic hydrocarbon by bacteria are initiated by aromatic hydrocarbon dioxygenases (Wammer and Peters 2006). The three-component naphthalene dioxygenase enzyme system catalyzes the first step in the aerobic degradation of naphthalene by *Pseudomonas* sp. strain NCIB 9816-4 (Resnick, Lee et al. 1996, Parales, Lee et al. 2000) : naphthalene + NADH + H^+ + O_2 ↔ (1R,2S)-1,2-dihydronaphthalene-1,2-diol + NAD^+ (Wammer and Peters 2006). C2,3O and *nahAc* genes are commonly found on the same plasmid (Phillips, Greer et al. 2012) with C2,3O often present in multiple copies (Sentchilo, Perebituk et al. 2000, Li, Shi et al. 2004). Phillips, Greer et al. (2012) observed a positive correlation between the copy numbers of the two genes and the PAH-mineralization rate, suggesting that plasmid transfer may have been a significant factor influencing the changes in degradation potential. The authors also observed that *nahAc* and C2,3O copy numbers are significantly impacted by exudate composition, with specific compounds associated with either increased (acetate, alanine) or decreased (malonate) degradative capacity. Organic acid acetate and the amino acid alanine have already been shown to directly increase lateral gene transfer events (Nielsen and van Elsas 2001).

Conclusions and perspectives

Rhizoremediation can provide a cost effective and environmentally sustainable remediation alternative for the breakdown of hydrocarbon contaminants from the soil (Martin, George et al. 2014). In this review, we clearly identified root exudates as a key ecological driver in the rhizosphere. Although numerous studies speculated that root exudation of organic compounds is the driving factor behind hydrocarbon rhizoremediation (Miya and Firestone 2001, Yoshitomi and Shann 2001, Germida, Frick et al. 2002, Joner, Corgie et al. 2002, Joner and Leyval 2003, Da Silva, Kamath et al. 2006, Dzantor 2007, Gao, Yang et al. 2011, Phillips, Greer et al. 2012, Martin, George et al. 2014), the extent to which biodegradation is achieved is

highly variable amongst plant species (Martin, George et al. 2014). Furthermore, studies directly linking the composition and quantity of root exudates to hydrocarbon biodegradation are scarce (Martin, George et al. 2014). Carboxylates (LMWOA) are a significant component of the root exudate mixture and are thought to improve hydrocarbon biodegradation by promoting microbial activity through the provisioning of an energy source, increasing phosphorus supply and/or enhancing the bioavailability of the contaminant (Martin, George et al. 2014). However, the impact of carboxylates on the soil microbial community has received considerably little attention and their influence on rhizoremediation of petroleum hydrocarbons so far lacks experimental study.

On the contrary, over the past ten years many studies have demonstrated the beneficial impacts of biostimulation (amendment) with root exudates on hydrocarbon degradation (Miya and Firestone 2001, Yang, Ratte et al. 2001, Yoshitomi and Shann 2001, White, Mattina et al. 2003, Zhao, Wang et al. 2006, Yi and Crowley 2007, Ling, Ren et al. 2009, Gao, Ren et al. 2010, Técher, Laval-Gilly et al. 2011, Xie, Liao et al. 2012, LeFevre, Hozalski et al. 2013, Ling, Sun et al. 2015). Furthermore, root exudates stimulate lateral gene transfer in the rhizosphere (Schwaner and Kroer 2001, Molbak, Molin et al. 2007), including hydrocarbon degrading genes (Phillips, Greer et al. 2012). However, the evolution of indigenous microorganisms – which implies mutational events and horizontal gene transfer – and the development of their hydrocarbon-degrading abilities are relatively slow processes (Mrozik and Piotrowska-Seget 2010). The improvement of the biodegrading potential of microbial communities can be resolved by promoting horizontal gene transfer between modified organisms and wild strains, allowing them to acquire new degradation functions. For example, hydrocarbon-degrading genes carried by plasmids in one or more donor strains can be transferred to a recipient indigenous microflora, i.e. bioaugmentation of contaminated soil with genetic modified microorganisms. Weyens, Schellingen et al. (2013) studied lateral transfer of toluene-degrading genes between the donor strain *Burkholderia vietnamiensis* BU61 (equipped with plasmid TOM-TEC coding for degradation of toluene and trichlorethylene (TCE)) and two recipient strains, *Burkholderia* sp. HU 001 (rhizosphere) and *Pseudomonas* sp. HU 002 (endophyte). Conjugation produced rhizospheric and endophytic strains able to degrade toluene, which were inoculated in willow rhizosphere. Inoculation with this consortium of plant-associated bacteria

equipped with the appropriate characteristics resulted in an improved phytoremediation of a toluene contaminated site: the degradation of toluene was improved leading to decreased toxicity and evapotranspiration for willows. Filonov, Akhmetov et al. (2005) constructed a *Pseudomonas putida* strain (KT 2442), with naphtalene-degrading genes carried by pNF142 plasmid. Twelve days after the bioaugmentation of a naphtalene-contaminated soil, the naphthalene concentration decreased from 2 to 0.2 mg/g of soil and pNF142 plasmid has been transferred to indigenous bacteria. The inoculated strain remained stable and competitive for 40 days. More recently, Wang, Jiang et al. (2014) inoculated *Pseudomonas fluorescens* TP13 strains, which contained C23O (catechol 2, 3-dioxygenase) genes carried by TOL plasmids, into the soil of a tomato farmland contaminated with hydroxybenze. Strain TP13 was able to colonize the tomato rhizosphere. The number of rhizosphere bacteria containing TOL plasmids with C23O gene increased gradually in the later stages of the experiment, and was strongly negatively correlated with phenol content. Furthermore, six strains of rhizosphere bacteria isolated were found to possess large plasmids containing identical C23O genes almost identical to those of strain TP13. Wang, Jiang et al. (2014) confirmed that plasmids were transferred from strain TP13 to rhizosphere bacteria, and that horizontal gene transfer stimulated hydroxybenzene degradation and plant growth in the contaminated farmland after 20 days.

It would be interesting to combine biostimulation with root exudates and bioaugmentation of hydrocarbon degrading genes carried by plasmids to improve hydrocarbon degradation, i.e. creating a microorganism that is able to degrade hydrocarbon and transfer its degradation capacities to others (with degradation genes carried by plasmid) and inoculating them in soil with root exudates amendments. At this time, to our knowledge, no experiment on this scale has yet been conducted.

Functional structure of rhizosphere plasmidome in a context of hydrocarbon contamination.

Fanny Rohrbacher, Sébastien Halary, Yves Terrat, Marc St-Arnaud

Article en préparation

Contribution des auteurs

Marc St-Arnaud, Yves Terrat et Sébastien Halary ont réalisé le design expérimental. Yves Terrat, Sébastien Halary et moi-même avons participé à l'échantillonnage. Yves Terrat a réalisé l'extraction de l'ADN, la préparation des librairies pour le séquençage et l'assemblage des reads en contigs. Je suis responsable des analyses bioinformatiques subséquentes, des résultats, de la rédaction de cet article, des figures et des tableaux. Marc St Arnaud, Yves Terrat et Sébastien Halary ont fourni des révisions, des commentaires et des suggestions tout au long de processus. Marc St-Arnaud a encadré la recherche.

Functional structure of rhizosphere plasmidome in a context of hydrocarbon contamination.

Introduction

In Canada alone, 30,000 sites have been identified as contaminated, due to the improper use, incorrect elimination or accidental losses of contaminants (De Sousa 2006). Among anthropogenic substances that cause serious ecotoxicological problems, polycyclic aromatic hydrocarbons (PAHs) are well known (Mrozik and Piotrowska-Seget 2010). PAHs are frequently found in the soil at relatively high concentrations (hundreds of mg/kg) (Nadal, Schuhmacher et al. 2004, Wehrer and Totsche 2008, An, Huang et al. 2011, Bourdel, Roy-Bolduc et al. 2016). Due to their toxicity, their mutagenic and carcinogenic properties, and their accumulation in the food web, these pollutants constitute a serious threat for the sustainability of various ecosystems and public health (Mrozik and Piotrowska-Seget 2010, Ling, Sun et al. 2015). Because most aromatic compounds persist in environment for a long time, their remediation represents a major issue.

Traditional remediation strategies (*e.g.* soil excavation, transportation to a landfill), may cost over $1 million per hectare. In addition to their prohibitive cost, the inaccessibility to excavation, the transportation and environmental impacts of soil treatments, a high proportion of contaminated sites are either not decontaminated or the rehabilitation is postponed, and other alternatives are clearly required (Das and Chandran 2011). Bioremediation is an alternative that directly benefits from the ability of organisms to clean up soils with shallow, low to moderate levels of hydrocarbon contamination (Miller 1996, Wenzel, Adriano et al. 1999, Vidali 2001, Khan 2005, Pilon-Smits 2005, Hassan, St-Arnaud et al. 2010, Das and Chandran 2011). Bioremediation advantages are numerous: preserving micro- and macro-organisms activities, improving soil structure and fertility, low carbon footprint (amount of CO_2 emitted) and cost reduction up to 80 percent over traditional strategies (Drake 1997, Glass 1998, Salt, Smith et al. 1998, EPA 2000, van Epps 2006). Nevertheless, bioremediation is a relatively slow process and may take several decades to decrease contaminant concentrations to safe levels (Khan 2005).

One avenue to accelerate decontamination is phytoremediation, *i.e.* the use of plants such as willows (Tesar, Reichenauer et al. 2002, Labrecque and Teodorescu 2005, Bissonnette, St-Arnaud et al. 2010, Guidi, Kadri et al. 2012, Desjardins, Pitre et al. 2015). In contaminated soils, plants can absorb, concentrate, sequester, transform and eliminate contaminants (Vidali 2001, Khan 2005, Favas, Pratas et al. 2014, Rohrbacher and St-Arnaud 2016). Although plants produce several enzymes degrading organic compounds, they are generally considered as a minor contributor to the dissipation of organic contaminants in soil (Gao, Yang et al. 2011). Because of their sessile lifestyle, plants need to adjust to biotic and abiotic stresses present in their immediate environment (Field, Jordán et al. 2006). For this purpose, a plant can promote the presence of certain microorganisms to improve its fitness in extreme environments such as contaminated soils (Vandenkoornhuyse, Quaiser et al. 2015). Thus, phytoremediation depends primarily on rhizoremediation, which takes place in the rhizosphere, *i.e.* the soil immediately adjacent to roots (a few millimeters thick).

Soil is considered to be the most microbially diverse environment and it is estimated that 1 g of soil can contain up to 10^9 microbial cells (Hamamura, Olson et al. 2006, Hansel, Fendorf et al. 2008). It is well known that the rhizosphere is highly influenced by plant roots and contains a higher microbial density than the bulk soil, described as the 'rhizosphere effect' (Smalla, Wieland et al. 2001, Yang, Ratte et al. 2001, Corgié, Joner et al. 2003, Morgan, Bending et al. 2005, Hartmann, Rothballer et al. 2008, Nihorimbere, Ongena et al. 2011, Bulgarelli, Schlaeppi et al. 2013, Favas, Pratas et al. 2014). Contaminant concentration can also influence the specific recruitment of particular microbes by the plant (Bell, Hassan et al. 2014, Yergeau, Sanschagrin et al. 2014). Numerous studies revealed that hydrocarbon contaminated soils are generally dominated by Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Acidobacteria (Popp, Schlömann et al. 2006, Militon, Boucher et al. 2010, Zhang, Mörtelmaier et al. 2012, Lu, Huggins et al. 2014). Microorganisms are able to degrade simple hydrocarbons (linear and branched alkanes) to complex (aromatic compounds), with the exception of polyaromatic hydrocarbons (PAHs) that cannot, in general, be fully degraded (Atlas and Bragg 2009, Rohrbacher and St-Arnaud 2016). Organic contaminants can provide microorganisms with a carbon source and electrons required in respiration (Germida, Frick et al. 2002). Most individual species are not equipped with all the appropriate enzymes, so degradation is mainly achieved

via a consortium of microorganisms with various and complementary enzyme systems (Macek, Mackova et al. 2000, Chaudhry, Blom-Zandstra et al. 2005, Yateem, Al-Sharrah et al. 2008).

