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Frankia as a Biodegrading Agent

Medhat Rehan, Erik Swanson and Louis S. Tisa

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Abstract

The *Frankia* actinorhizal plant symbiosis plays an important role in colonization of soils contaminated with toxic aromatic hydrocarbons. Our understanding of the bacterial partner, *Frankia*, in the actinorhizal symbiosis has been greatly facilitated by the availability of sequenced genomes. The analysis of these *Frankia* genomes has suggested that these bacteria are metabolically diverse and have potential for toxic aromatic hydrocarbon degradation. In this chapter, we explore what is known about that metabolic potential.

Keywords: *Frankias*-triazines, aromatic hydrocarbon degradation, PAH, bioremediation, bioinformatics, actinobacteria

1. Introduction

Frankia are filamentous nitrogen-fixing Gram-positive actinobacteria that are found as free-living microbes in the soil and in symbiotic associations with actinorhizal plants [1-5]. These bacteria fix nitrogen by converting atmospheric N₂ into biologically useful ammonia and supply the host plants with a source of reduced nitrogen. *Frankia* are developmentally complex and form three cell types: vegetative hyphae, spores located in sporangia, and vesicles. Hyphae are septate structures and form the growing state of this microbe. Under appropriate conditions, either terminal or intercalary multilocular sporangia are produced and contain many spores. When mature, the spores are released from the sporangia. The spores are presumed to aid in the survival and dispersal of *Frankia* in the environment. Vesicles are produced under nitrogen-limited conditions and consist of unique lipid-enveloped cellular structures that contain the enzymes responsible for nitrogen fixation. Thus, vesicles act as specialized structures for the nitrogen fixation process. *Frankia* are able to establish symbiotic nitrogen-

fixing associations with over 220 species of woody dicotyledonous plants, termed actinorhizal plants, that are found in eight families of angiosperms [1, 3-6]. The symbiosis with *Frankia* allows these actinorhizal host plants to colonize nutrient-poor soil and harsh environments. Actinorhizal plants have been used to recolonize and reclaim industrial wastelands and environments contaminated with heavy metals and toxic aromatic hydrocarbon [7-15]. The metabolic potential of these bacteria has only recently been investigated in the context of bioremediation [16-18].

1.1. *Frankia* genomics and identification of metabolic potential

Based on phylogenetic analysis, *Frankia* strains have been classified into four main lineages [19-23]. Members of lineage 1 are found infective on host plants of the Betulaceae (*Alnus*), Myricaceae, and Casuarinaceae families, while lineage 2 represents strains that are infective on Rosaceae (*Dryas*, etc.), Coriariaceae (*Coriaria*), Datisceae (*Datisca*), and the genus *Ceanothus* (Rhamnaceae). Members of lineage 3 are the most promiscuous and are infective on Eleagnaceae, Rhamnaceae, Myricaceae, *Gymnostoma*, and occasionally *Alnus*. The fourth *Frankia* lineage consists of the “atypical” strains which are unable to reinfect actinorhizal host plants or form ineffective root nodule structures that are unable to fix nitrogen. Our understanding of this genus has been greatly enhanced by the sequencing of several *Frankia* genomes from the different *Frankia* lineages [24-33]. Analysis of *Frankia* genomes has revealed new potential with respect to metabolic diversity, natural product biosynthesis, and stress tolerance, which may help aid the cosmopolitan nature of the actinorhizal symbiosis [31, 34].

In this chapter, we will describe what is known about the degradation properties of these bacteria.

2. Rhizodegradation

Among bacteria with bioremediation potential, *Frankia* are unique in that these bacteria form a symbiosis with actinorhizal plants. The implications of this trait for bioremediation efforts have only recently been explored. In the context of bioremediation, the most extensively studied system is the *Frankia*–*Alnus* association. Diverse assemblages of free-living *Frankia* strains are present in soils with polyaromatic hydrocarbon (PAH) contamination [8-10, 15, 35-38]. These *Frankia* strains readily form symbioses with alders, resulting in greatly increased alder fitness in harsh environments. The *Frankia*–alder symbiosis also increases the mineralization of representative organic pollutants in oil-sands reclamation sites. The *Frankia*–alder symbiosis has been used in reclamation projects because of these traits [5, 8, 36-38]. Free-living *Frankia* also appears to be part of natural degradation communities. Specifically, *Frankia* has been found to be one of the most abundant genera in wastewater treatment communities [35]. Based on these findings, *Frankia* appears