The adaptation of bacteria is accelerated by their ability to acquire genes directly from other organisms (Cohan and Koeppel 2008, Dagan, Artzy-Randrup et al. 2008). Horizontal acquisition of catabolic genes allows microbial populations having different physiological properties, cellular structures or ecological niches, to acquire new capabilities to improve their performance in their current ecological niche or to colonize a new niche (Gogarten, Doolittle et al. 2002, Cohan and Koeppel 2008). Lateral gene transfer is a major driver of bacterial evolution; the amount of acquired genes would represent between 66 and 96% of the bacterial genome (Dagan, Artzy-Randrup et al. 2008). Conjugation, *i.e.* transfer of genes carried by plasmids (de la Cruz and Davies 2000, Popa and Dagan 2011), is one of the most important mechanism for exchanging genes (Halary, Leigh et al. 2010, Zhang, Pereira e Silva Mde et al. 2014). The plasmidome is defined as the overall plasmid content in a given environment (Walker 2012). Plasmids are extrachromosomal and mostly circular DNA molecules, which are transmitted both vertically (from mother cell to daughter during bacterial division) and horizontally (from a donor cell to a recipient cell) (Mela, Fritsche et al. 2008, Walker 2012). Plasmids include two distinct regions (Thomas 2000): (1) the first is composed by skeleton genes, often conserved among members of the a plasmid family, which ensure plasmid replication, maintenance and transfer; (2) the second region gathers accessory genes, encoding adaptive functions such as environmental pollutants metabolism, resistance to antibiotics or trace elements (Rhodes, Parkhill et al. 2004, Ono, Miyazaki et al. 2007, Schluter, Krause et al. 2008, Li, Top et al. 2015). Conjugative plasmids, which are self-transmissible, contain two modules: MOB (Mobility system) and MPF (Mating Pair Formation system) (Smillie, Garcillan-Barcia et al. 2010). The MOB module contains an origin of transfer (oriT), a relaxase (MOB genes) and a facultative type IV coupling protein (T4CP). The T4CP connects the relaxosome (relaxase-DNA complex) to the transport channel of the type IV secretion system (T4SS) coded by the MPF module (Smillie, Garcillan-Barcia et al. 2010). Mobilizable plasmids, which possess only the MOB module, need the MPF module of a co-resident conjugative plasmid to become transmissible by conjugation (Smillie, Garcillan-Barcia et al. 2010).

This research aims to describe and compare the plasmidomes of willow rhizospheres in hydrocarbon contaminated soils, as compared with unplanted soil. We harvested the rhizosphere of willows planted in pots containing contaminated and non-contaminated soil, as well as soil from non-planted comparable pots. Total DNA was extracted and sequenced using the Illumina Hiseq 2500 platform. To highlight functions carried by plasmids, the metagenomes were assembled and the ORFs carried by plasmids were identified by their backbone genes. Our results indicate a strong effect of hydrocarbon contamination on plasmid composition. Furthermore, plasmids harbored genes involved in several metabolic pathways, including xenobiotic biodegradation, energy production, signal transduction, chemotaxis and metabolisms of carbohydrates, amino acids and secondary metabolites. To date, this is the first comparative soil metagenomic study documenting the plasmidome diversity in a phytoremediation system. The results provide support to the role of lateral gene transfer in rhizosphere bacteria adaptation to hydrocarbon contamination.

Materials and methods

Experimental design

Approximately 240 L of contaminated and of non-contaminated soil was collected from two nearby area of the site of a former petrochemical plant in Varennes (QC, Canada), a small township located Southeast of Montréal (geographic coordinates: contaminated: 45.699145, -73.430997; non-contaminated: 45.700788, -73.430302). Decades of industrial activity (1953–2008) resulted in the accumulation of mixed petrochemical residues. The two soils (contaminated and non-contaminated) were separately homogenized, distributed in 20 L pots. The present study used a subset of a larger experiment intended to study a phytoremediation system based on willows and microbes. The whole experiment was a repeated block design with six blocks, each containing a combination of the following factors: the two soils, planted or not with willows, and the planted pots inoculated or not with the mycorrhizal fungus *Rhizoglomus irregulare* (see Cloutier-Hurteau, Turmel et al. (2014) and Yergeau et al. 2014 for details). In the present study, focusing on microbial plasmids, three blocks out of the six available were sampled, with 12 pots that were used, i.e. one planted and one unplanted pot per soil type in each block. The contaminated soil initially contained a mean of 1497 mg/kg total petroleum

hydrocarbons (TPHs) and 73,5 mg/kg polyaromatic hydrocarbons (PAHs), while concentrations were lower at the end of the experiment (432 mg/kg TPHs, 9,9 mg/kg PAHs); the non-contaminated soil contained <100 mg/kg TPHs and < 0,1 mg/kg PAHs at both time points (see **Supplementary Table S1** of Yergeau et al. 2014 for detailed soil analyses). Willow cuttings (*Salix purpurea* var. Fish Creek) were first grown in a sterile medium for eight weeks and were then transferred to pots after stems had grown to about 20 cm (early October 2011). Plants were placed in a greenhouse at the Institut de recherche en biologie végétale, Montréal, under natural daylight supplemented with high pressure sodium-vapor lamps, and maintained at 18–20°C (night/day) for 6 months. Pots were watered frequently to maintain soil moisture near field capacity, and saucers were used under pots to prevent leaching of contaminants.

Soil sampling

For the metagenomic analysis, the soil was sampled six months after planting (April 2012). The plants were completely extracted from the pots and shaken vigorously to remove excess soil. The remaining soil attached to the roots was considered the rhizospheric soil. Soil from unplanted soil pots was collected at a depth of 5 cm. At least five different soil subsamples were collected and homogenized in 50 ml Falcon tubes and immediately flash frozen in liquid nitrogen (Yergeau, Sanschagrin et al. 2014). Tubes were kept frozen at -80°C until the DNA was extracted. Additional soil samples were harvested from each pot at the beginning and end of experiment, and sent to Maxxam Analytics (Montreal, QC, Canada), where soil was analyzed for C10–C50 hydrocarbons (sum of all aliphatic hydrocarbon compounds with chain lengths from C10–C50) and polycyclic aromatic hydrocarbons (PAHs) according to standard protocols.

DNA extraction, sequencing and assembly

DNA was extracted from 2-g soil samples using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). Three µg of genomic DNA per sample were subjected to high-throughput sequencing using a paired-end 2×150 bp protocol on a Illumina Hiseq 2500 platform with 6 samples multiplexed per line, at the McGill University and Genome Québec Innovation Centre. Approximately 10 Gigabases were generated for each sample. Reads were quality filtered using the Trimmomatic program (Bolger, Lohse et al. 2014), and assembled

into contigs using the IDBA-UD assembler (Iterative de Bruijn graph de novo assembler – Uneven sequencing depth) with default parameters on a high performance server of the Compute Canada computing network (Peng, Leung et al. 2012).

Plasmidic sequences characterization and annotation

Open Reading Frames (ORFs) were predicted from contigs using FragGeneScan (Rho, Tang et al. 2010), with default parameters. ORFs carried by plasmids were identified by their backbone genes in the ACLAME database using Blastp, with the e-value was set to $1e^{-05}$ (Altschul, Gish et al. 1990, Leplae, Hebrant et al. 2004). To limit false positives, contigs were filtered out with a minimum of 5 hits and a minimum length characteristic of 10 ORFs by contig. All ORFs present on these filtered contigs were considered as dominant plasmidic ORFs and were subsequently analyzed.

Protein sequences of dominant plasmidic ORFs were first aligned using BLASTP (Altschul, Gish et al. 1990) against a custom prokaryotic proteins database (made from all bacterial protein in nr) with an e-value threshold of $1e^{-03}$. The alignments were analysed using MEGAN v. 6.4.0. (MEtaGenome ANalyzer, Center for Bioinformatics, Tübingen, Germany) (Huson, Beier et al. 2016) with default parameters, to perform taxonomical and KEGG pathways annotations (Kanehisa and Goto 2000).

The identification of genes involved in conjugation, such as MOB genes, T4CP genes, ATPase genes and MPF genes, was performed using CONJscan-T4SSscan with default parameters (http://mobyle.pasteur.fr/cgi-bin/portal.py#forms::CONJscan-T4SSscan) (Guglielmini, Quintais et al. 2011). CONJscan-T4SSscan is a database regrouping T4SS, conjugative and mobilization systems. This web server used hidden Markov model (HMM) profiles to scan a set of protein sequences for T4SS components (the server was turned off the 30th December 2016).

Clustering and statistical analysis

Statistical analyses were conducted in R (v 3.3.2, The R Foundation for Statistical Computing). Because of the large difference in the number of plasmidic sequences between samples from the contaminated and uncontaminated pots, statistical analysis were performed per contamination treatment. Normal distribution and variance homogeneity of the residuals

were tested using the 'shapiro.test' and 'bartlett.test' functions, respectively, and data were log transformed before analysis of variance. ANOVA were carried out using the 'Two factor ANOVA with replication' function to test differences in the number of genes involved in conjugation (identified with CONJscan-T4Ssscan) and genes of various functions (identified with KEGG level 2), between contaminated rhizosphere and contaminated unplanted soil.

In MEGAN v6.4.0, an unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm (Bray-Curtis similarity) was used to create a hierarchical dendrogram of the 11 samples (Sokal 1958). Only two replicates represent the non-contaminated unplanted soil condition, because no dominant plasmidic ORF were found in the third replicate.

Plasmidic proteome networks

EGN (Evolutionary Gene and Genome Network) (Halary, Leigh et al. 2010, Halary, McInerney et al. 2013)was used to construct protein similarity networks using the following parameters: protein sequence identity threshold ≥40%, hit length threshold ≥20% of the smallest sequence, hit coverage ≥90% of both sequence length, and E-value ≤$1e^{-05}$ (Cheng, Karkar et al. 2014, Corel, Lopez et al. 2016). Networks were visualized in Cytoscape (Shannon, Markiel et al. 2003). Only the first 40 connected components, containing more than 20 nodes, were characterized against KEGG database (levels 2 and 3). In each connected component, the unique annotated sequences were removed.

Results

Plasmidome datasets

Sequencing of the 12 soil genomic DNA libraries resulted in a mean of 50 million filtered reads per sample. Numbers of contigs, open reading frames (ORFs), plasmidic ORFs, and KEGG pathways are provided in **Table 2.1**, and are grouped per the four following conditions: (1) contaminated unplanted soil, (2) contaminated rhizosphere, (3) non-contaminated unplanted soil, and (4) non-contaminated rhizosphere.

Tableau 2.1. Effect of hydrocarbon contamination and presence of willows on the amount of different genetic elements in the metagenomic dataset.

Steps	Mean per condition [1]				Total number
	Contaminated soil		Non contaminated soil		
	Unplanted soil	Rhizospheric soil	Unplanted soil	Rhizospheric soil	
Contigs	834 081 (±221 912)	932 845 (±80 881)	650 616 (±274 853)	954 289 (±9 425)	10 115 496
ORFs[2]	1 135 469 (±308 111)	1 239 540 (±84 041)	788 231 (±317 414)	1 219 281 (±9 791)	13 147 561
Plasmidic ORFs[3]	32 289 (±9 565)	29 887 (±4 519)	9 834 (±1 887)	19 598 (±1 112)	274 822
Dominant plasmidic ORFs[4]	4 239 (±3 423)	3 277 (±3 228)	13 (±13)	162 (±80)	23 072
Protein sequences[5]	3 723 (±3 015)	2 886 (±2 851)	11 (±11)	142 (±70)	20 285
Functional annotation :					
- conjugation mechanism[6]	242 (±141)	143 (±64)	7 (±7)	11 (±6)	1 216
- KEGG pathways [7]	1 461 (±1 240)	991 (±877)	1 (±1)	59 (±37)	7 540

[1] Numbers are means of three replicates of each condition ± standard deviation.
[2] ORF : Open Reading Frame. All ORFs predicted from contigs by FragGeneScan.
[3] ORFs carried by plasmids, identified from their skeleton genes in ACLAME database.
[4] ORFs present on filtered contigs (≥ 5 hits per ORF and from contig with ≥10 ORFs).
[5] Sequences annotated with Blastp
[6] CONJscan-T4SSscan: Identified from the database of conjugative and mobilization systems, and T4SS (Guglielmini, Quintais et al. 2011).
[7] Identified from the Kyoto Encyclopedia of Genes and Genomes database.

More than 10 million of ORFs were identified in the 12 samples and were almost equally distributed between contaminated (54,2%) and non-contaminated soil conditions (45,8%). Plasmidic ORFs represented only 2% of all ORFs. Two-third of plasmidic ORFs were found in contaminated soil and were almost equally distributed between rhizospheric and unplanted soil, while the remaining third was found in non-contaminated conditions. Dominant plasmidic ORFs (≥5 hits per ORF and from contig with ≥10 ORFs) represented 8,4% of all plasmidic ORFs. They were mainly distributed among contaminated conditions: 55% belonged to unplanted soil samples, and 42,6% to rhizospheric soil samples. The remaining 2,3% were mostly attributed to rhizospheric soil samples from non-contaminated pots. Approximately 90% of these dominant plasmidic ORFs were assigned in the custom prokaryotic protein database using BlastP. Then, two different functional annotations were performed: CONJscan-T4SSscan and KEGG. Only 5,3% of dominant plasmidic ORFs were annotated with CONJscan-T4SSscan. Among these, 95,6% were found in contaminated soil conditions (59,7% belonged to unplanted soil condition and 35,9% belonged to rhizospheric soil). The KEGG database allowed to annotate 32,7% of dominant plasmidic ORFs. More than 97,7% of annotated sequences with KEGG database belonged to contaminated conditions.

Taxonomic affiliation of dominant plasmidic ORFs

Dominant plasmidic ORFs annotated as Proteobacteria were present in all four conditions (**Figure 2.1**) but were more prevalent in contaminated soil. In unplanted contaminated soil, dominant plasmidic ORFs were mainly annotated as Proteobacteria (43,2%), Chloroflexi (31,6%) and Firmicutes (6,9%), while the remaining ORFs belonged to 16 others additional phyla. Interestingly, those annotated as Chloroflexi were found exclusively in this condition. In contaminated rhizospheric soil, dominant plasmidic ORFs were annotated as Cyanobacteria (27,9%), Proteobacteria (20,8%), Actinobacteria (20,4%), Bacteroidetes/Chlorobi (17,5%) and Fibrobacteres/ Acidobacteria (8,3%), with the remaining belonging to 10 additional phyla. In non-contaminated soil, dominant plasmidic ORFs were mainly annotated as Actinobacteria, with 83,3% in unplanted soil and 64,2% in rhizospheric soil. Furthermore, Gemmatimonadetes represented 13% of the ORFs from non-contaminated rhizospheres while this group was only 1,54% in contaminated rhizospheric soil and lower than 0,6% in non-planted pots.

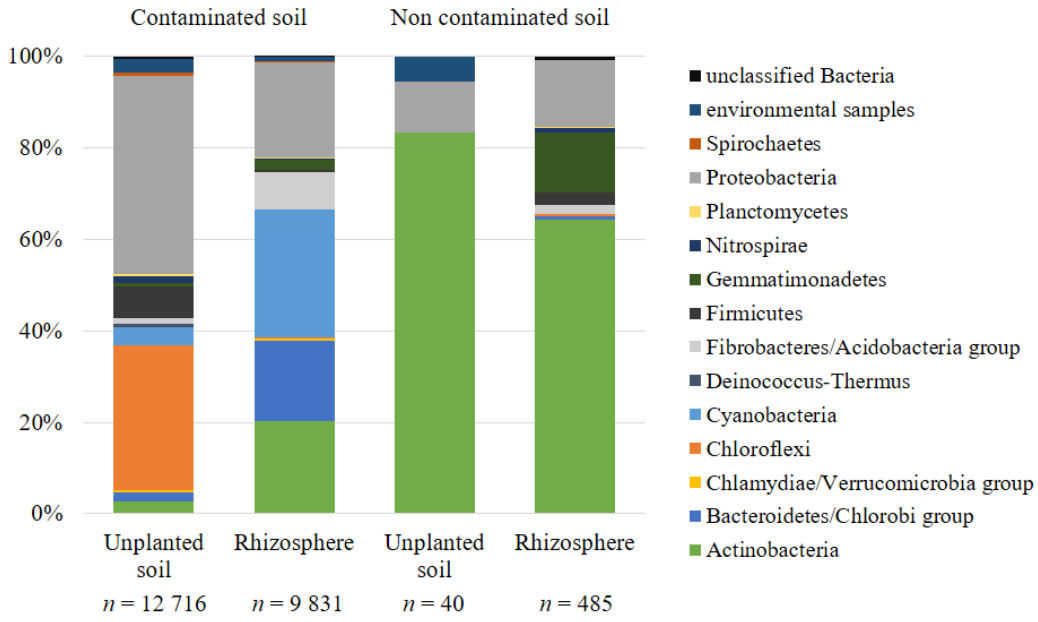

Figure 2.1. Taxonomic annotation of dominant plasmidic ORFs with regards to hydrocarbon contamination and presence of plants.

Functional analysis of the plasmidome

UPGMA tree of the dominant plasmidic ORFs identified with the KEGG database revealed clustering of contaminated samples, while the most distant condition was non-contaminated unplanted soil (**Figure 2.2**). Level 1 annotation was represented by four categories, which were from the most to the least abundant: “Metabolism”, “Genetic information processing”, “Environmental information processing” and “Cellular processes". Because of the high difference in the number of sequences retrieved from contaminated and non-contaminated conditions, ANOVA comparisons of the sequences belonging to each functional category were performed only within samples from the contaminated soil.

Metabolism

“Metabolism” contained eleven Level 2 categories. “Amino acid metabolism” category did not show significant difference between contaminated rhizospheric soil and contaminated unplanted soil (F=0,698; *P*=0,407). Within “amino acid metabolism”, “alanine, aspartate and glutamate metabolism”, “arginine and proline metabolism” and “glycine, serine and threonine metabolism” were the three most abundant subcategories.

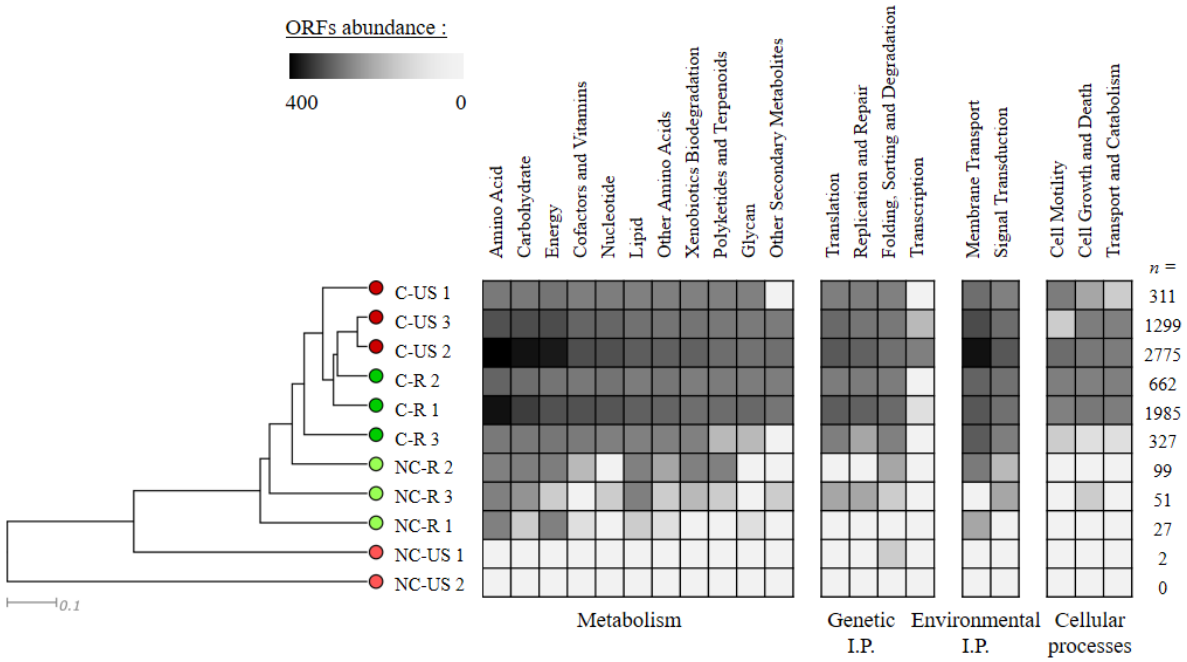

Figure 2.2. Dominant plasmidic ORFs identified with the KEGG database (bottom groups: level 1, top groups: level 2) for contaminated (C) or non-contaminated (NC), rhizosphere (R) or unplanted soil (US). The heatmaps were drawn for each replicate of the four treatments. The MEGAN hierarchical classification was used with the level 2 of KEGG database. I.P.: Information Processing. *n*: number of dominant plasmidic ORFs in a replicate. Only two replicates represent the non-contaminated unplanted soil condition, because no dominant plasmidic ORF were found in the third replicate.

"Carbohydrate metabolism" category regrouped six subcategories. "Amino sugar and nucleotide sugar metabolism" and "pyruvate metabolism" subcategories were the most abundant. The other subcategories were "propanoate metabolism", "glycolysis/ gluconeogenesis", "citrate cycle (TCA cycle)" and "butanoate metabolism". Sequences related to "carbohydrates metabolism" category were significantly more abundant in contaminated unplanted soil (F=4,102; *P*=0,047).

"Energy metabolism" category did not show significant difference between contaminated rhizospheric soil and contaminated unplanted soil (F=2,942; *P*=0,097). More than half of these sequences were related to the "oxidative phosphorylation" subcategory. The other most abundant subcategories were "photosynthesis", "methane metabolism", "carbon fixation pathways in prokaryotes", "nitrogen metabolism" and "sulfur metabolism".

“Xenobiotics degradation and metabolism” functions were more abundant in contaminated soil (239 against 11 in non-contaminated soil), and also significantly more abundant in contaminated unplanted soil (F=5,298; *P*=0,023). The main degradation genes identified were those involved in “benzoate degradation”, “chlorocyclohexane and chlorobenzene degradation”, “metabolism of xenobiotics by cytochrome P450”, “polycyclic aromatic hydrocarbon”, “aminobenzoate degradation”, “fluorobenzoate degradation”, “nitrotoluene degradation”, “atrazine degradation”, “naphthalene degradation”, “toluene degradation” and “ethylbenzene degradation”. However, four degradation genes were more abundant in rhizospheric soil: “styrene degradation”, “xylene degradation” and “bisphenol degradation”.

In “biosynthesis of other secondary metabolism” category, no significant difference was found between contaminated rhizospheric soil and contaminated unplanted soil (F=1,126; *P*=0,317). The main subcategory was “streptomycin biosynthesis”. Others main subcategories were also related to antibiotic biosynthesis, such as “novobiocin biosynthesis”, and “penicillin and cephalosporin biosynthesis”. “Tropane, piperidine and pyridine alkaloid biosynthesis”, “phenylpropanoid biosynthesis”, “isoquinoline alkaloid biosynthesis”, “stilbenoid, diarylheptanoid and gingerol biosynthesis”, and “butirosin and neomycin biosynthesis” were also detected.

“Biosynthesis of polyketides and terpenoids” category did not show significant difference between contaminated rhizospheric soil and contaminated unplanted soil (F=0,503; *P*=0,482). The main subcategory was “terpenoid backbone biosynthesis” that represented almost the half of this category.

Environmental information processing

This category regrouped two level 2 categories: “membrane transport” and “signal transduction”. For “membrane transport” category, no significant difference was observed between contaminated rhizospheric soil and contaminated unplanted soil (F=0,024; *P*=0,879). Two subcategories were represented: “ABC transporters” and “bacterial secretion systems”. ABC transporters were involved in transport of a multitude of substrates, such as branched-chain amino acid, cobalt, nickel, iron, molybdate, phosphate, sulfate and lipopolysaccharides.

The two main bacterial secretion systems represented in this category were “general secretion pathway” (Type II secretion system) and Vir proteins of “type IV secretion system”.

“Signal transduction” category was only represented by “two-component systems” and this subcategory was significantly more abundant in contaminated unplanted soil (F=4,748; *P*=0,03). A two-component system is composed of a sensor histidine kinase and a response regulator. “OmpR family” was the main two-component system family (40%). The main two-component system of the “OmpR family” were KdpD/KdpE (and KdpA,B,C) involved in potassium transport, and PhoR/PhoBP involved in phosphate assimilation. The second one was mainly present in unplanted soil. “NtrC family” was the second main two-component system family. The two two-component systems GlnL/GlnG (and GlnA,B,D) and NtrY/NtrX, belonging to the “NtrC family”, were both involved in nitrogen assimilation and represented the main subcategories of “NtrC family”. The two component system GlnL/GlnG (and GlnA,B,D) was mainly found in unplanted soil. The two-component system AtoB/AtoC, involved in short chain fatty acid metabolism was also an important two-component system of the “NtrC family”. The two-component system PilR/PilS (plus RpoN), involved in the expression of type IV pili was only present in the rhizospheric soil. “Chemotaxis family” was the third most important two-component system family and was mainly present in the rhizospheric soil. “Chemotaxis family” were related to “bacterial chemotaxis two-component system” (MCP,CheARW/CheYB) and to “twitching mobility” (PilJ,ChpA/PilGH). “NarL family”, the last two-component system, was only present in unplanted soil. The two-component system LiaS/LiaR, an antibiotic-sensing system that coordinate the genetic response to cell wall-active antibiotics, was the most important two-component system of “NarL family”. The two-component system NarX/NarL was involved in nitrate-sensing regulation.

Cellular processes

This category regrouped three level 2 categories: “cell motility”, “cell growth and death” and “transport and catabolism”. “Cell motility” did not show significant difference between contaminated rhizospheric soil and contaminated unplanted soil (F=1,939; *P*=0,201). This subcategory was divided in two subcategories: “bacterial chemotaxis” and “flagellar assembly”. “Bacterial chemotaxis” was mainly found in contaminated rhizospheric soil.

Genes involved in conjugation

Gene families involved in conjugation mechanism, *i.e.* relaxases (MOB genes), T4SS (MPF genes), T4CP and ATPases coding-genes, were analyzed using CONJscan-T4SSscan (**Figure 2.3**). There was twenty times less annotated sequences with CONJscan-T4SSscan in the non-contaminated soil condition. Because of the large difference in the number of dominant plasmidic ORFs in contaminated and non-contaminated soils, only gene families identified from the contaminated soil condition were analyzed.

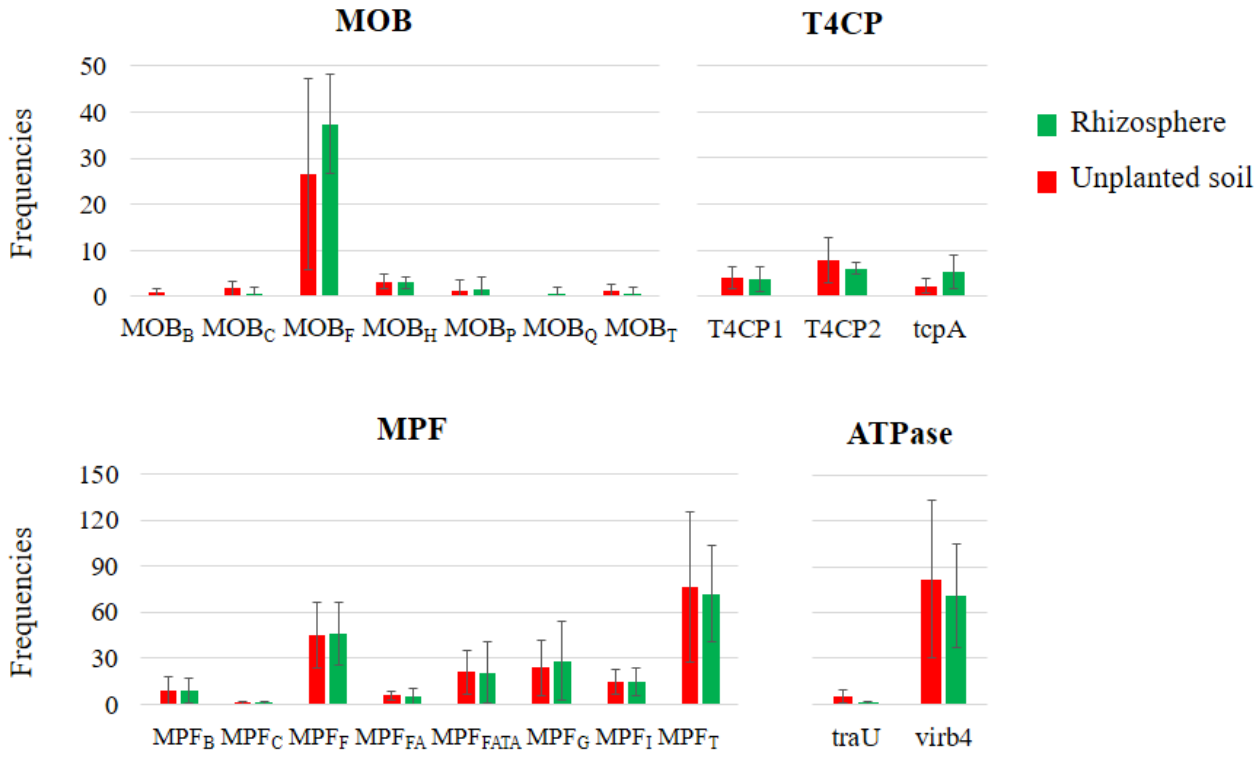

Figure 2.3. Gene families involved in conjugation mechanism carried by dominant plasmidic ORFs, in contaminated rhizospheric soil and contaminated unplanted soil. MOB: relaxase gene; T4CP: Type IV Coupling Protein; MPF: Mating Pair Formation; FA: Firmicutes Actinobacteria, FATA: Firmicutes Actinobacteria, Tenericutes Archaea.

The distribution of gene families involved in conjugation mechanisms did not shown significant difference between contaminated rhizospheric soil and contaminated unplanted soil (MOB: F=0,02 and *P*=0,89; T4CP: F=0,57 and *P*=0,46; MPF: F=0,002 and *P*=0,97; ATPase: F=2,13 and *P*=0,18). Among T4SS (Type IV Secretion System), the two dominant MPF types were MPF_T (37,2%) and MPF_F (22,8%), followed by MPF_G, MPF_{FATA} and MPF_I. Among relaxase genes, MOB_F represented 80,4%, of all MOB genes. T4CP genes represented only 4,6% of all genes involved in conjugation. ATPase genes represented almost a quarter of all genes involved in conjugation mechanisms. Among the two ATPase genes, the virB4 one was mainly found (96,1%) compared to traU.

Plasmidic proteome network

The sequence similarity network constructed using the EGN contained 3242 connected components (CC), with 87% of these CC containing less than 5 nodes. Therefore, only the largest 35 connected components, which included at least 20 nodes, were annotated with KEGG database levels 2 and 3 (**Figure 2.4**, **Table 2**). These 35 connected components represented 11,5% of all dominant plasmidic ORFs. The first connected component, which contained more than 600 nodes, was identified as a group of genes involved in signal transduction. It contained two-component systems involved in phosphate assimilation (OmpR family), nitrogen assimilation (Ntr family) and nitrate regulation (NarL family). The second largest CC contained 524 nodes and regrouped ABC transporters (Membrane transport). They were involved in transport of amino acid, lipopolysaccharides, phosphate, iron, macrolide, sulfate, cobalt and nickel. Two other connected components (CC3 and CC4) were also identified as “membrane transport”. They contained functions involved in “type IV pilus”, which were distributed among the four environmental conditions. Functions of connected components CC5 and CC6 were more abundant in rhizospheric soil (contaminated and non-contaminated). These functions were involved in “fatty acid biosynthesis/degradation”, “glycolysis/gluconeogenesis” and “biotin biosynthesis”. Eight connected components (CC6, CC9, CC16, CC18, CC21, CC25, CC31 and CC32) were related to “carbohydrate metabolism” and were mainly related to contaminated samples. Other main annotations were “motility and chemotaxis” (CC17 and CC30), “lipid metabolism” (CC5, CC12, CC25), “amino acids metabolism” (CC6, CC9, CC16, CC18, CC25, CC26) and “energy metabolism” (CC8, CC17, CC21, CC29).

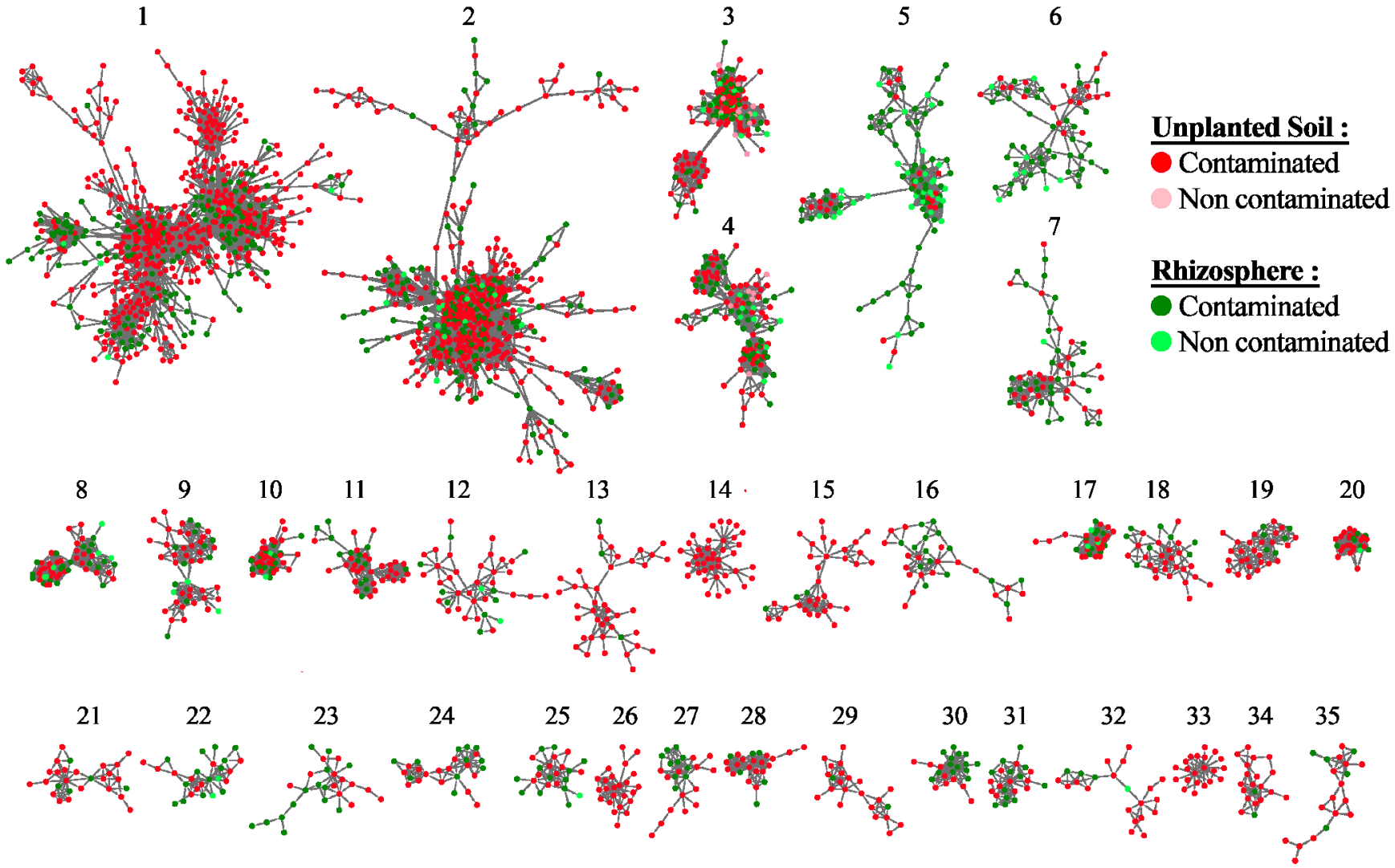

Figure 2.4. Sequence similarity networks of the largest 35 connected components of dominant plasmidic ORFs, with regards to hydrocarbon contamination and presence of plant.

Tableau 2.2. Characteristics of the largest connected components produced using EGN and annotated with KEGG database levels 2 and 3.

CC[1]	Nodes	KEGG[2] Levels 2 and 3	% annot.[3]	C-US	C-R	NC-US	NC-R[4]
1	607	Signal Transduction - Two-component system	19,3	72,2	27		0,8
2	524	Membrane Transport - ABC transporters	25,2	67,6	30		2,5
3	170	Membrane Transport - Bacterial secretion system	38,2	44,1	40	8,8	7,1
4	123	Membrane Transport - Bacterial secretion system	69,9	40,7	47,2	4,9	7,3
5	116	Lipid Metabolism - Fatty acid biosynthesis	22,4	8,6	63,8		27,6
6	69	Carbohydrate Metabolism - Glycolysis / Gluconeogenesis Lipid Metabolism - Fatty acid degradation	20,3 14,5	29	59,4		11,6
7	59	Membrane Transport - Bacterial secretion system	76,3	42,4	55,9		1,7
8	58	Energy Metabolism - Oxidative phosphorylation	91,4	53,4	36,2		10,3
9	49	Energy metabolism - Nitrogen metabolism Carbohydrate Metabolism - Amino sugar and nucleotide sugar	36,7 59,2	71,4	22,4		6,1
10	45						
11	44	Signal Transduction - Two-component system	4,5	70,5	29,5		
12	42	Lipid Metabolism - Fatty acid biosynthesis	42,8	71,4	23,8		4,8
13	42						
14	40						
15	35						
16	34	Carbohydrate Metabolism - Glycolysis / Gluconeogenesis - Propanoate metabolism - Butanoate metabolism Amino Acid Metabolism - Glycine, serine and threonine metabolism	41,2 11,8 8,8 11,8	50	50		
17	33	Energy Metabolism - Oxidative phosphorylation Cell Motility - Flagellar assembly	84,8 9,1	60,6	30,3		9,1
18	32	Amino Acid Metabolism - Arginine and proline metabolism - Lysine biosynthesis Carbohydrate Metabolism - Propanoate metabolism	31,3 6,3 25,0	75	25		
19	32						

20	29					
21	27	Carbohydrate Metabolism - Glyoxylate and dicarboxylate metabolism Energy Metabolism - Oxidative phosphorylation	59,2 25,9	81,5	18,5	
22	26	Folding, Sorting and Degradation - Protein export	46,2	42,3	50	7,7
23	26					
24	26	Glycan Biosynthesis and Metabolism - Lipopolysaccharide biosynthesis	19,2	42,3	50	7,7
25	24	Carbohydrate Metabolism - Propanoate metabolism Amino Acid Metabolism - Valine, leucine and isoleucine degradation Lipid Metabolism - Fatty acid degradation	16,7 8,3 8,3	45,8	50	4,2
26	24					
27	24					
28	24					
29	23	Energy Metabolism - Oxidative phosphorylation	95,6	91,3	8,7	
30	23	Cell Motility - Bacterial chemotaxis Membrane Transport - Bacterial secretion system ImpK	8,7 8,7	91,3	8,7	
31	23					
32	22	Biosynthesis of Polyketides and Terpenoids - Polyketide sugar unit biosynthesis Carbohydrate Metabolism - Galactose metabolism	45,5 31,8	81,8	13,6	4,5
33	21					
34	20	Translation - Aminoacyl-tRNA biosynthesis Amino Acid Metabolism - Arginine and proline metabolism	75,0 15,0	90	10	
35	20	Nucleotide Metabolism - Purine metabolism Translation - Ribosome	40,0 30,0	90	10	

[1] Connected components.

[2] Kyoto Encyclopedia of Genes and Genomes

[3] percentage of annotated nodes with KEGG database Level 1 and 2

[4] contaminated (C) or non-contaminated (NC) soil, rhizosphere (R) or unplanted soil (US)

Discussion

In this study, using a bioinformatic approach, we identified the ORFs carried by plasmids among a metagenomic dataset gererated from willow rhizosphere or bulk soil samples, contaminated or not with petroleum hydrocarbons. We further characterized the dominant plasmidic ORFs and compared the frequency of the main gene families and functions with regards to contamination and presence of willows. Our study revealed that the composition of plasmids was strongly determined by hydrocarbon contamination. In contaminated soil, sequences related to conjugation, metabolisms of carbohydrates, amino acids, energy, membrane transport, signal transduction and xenobiotics degradation were the most abundant categories. Moreover, the presence of willows significantly modified the frequency of gene belonging to "xenobiotics degradation", "carbohydrates metabolism" and "signal transduction".

Gene transfer between soil bacteria

The most abundant genes involved in conjugation mechanism were MPF_T, MPF_F, MOB_F and VirB4 in CONJscan-T4SSscan (**Figure 2.3**), and "Type IV secretion system" of "Membrane transport" in KEGG. Type IV secretion systems (T4SS), containing two key components VirB4 and MPF, is a versatile system secreting a wide range of substrates from proteins to DNA, such as plasmid in the mechanism of conjugation (Fronzes, Christie et al. 2009). MPF_T, MPF_G, MPF_I and MPF_F are almost exclusively found in Proteobacteria, and (Smillie, Garcillan-Barcia et al. 2010, Guglielmini, Néron et al. 2014). Guglielmini, Quintais et al. (2011) discovered that many MOB/MPF combinations are found among conjugative elements, and suggested that the MOB and MPF modules can shuffle over long evolutionary distances. For example, they detected that the most relevant association was MPF_F/MOB_F (Garcillán-Barcia, Francia et al. 2009, Smillie, Garcillan-Barcia et al. 2010). Regarding VirB4, this is an ATPase gene found in Proteobacteria, FATA (Firmicutes, Actinobacteria, Tenericutes, Archaea), Bacteroidetes and Cyanobacteria. The preponderance of MPF_T, MPF_F, MOB_F and VirB4 suggest that the bacteria involved in conjugation in contaminated soil were mostly Proteobacteria. This agree with Yergeau, Sanschagrin et al. (2014) findings showing that Proteobacteria also formed the main proportion of the active microbial community composition in contaminated pots, based on 16S rRNA gene sequencing.

Similarly, taxonomic annotation of the plasmid-carried genes characterized in KEGG database (**Figure 2.1**) have shown that they mainly belong to Proteobacteria and Actinobacteria. These results support that Proteobacteria and Actinobacteria are the main phyla for exchanging genes in soil (Klümper, Riber et al. 2015). Musovic, Oregaard et al. (2006) detected transfer between Proteobacteria and Actinobacteria in the barley rhizosphere, via broad host range plasmids. In contaminated unplanted soil, dominant plasmidic ORFs were associated to a higher taxonomic diversity than in contaminated rhizospheric soil. These results support the hypothesis that a typical 'core' microbiome is recruited by the plant from common soil bacteria and selected by the ability of community members to grow in root exudates (Hirsch and Mauchline 2012). In contaminated unplanted soil, dominant plasmidic ORFs were mainly annotated as Proteobacteria and Cloroflexi. Proteobacteria are commonly found in rhizosphere and are also well known to degrade hydrocarbons (Watanabe 2001, Bulgarelli, Rott et al. 2012, Lundberg, Lebeis et al. 2012). In contaminated rhizosphere, dominant plasmidic ORFs were less taxonomically diverse than in contaminated unplanted soil, and were annotated to four major phyla: Cyanobacteria, Actinobacteria, Proteobacteria and Bacteroidetes. With the exception of Cyanobacteria that were not detected in their study, Bell, Hassan et al. (2014) reported the same phyla in hydrocarbon contaminated rhizosphere of willows but with a higher dominance of Proteobacteria. Cyanobacteria form symbiotic associations with a broad range of organisms including angiosperms (Bergman 2002, Cuddy, Neilan et al. 2012). In all situations, the cyanobiont is intimately associated with plant tissues and is responsible for supplying the host with fixed nitrogen (Bergman, Rai et al. 2007). Cyanobacteria are also known hydrocarbon degraders (Raghukumar, Vipparty et al. 2001, Prince, Gramain et al. 2010). Bell, Hassan et al. (2014) and Yergeau, Sanschagrin et al. (2014) studied bacterial communities in the same samples and did not detected cyanobacteria. The presence of genes belonging to cyanobacteria in our samples is probably due to a lateral acquisition from cyanobacteria.

Adaptation to hydrocarbon contamination

Here, samples from contaminated soil contained more plasmids and a higher taxonomy diversity than those from non-contaminated soil, suggesting an important dynamic of conjugation mechanism in this environment. This is obviously induced by hydrocarbon contamination, where plasmids play a major role in the bacterial adaptation.

Hydrocarbons are a carbon source for bacteria, therefore they need to adapt by acquiring new genes to degrade them and use them as nutrients. Degradation functions related to monoaromatic hydrocarbon - such as BTEX (benzene, toluene, ethylene, xylene) and their derivatives benzoate, ethylbenzene, chlorobenzene, styrene -, and polycyclic aromatic hydrocarbon (such as naphthalene) were present in plasmids of contaminated soil. Benzene is considered as the simplest aromatic hydrocarbon. Chlorobenzene, toluene, styrene and ethylbenzene (precursor of styrene) are considered as simple benzene derivatives. Further loss of hydrogen in benzene gives "fused" aromatic hydrocarbons, such as naphthalene, a polycyclic aromatic hydrocarbon (PAH).

Energetically favorable metabolic pathways of hydrocarbon degradation were found in plasmid of contaminated soil. They include aerobic degradation (oxidative phosphorylation and cytochrome P450), nitrate reduction (denitrifiers), sulfate reduction and methanogenic degradation. Oxidative phosphorylation (or aerobic respiration) was identified here as the main subcategory of “energy”. This process utilizes hydrocarbons as a carbon source to produce energy, while subsequently degrading the long-chained molecules. This is the most energetically favorable metabolic pathway to make energy. In this pathway, energy in the form of ATP (adenosine triphosphate) is generated from the NADH (nicotinamide adenine dinucleotide) produced in the breakdown of hydrocarbons (Committee on Oil in the Sea 2003, Noone, Sumaila et al. 2013). Cytochrome P450, which is a detoxification enzyme system, was identified among “xenobiotics degradation” functions. In this degradation pathway, hydrophobic PAH are oxidized to smaller, and more water soluble molecules that can be excreted by cells (Das and Chandran 2011). Anaerobic mechanism of hydrocarbon degradation were also found in plasmids from contaminated soil samples. It has been shown that monoaromatic aromatic compounds such as benzene, toluene, ethylbenzene and xylene can be biodegraded in the absence of oxygen by a large diversity of organisms (Chakraborty and Coates 2004). These hydrocarbons have been shown to serve as carbon and energy sources for bacteria growing phototrophically, or respiratorily with nitrate, sulfate, or carbon dioxide as the sole electron acceptor (Chakraborty and Coates 2004).

"Benzoate degradation via CoA ligation" was the most abundant subcategory of "xenobiotics degradation". Benzoate has been consistently detected as intermediates of anaerobic benzene (and BTEX) degradation (Chakraborty and Coates 2004). Mechanistically, BTEX are initially activated through hydroxylation, and ultimately produce benzoate or its CoA derivatives as a central common intermediate (Chakraborty and Coates 2004). The aerobic route of benzoate oxidation, which is initially activated by benzoate–CoA ligase, occurs in ~5% of bacteria, particularly in Actinobacteria and Proteobacteria (Fuchs, Boll et al. 2011). This route is considered either as the only pathway or as an additional strategy that is used under low O_2 pressure (Fuchs, Boll et al. 2011).

Enzymes involved in the metabolism of succinate and fumarate were also found in plasmid from the contaminated soil samples. Fumarate addition to hydrocarbon seems to be the best understood and the most widespread of anaerobic mechanisms of hydrocarbon degradation. It is used as the activation step of the catabolic process of monoaromatic hydrocarbons and yields substituted succinate derivatives (Chakraborty and Coates 2004). This reaction has been recognized for the activation of several alkyl-substituted benzenes as well for n-alkanes (Kube, Heider et al. 2004). Furthermore, genes involved in methanogenic degradation, sufate- and nitrate- reduction, were found among plasmids in contaminated soil. Methanogenic, sulfate- and nitrate-reducing bacteria has been measured in hydrocarbon contaminated soil many times and linked to hydrocarbon degradation (Fukui, Harms et al. 1999, Chakraborty and Coates 2004, Gray, Sherry et al. 2010, Jimenez, Richnow et al. 2016). In the case of toluene, fumarate addition appears to be the universal mechanism of activation and is now known to be utilized by anoxygenic phototrophs, nitrate- and sulfate-reducing, and methanogenic bacteria (Chakraborty and Coates 2004).

These results highlight that hydrocarbon degradation genes are exchanged between bacteria, suggesting that they are primordial for bacterial adaptation in hydrocarbon contaminated soil. The fact that hydrocarbon degradation functions carried by plasmids were significantly more abundant in unplanted soil was very surprising. On the contrary, in a metatransciptomics study of samples from the same experiment, Yergeau, Sanschagrin et al. (2014) revealed a generally higher expression of many hydrocarbon-degrading genes in the rhizosphere of willows. They concluded that root exudation stimulated rhizospheric bacteria to degrade hydrocarbons.

Adaptation to the rhizosphere environment

Functions related to nutrient assimilation, chemotaxis, metabolisms of amino acids, carbohydrates and secondary metabolites, were all detected in plasmids from the rhizosphere samples.

Nitrogen and phosphate are essential elements for bacteria in rhizosphere. On one hand, nitrogen compose most of macromolecules in a bacterial cell, including proteins, nucleic acids and cell wall components. On the other hand, phosphorus is an important fitness factor for plant-associated bacteria, controlling aggregation and surface attachment. When these nutrients become limiting, bacteria can accommodate their physiology either to use them at lower concentration or to use energetically less favorable nutrients. Mechanisms involved in such nutritional adaptation include the two-component systems (TCSs)(Ramos 2012). TCSs, the major signal transduction pathway used in wide varieties of bacteria, is composed of a sensor kinase that monitors external signals, and a response regulator that controls physiological activities for response against external signals (Itou and Tanaka 2001, Yoshida, Ishihama et al. 2015).

TCSs related to phosphate (PhoR/PhoBP), nitrogen (NtrC family) and potassium (KdpD/KdpE) assimilation were detected in plasmids from rhizosphere samples. Under phosphate limitation, PhoR senses the phosphate concentration as a sensor kinase, and PhoB responds to phosphate starvation as a response regulator or transcriptional activator (Baek and Lee 2007). Phosphate-solubilization ability of rhizosphere microorganisms is considered one of the most important traits associated with plant phosphate nutrition. Regarding nitrogen limitation, bacteria respond by phosphorylating the response regulator NtrC (product of *glnG*) by its cognate sensor kinase NtrB (product of *glnL*) (Brown, Barton et al. 2014).

Chemotaxis is also a required behavior for rhizosphere bacteria that generally live under conditions of “nutrient starvation”, and are thus constantly looking for nutrients. Plant roots release 5%–21% of their photosynthetically fixed carbon as soluble sugars, amino acids, or secondary metabolites. Microorganisms are attracted to root exudates through chemotaxis, carbohydrates and amino acids predominantly acting as chemoattractants (Huang, Chaparro et al. 2014). Chemotaxis, the sensory system, allows bacteria to swim toward the highest

concentration of roots exudates, in order to provide the required nutrients and energy for their metabolism, survival and reproduction (Ryan, Delhaize et al. 2001, Bertin, Yang et al. 2003, Narasimhan, Basheer et al. 2003, Bais, Weir et al. 2006, Lioussanne, Jolicoeur et al. 2008, Badri and Vivanco 2009, Huang, Chaparro et al. 2014, Ben 2015, Rohrbacher and St-Arnaud 2016). Genes controlling the chemotaxis behaviors of bacteria, such as MCP (methyl-accepting chemotaxis protein) and the two-component system CheARW/CheYB, were detected in the plasmids of rhizosphere. MCP is a transmembrane sensor protein which allows bacteria to detect concentrations of molecules in the extracellular matrix (Wadhams and Armitage 2004). Binding a ligand causes a conformational change in the MCP and activates CheA, a sensor kinase, via the signal transducer CheW (Wadhams and Armitage 2004). Activated CheA transfers its phosphoryl group to the response regulator CheY, which in turn controls the flagellar switch and changes the direction of rotation of the flagellum (Wadhams and Armitage 2004). The bacteria may swim smooth or tumble accordingly to re-orient itself toward attractants.

Genes related to metabolism of amino acids, such as alanine, aspartate, glutamate, arginine and proline, were detected in plasmids. Amino acids serve as the functional units in proteins, but also as metabolic intermediates, playing structural roles in bioactive natural products, acting as co-substrates in enzymatic transformations, and as key regulators of cellular physiology (Moe 2013). In the rhizosphere, amino acids are present as a result of lysis of plant root cells. Vranova, Rejsek et al. (2011) demonstrated that alanine and glutamine represented more than 10% of the total free amino acid content in soil. Bacteria are able to use free amino acids as biological sources of both carbon and organic nitrogen, even if they were shown to prefer to take up inorganic nitrogen (Forsum, Svennerstam et al. 2008, Moe 2013). Certain amino acids (*e.g.*, glutamate and proline) and their betaines (*e.g.*, glycine betaine) serve as osmoprotectants (Moe 2013). This ability is particularly important in the rhizosphere, which is prone to significant variations in solute concentrations. Amino acids can also alter key phenotypes related to plant root growth and microbial colonization, symbiotic interactions, and pathogenesis in the rhizosphere (Moe 2013).

Genes involved in metabolisms of carbohydrates (such as glucose, pentose, fructose, galactose, starch and sucrose) and of organic acids were present in plasmids from contaminated rhizospheric soil samples. Brimecombe, De Leij et al. (2007) estimated that 12-40% of the total

amount of carbohydrates produced by plant photosynthesis is released in root exudates. Carbohydrates, amino acids and organic acid represent the largest proportion of low molecular weight root exudates, and are fundamental to the provision of easily degradable energy and nutrient sources to the rhizosphere (Bertin, Yang et al. 2003, van Hees, Jones et al. 2005, Badri and Vivanco 2009, Krauss and Nies 2014, Rohrbacher and St-Arnaud 2016).

In rhizosphere, genes related to secondary metabolites, such as flavonoids, terpenoids, vitamins, carotenoids, polyketides, antibiotics, were also detected in plasmids. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. In addition to carbohydrates and amino acids, plants produce and release numerous secondary metabolites and hormones into the rhizosphere, many of which playing a role in plant-bacteria interactions. Plants use these compounds to attract beneficial soil microorganisms and defend themselves against pathogens (Huang, Chaparro et al. 2014). For example, flavonoids not only act as chemoattractants to lead rhizobia to the root surface, but also regulate expression of the nod genes involved in nodule formation, in which bacteria fix atmospheric nitrogen for plants. Furthermore, vitamins, such as biotin (B7), thiamin (B1), riboflavin (B2), folate (B9) and pantothenate (B4), are commonly released by plant roots and are considered as cofactors for microbial enzymes (Guillén-Navarro, Encarnación et al. 2005). Biotin is important for the establishment of symbiosis in some rhizobia, and they were the only prokaryotes in which genes encoding biotin have been identified (Guillén-Navarro, Encarnación et al. 2005). Genes related to the biosynthesis of phytohormones, such as zeatin (which is a cytokinine) and auxin, were also detected in plasmids of rhizosphere. Auxins (indole-3-acetic acid (IAA)) and cytokinins, signaling molecules, are known to play a vital role in plant growth and development. They are involved in apical dominance, shoot and root branching, leaf expansion, growth of lateral buds, photosynthesis, seed germination, floral transition, and leaf senescence (Mok and Mok 2001). Cytokinines also affect nutrient mobilization and biomass distribution, and participate in the maintenance of the meristem function and response to environmental stimuli (Takei, Sakakibara et al. 2001, Frébort, Kowalska et al. 2011). Auxin can be synthesized through the precursor tryptophan (Trp), which is found in our results (Wang, Chu et al. 2015). The bacterial production of phytohormones is well known among plant-growth promoting rhizobacteria (PGPR). This symbiotic interactions between plant and PGPR, allow

the plant to adapt to many types of environmental conditions and changes (Bulgarelli, Schlaeppi et al. 2013, Rohrbacher and St-Arnaud 2016). Morever, bacteria, in particular Actinomycetes and Cyanobacteria, are prolific sources of polyketides (such as tetracycline, vancomycin, ansamycin), many possessing antibiotic activity (Ridley, Lee et al. 2008). Antibiosis by rhizobacteria is known to play an active role in the biocontrol of plant disease (disease suppression).

Conclusion

This study provided the first in-depth metagenomic analysis of plasmidic genes in the rhizosphere of plants grown in hydrocarbon-contaminated soil. Firstly, plasmid-carried genes represented a large taxonomic diversity, corresponding to the known taxonomic diversity in soil. For example, Lundberg, Lebeis et al. (2012) and Bulgarelli, Rott et al. (2012) reported that in *Arabidopsis* rhizosphere, community structure was dominated by Proteobacteria and Actinobacteria, with Acidobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes and Cyanobacteria forming substantial proportions. Secondly, plasmid-carried genes came mainly from Proteobacteria, suggesting that this phyla is highly involved in conjugation in soil. Thirdly, our results indicated a strong effect of hydrocarbon contamination, suggesting that hydrocarbon degradation genes are exchanged between bacteria in contaminated soil and are primordial for their adaptation in this environment. Fourthly, our study highlighted that genes involved in adaptation in the rhizosphere and association with the plant were also carried by plasmids. Surprisingly, no significant differences were detected for genes involved in conjugation and for many KEGG categories between rhizosphere and unplanted soil. Using metatranscriptomics, Yergeau, Sanschagrin et al. (2014) studied samples from the same experiment, and revealed a strong rhizosphere effect on microbial activity that was more pronounced in contaminated soil. They detected higher expression of many genes in rhizosphere, related to carbon and amino-acid utilization, nitrogen cycling and hydrocarbon degradation. Taken together, these results suggest that a bacterial community may harbor all the required information for its adaptation to new substrates in a portion of the community, and mobilize the functions when required through conjugaison when it is required, for example after hydrocarbon contamination or in association with plants.

Conflict of interest

The GenoRem project contains several industrial partners, including ConocoPhillips, the company that provided us with access to their site for this study. Our manuscript has in no way been modified by ConocoPhillips, nor has any industrial partner commented on, or had any influence in, the analysis of our results. The authors declare no conflict of interest.

Discussion générale et conclusion

Depuis les années 2000, de nombreuses études ont démontré les impacts bénéfiques de la biostimulation avec des exsudats racinaires (Miya and Firestone 2001, Yang, Ratte et al. 2001, Yoshitomi and Shann 2001, White, Mattina et al. 2003, Zhao, Wang et al. 2006, Yi and Crowley 2007, Ling, Ren et al. 2009, Gao, Ren et al. 2010, Técher, Laval-Gilly et al. 2011, Xie, Liao et al. 2012, LeFevre, Hozalski et al. 2013, Ling, Sun et al. 2015). De plus, Schwaner and Kroer (2001), Molbak, Molin et al. (2007) et Phillips, Greer et al. (2012) ont démontré que les exsudats racinaires stimulaient le transfert latéral de gènes dans la rhizosphère, notamment des gènes de dégradation aux hydrocarbures. Cependant, l'évolution des microorganismes indigènes – qui implique à la fois des évènements mutationnels et le transfert latéral de gènes – et le développement de leurs capacités biodégradantes d'hydrocarbures sont des processus relativement lents (Mrozik and Piotrowska-Seget 2010). L'amélioration du potentiel biodégradant des communautés bactériennes peut être résolue par la bioaugmentation du sol contaminé avec des microorganismes génétiquement modifiés.

Biologie synthétique et modification génétique

Les bactéries étant capables de s'échanger des plasmides, l'intégration des gènes de dégradation d'hydrocarbures dans les plasmides de bactéries donneuses semble la solution tout indiquée pour améliorer la biodégradation. Ces bactéries donneuses peuvent alors transférer leurs gènes de dégradation aux bactéries indigènes receveuses. Peu importe si les souches ensemencées disparaissent, puisque les plasmides possédant les gènes de dégradation sont transférés aux bactéries indigènes et perdurent donc dans le milieu. Filonov, Akhmetov et al. (2005) ont construit une souche de *Pseudomonas putida* (KT2442), contenant des gènes de dégradation du naphtalène portés par le plasmide pNF142. Douze jours après la bioaugmentation dans un sol contaminé au naphtalène, la concentration en naphtalène a diminué de 2 à 0,2mg/g de sol, et le plasmide pNF142 a été transféré aux bactéries indigènes. La souche inoculée est restée stable et compétitive durant 40 jours. De même, Weyens, Schellingen et al. (2013) ont étudié le transfert latéral de gènes de dégradation du toluène entre la souche donneuse *Burkholderia vietnamiensis* BU61 (équipée du plasmide TOM-TEC codant pour la dégradation du toluène et trochloroéthylène (TCE)) et deux souches receveuses *Burkholderia* sp. HU 001

(rhizosphère) et *Pseudomonas* sp. HU 002 (endophyte). La conjugaison a produit des souches rhizosphériques et endophytiques capables de dégrader le toluène, qui ont été inoculées dans la rhizosphère de saule. L'inoculation avec ce consortium de bactéries associées aux plantes équipées des caractéristiques appropriées a résulté en une amélioration de la phytoremédiation du site contaminé au toluène : la dégradation du toluène a été améliorée, ce qui a mené à une diminution de la toxicité et une évapotranspiration par les saules. Plus récemment, Wang, Jiang et al. (2014) ont génétiquement modifié une souche de *Pseudomonas fluorescens* TP13 : ils lui ont intégré des plasmides TOL possédant des gènes C23O (cathécol 2,3-dioxygénase) de dégradation de l'hydroxybenzène. Après avoir inoculé cette souche dans le sol d'un champ de tomates contaminé à l'hydroxybenzène, ils ont non seulement remarqué que la souche TP13 avait été capable de de coloniser la rhizosphère de tomates, mais aussi que le nombre de bactéries contenant les plasmides TOL avec les gènes C23O avait augmenté graduellement. De plus, ils ont observé une augmentation du taux de dégradation de l'hydroxybenzène. Wang, Jiang et al. (2014) ont alors confirmé que les plasmides possédant les propriétés biodégradantes avaient bien été transférés au sein de la communauté indigène, et que le transfert horizontal de ces plasmides avait stimulé la dégradation de l'hydroxybenzène et la croissance de la plante dans le champ contaminé après 20 jours. Des résultats similaires ont également été observés par Ling, Sun et al. (2015) en inoculant des souches de *Sphingobacterium* sp. D-6 hébergeant des gènes de dégradation du DDT (dichlorodiphényltrichloroéthane), un hydrocarbure aromatique chloré, sur des plasmides pDOD. Le transfert latéral de gènes de dégradation entre des organismes modifiés et les souches sauvages permet ainsi d'acquérir de nouvelles fonctions de dégradation.

Le forçage génétique, une des technologies les plus récentes et des plus fascinantes de modification génétique, peut être utilisée pour s'attaquer à des vecteurs de maladies ou pour propager des traits bénéfiques. Techniquement, le principe est d'introduire une séquence modifiée dans une séquence cible (Schmidt and de Lorenzo 2016). La séquence modifiée exprime une nucléase Cas9, et un ARN guide, permettant de couper la séquence cible. La coupure d'ADN pourra être réparée uniquement en recombinant la séquence d'ADN modifiée (Esvelt, Smidler et al. 2014). Le forçage génétique permet à des changements génomiques d'un donneur d'être copiés dans son partenaire à chaque génération et d'être propagés

exponentiellement à travers sa lignée. Le forçage génétique nécessite que l'agent actif soit diploïde. Bien que les bactéries soient haploïdes, certaines portions du génome acquièrent une diploïdie transitoire lorsque la cellule contient des éléments génétiques mobiles (tels que les plasmides) portant des segments de chromosome correspondants (Lorenzo, Marlière et al. 2016). Actuellement, la modification délibérée d'une population bactérienne avec CRISPR/Cas9 a principalement été réalisée pour supprimer des facteurs de virulence et la résistance aux antibiotiques portés par les plasmides (Bikard, Euler et al. 2014, Citorik, Mimee et al. 2014, Yosef, Manor et al. 2015). On peut facilement imaginer de modifier délibérément une population bactérienne entière avec la stratégie CRISPR/Cas9 pour la bioremédiation, en propageant des gènes de dégradation d'hydrocarbures (Lorenzo, Marlière et al. 2016).

Bien que l'utilisation des bactéries génétiquement modifiées (BGM) ait franchi une belle frontière avec de vastes implications futures, deux obstacles majeurs limitent la validation de cette technique. En effet, la modification d'une espèce ou une fonction biologique dans un écosystème complexe peut avoir des conséquences inattendues (Oye, Esvelt et al. 2014, Akbari, Bellen et al. 2015). De plus, du fait de la croissance non contrôlée des BGM et leur fort potentiel à propager de nouvelles informations génétiques aux microorganismes receveurs (surtout des gènes nuisibles, tels que la résistance aux antibiotiques ou la virulence), il est nécessaire de trouver des moyens afin de limiter l'expansion des bactéries modifiées ou reprogrammées.

Options de confinement pour les bactéries génétiquement modifiées

La capacité croissante de l'édition de génomes bactériens pour la bioremédation ouvre des opportunités biotechnologiques sans précédent. Cependant, les inquiétudes concernant les rejets accidentels ou délibérés dans le milieu naturel grandissent elles aussi. Les approches pour remédier à ces inquiétudes impliquent la mise en place de dispositifs de confinement des BGM pour arrêter à la fois le transfert latéral des gènes et la survie des BGM une fois leurs rôles remplis. Le confinement d'une BGM dans un cadre spatiotemporel peut être réalisé grâce à l'auxotrophie synthétique ou la création de circuits génétiques limitants (Schmidt and de Lorenzo 2016). Malgré l'avancée significative dans le domaine de la biologie synthétique, de sérieux obstacles doivent encore être surmontés.

L'auxotrophie synthétique, la première approche, permet de restreindre la croissance bactérienne à l'expression de gènes essentiels qui dépendent d'acides aminés synthétiques (Ravikumar and Liu 2015, Schmidt and de Lorenzo 2016). Par exemple, Lajoie, Rovner et al. (2013) ont remplacé tous les codons stop UAG par un autre codon stop UAA dans la souche d'*E.coli* MG1655. Cela a permis de recoder le codon UAG en le réassignant à un acide aminé non standard (NSAA), tout en évitant une incorporation délétère aux positions UAG initiales. La souche *E.coli* MG1655 est donc devenue auxotrophe synthétique, c'est-à-dire dépendante aux enzymes modifiées essentielles pour sa survie. De plus, étant donné que la conservation du code génétique permet aux bactéries de partager des gènes par transfert latéral, la modification du code génétique a effacé cette capacité de transfert. Une approche identique a été suivie par Rovner, Haimovich et al. (2015). Des codons TAG ont été intégrés dans 22 gènes essentiels, liant l'expression et de la fonctionnalité de ces gènes à l'incorporation d'acides aminés synthétiques dérivés de phénylalanine. Ces deux stratégies d'auxotrophie synthétique ne contenaient pas seulement des organismes modifiés, mais rendaient impossible le transfert latéral des gènes recodés tant que le code génétique n'était pas compris par d'autres hôtes non recodés (Mandell, Lajoie et al. 2015, Rovner, Haimovich et al. 2015). Dans ce cas, le confinement trophique est amplifié par le confinement sémantique (Rovner, Haimovich et al. 2015).

La seconde approche implique les circuits génétiques qui limitent la survie des BGM au-delà des niches physico-chimiques et/ou nutritionnelles. Typiquement, ces circuits génétiques impliquent un réseau plus ou moins complexe de facteurs de transcription, des promoteurs apparentés, des gènes de suicide cellulaire et antidotes, la réponse à un signal environnemental déterminant une existence continue ou une mort programmée (Ramos, Anderson et al. 1995, de Lorenzo 2010, Schmidt and de Lorenzo 2012). Malheureusement, les mutations et insertions de séquences spontanées finissent par détruire la connectivité et permettent à nouveau la prolifération des BGM (Schmidt and de Lorenzo 2016). La biologie synthétique a cependant permis de revitaliser cette approche et de la sophistiquer. Wright, Delmans et al. (2014) ont développé la stratégie *Gene Guard* qui rend des plasmides dépendants entre eux dans une souche bactérienne spécifique mais nuisible pour une autre. Les souches hôtes ne peuvent pas survivre sans le plasmide et le plasmide ne peut pas être pris par une autre bactérie. Un autre système de

confinement nommé *Dead Man*, développé par Chan, Lee et al. (2016), implique un circuit dans lequel deux répresseurs (TetR et LacI) inhibent la transcription l'un de l'autre. L'anhydrotetracycline est nécessaire pour l'expression de LacI, qui à son tour réprime l'expression d'une toxine et/ou active la transcription d'un gène essentiel. L'autre stratégie développé par Chan, Lee et al. (2016), *Pass Code*, permet à des protéines régulatrices chimériques de lier la même séquence ADN mais de répondre à différents signaux. De ce fait, ces protéines peuvent évaluer l'absence ou la présence de différents signaux environnementaux et ainsi exprimer ou non des gènes de suicide cellulaire.

Conclusion

Comme le séquençage de nouveaux métagénomes ne cesse de croitre, de nouveaux gènes létaux et éléments régulateurs deviennent disponibles pour construire des systèmes de suicides cellulaires encore plus complexes et augmenter l'efficacité de confinement du transfert latéral de gènes. Les modifications génétiques ne seront cependant jamais absolues, puisque des mutations mèneront inévitablement à l'apparition de sous-populations survivantes (García and Díaz 2014). Les approches de la biologie de synthèse sont prometteuses pour construire des « murs » qui permettent un haut niveau de confinement des organismes synthétiques et modifiés (Schmidt and de Lorenzo 2016). Les effets toxicologiques de l'introduction de composés synthétiques (biomolécules telles que les acides aminés synthétiques, etc) dans les êtres vivants ne sont pas non plus connus (Schmidt and Pei 2011). D'autres études sont alors requises pour évaluer les risques de l'implémentation de telles stratégies de biologie de synthèse pour limiter le confinement du transfert latéral de gènes de bactéries génétiquement modifiées pour la bioremédiation.

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Annexe

Les bactéries au secours de l'environnement

Fanny Rohrbacher

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Contribution des auteurs

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Les bactéries au secours de l'environnement

L'« or noir », autrefois abondant et abordable, a permis une prodigieuse croissance économique et a élevé le niveau de vie de nombreux pays, dont le Canada, au cours du siècle dernier. Or son utilisation a aussi entraîné des conséquences dévastatrices pour la planète. Afin de restaurer un équilibre environnemental, la dépollution des milieux contaminés aux hydrocarbures devient plus que nécessaire. La bioremédiation, c'est-à-dire l'utilisation d'organismes vivants, constitue maintenant une méthode privilégiée de décontamination. Cette solution « verte » est bien plus économique que les techniques traditionnelles de décontamination, telles que les traitements chimiques, l'excavation ou l'incinération.

La production industrielle d'hydrocarbures – autant que leur utilisation inappropriée, leur élimination incorrecte et les pertes accidentelles liées à leur extraction ou à leur transport – entraîne la pollution de nombreux environnements, et ce, depuis le 19^{e} siècle. Les marées noires figurent parmi les exemples les plus flagrants de pollution liée aux hydrocarbures. Celle survenue dans le golfe du Mexique en 2010 en raison de l'explosion de la plate-forme pétrolière Deepwater Horizon a été décrite comme le pire désastre environnemental aux États-Unis. Plus de 800 millions de litres de pétrole brut ont été déversés dans le golfe, causant des dégâts irréversibles sur la faune et la flore et menaçant plus d'une cinquantaine d'espèces marines, sans compter les oiseaux et animaux englués dans le pétrole sur les plages. À la suite de cette marée noire, les scientifiques et les pêcheurs ont remarqué des mutations et des difformités chez de nombreuses espèces marines (crevettes sans yeux, ou encore crabes à carapace molle et plus petits que la normale). Sur la terre ferme, les friches industrielles sont des exemples de contamination aux hydrocarbures moins connus, mais aux conséquences tout aussi importantes que les marées noires. Des déchets dangereux, tels que des hydrocarbures, y sont fréquemment oubliés ou cachés et deviennent une source durable de pollution des sols. En 2009, la société de technologies environnementales Ventix a réalisé une étude sur les friches industrielles à Montréal. Elle a ainsi évalué à 135 km^2 la superficie des friches industrielles contaminées dans la métropole, soit plus du tiers de l'île (Joncas 2013). Montréal a déjà été le plus important centre de raffinage au Canada, mais sur les six raffineries montréalaises existant il y a quelques dizaines d'années, quatre ont cessé leurs activités dans les années 1980, devenant des friches industrielles contaminées.

La présence d'hydrocarbures dans l'environnement non seulement nuit gravement à la pérennité des écosystèmes, mais constitue aussi un danger pour la santé publique du fait de leur toxicité ainsi que de leurs propriétés mutagènes et cancérigènes**.** Les hydrocarbures peuvent également s'accumuler dans la chaîne alimentaire, finissant par se retrouver dans les assiettes de chacun d'entre nous (Kim, Jahan et al. 2013). Parmi les effets toxiques des hydrocarbures, les troubles digestifs, respiratoires et neurologiques représentent les plus importants. Certains hydrocarbures, tels que le benzène, peuvent aussi affecter le développement d'un nourrisson, puisqu'ils sont présents dans le lait maternel d'une femme qui y est exposée ou traversent son placenta. La dépollution des milieux contaminés aux hydrocarbures, qu'ils soient marins ou terrestres, est donc devenue un enjeu écologique et sanitaire majeur.

Adaptation des bactéries à l'extrême

Depuis plus de 3,5 milliards d'années, les bactéries sont capables de s'adapter et de coloniser tous les types d'environnements jusqu'aux plus extrêmes, des eaux hypersalines de la mer Morte aux sources bouillantes et acides de Yellowstone, en passant par la désastreuse marée noire du golfe du Mexique et les friches industrielles montréalaises (Canganella and Wiegel 2011). Les bactéries sont capables de vivre dans ces milieux extrêmes parce qu'elles s'y adaptent rapidement. Cette adaptation repose sur ce qui pourrait paraître un superpouvoir, celui de s'échanger des gènes par simple contact (Soucy, Huang et al. 2015). En effet, les bactéries n'acquièrent pas seulement leurs gènes de leur mère, mais aussi de leurs sœurs, cousines et voisines. Ce phénomène, appelé *transfert latéral de gènes,* consiste principalement en la transmission de gènes portés par des plasmides (petites molécules circulaires d'ADN) entre une bactérie donneuse et une bactérie receveuse. Des bactéries aux propriétés physiologiques, aux structures cellulaires ou aux milieux de vie différents peuvent alors s'échanger des gènes pour améliorer leurs performances dans leur milieu actuel ou pour en envahir un nouveau (Gogarten, Doolittle et al. 2002). Bien que les plasmides ne soient pas essentiels à la survie de la bactérie, ils contiennent des gènes qui peuvent lui être grandement bénéfiques, les plus connus étant ceux de résistance aux antibiotiques, de virulence ou de biodégradation de contaminants tels que les hydrocarbures (de la Cruz and Davies 2000, Nojiri, Shintani et al. 2004, Obayori and Salam 2010).

Hydrocarbures au menu

Grâce à leur pouvoir adaptatif, les bactéries indigènes (initialement présentes dans un milieu) exposées à long terme à une contamination finissent par développer la capacité de dégrader les contaminants. Pour elles, les hydrocarbures constituent une source de carbone et d'énergie pour leur respiration et leur croissance, ce qui leur permet de dégrader les hydrocarbures, des plus simples aux plus complexes, puis de les assimiler (Das and Chandran 2011). Dans un milieu contaminé, une bactérie est rarement équipée de tous les mécanismes appropriés pour dégrader un hydrocarbure. Une dégradation totale devient donc possible grâce à un ensemble de bactéries possédant des mécanismes de dégradation variés. La dégradation aérobie (en présence d'oxygène) représente la voie la plus rapide et la plus complète de dégradation des hydrocarbures (Rohrbacher and St-Arnaud 2016). De nombreux sites contaminés non oxygénés, tels que les puits de pétrole ou les sols profonds des friches industrielles, se révèlent à priori peu favorables au développement de la vie. Pourtant, ces milieux abritent eux aussi une communauté bactérienne riche et variée, qui, en l'absence d'oxygène, est malgré tout capable de dégrader les hydrocarbures. Même si cette dégradation anaérobie n'est pas aussi efficace que la dégradation aérobie, l'intérêt grandit pour son utilisation dans la dépollution de sites contaminés.

Biodégradation accélérée

Bien que la bioremédiation constitue la meilleure solution à la décontamination de sites pollués aux hydrocarbures, elle est relativement lente et peut durer plusieurs années. De nombreuses stratégies visent alors à l'accélérer. Parmi elles, l'ajout de dispersant permet au pétrole, qui devrait normalement remonter à la surface de l'eau, d'être émulsionné en de minuscules gouttelettes et de rester en suspension dans l'eau. Ainsi, ces gouttelettes peuvent être attrapées plus facilement par les bactéries indigènes, qui les dégradent. Cette stratégie a été utilisée pour la bioremédiation de la marée du golfe du Mexique. Cependant, le fait de disperser les hydrocarbures entraîne temporairement et localement une forte augmentation de leur toxicité, jusqu'à ce que le pétrole dispersé se dissémine dans un vaste volume d'eau et soit dégradé par les bactéries indigènes (Rico-Martínez, Snell et al. 2013). Cet effet implique une certaine limitation de l'usage de la dispersion près des côtes et des zones sensibles ou lorsque les conditions de dilution semblent défavorables.

La bioaugmentation, quant à elle, consiste à enrichir un milieu pollué aquatique ou terrestre avec une ou plusieurs espèces bactériennes. Cette approche est utilisée lorsque les bactéries indigènes ne

sont pas capables de dégrader les hydrocarbures parce qu'elles se trouvent dans des conditions stressantes de contamination soudaine. Bien que la bioaugmentation de bactéries exogènes (étrangères) ait été appliquée avec succès dans le nettoyage de sites contaminés, de nombreux facteurs influencent son efficacité au fil du temps ainsi que la survie des souches microbiennes introduites dans le sol ou l'eau. Non seulement les caractéristiques mêmes du milieu (telles que la température, le type de sol, la concentration en oxygène) ont une influence considérable sur son efficacité, mais la compétition entre bactéries exogènes et indigènes explique son échec à long terme (Thompson, Van Der Gast et al. 2005). En effet, les bactéries exogènes, habituées à croître en laboratoire dans des conditions optimales de croissance, entrent en compétition avec les indigènes, souvent beaucoup plus robustes et parfaitement adaptées aux conditions environnementales du milieu contaminé. La population exogène ensemencée finit donc par s'affaiblir et disparaître, son pouvoir biodégradant envolé en même temps qu'elle.

Bactéries génétiquement modifiées

Actuellement, les chercheurs s'intéressent à modifier génétiquement les bactéries afin de pallier ce phénomène de compétition. Les bactéries étant capables de s'échanger des plasmides, l'intégration des gènes de dégradation d'hydrocarbures dans les plasmides des bactéries donneuses semble la solution tout indiquée. Ces bactéries donneuses peuvent alors transférer leurs gènes de dégradation aux bactéries indigènes receveuses. Ainsi, la disparition des bactéries donneuses importe peu, puisque les plasmides possédant les gènes de dégradation ont été transférés aux bactéries receveuses et perdurent donc dans le milieu (Wang, Jiang et al. 2014). Cependant, la croissance non contrôlée de bactéries génétiquement modifiées et leur fort potentiel de propager de nouvelles informations génétiques aux bactéries receveuses représentent des obstacles majeurs à la validation de cette technique (Benjamin, de Lima et al. 2015). En effet, la modification génétique des bactéries peut s'avérer dangereuse si elle n'est pas pleinement maîtrisée. Des bactéries nuisibles pourraient alors prendre de l'ampleur dans un écosystème et entraîner des conséquences dévastatrices pour la faune et la flore indigènes. Créer une nouvelle bactérie « kamikaze », c'est-à-dire contenant des gènes de suicide cellulaire, pourrait pallier cet inconvénient (Paul, Pandey et al. 2005). Cette bactérie serait non seulement capable de dégrader les hydrocarbures, mais aussi de contrôler sa croissance et son transfert latéral de gènes. L'apparition de traits génétiques indésirables, comme la résistance aux antibiotiques ou la virulence, serait alors évitée.

Bien que les chercheurs soient encore dans une phase de recherche et d'expérimentation, l'utilisation de bactéries génétiquement modifiées a permis de franchir une frontière importante dans la bioremédiation de sites contaminés aux hydrocarbures.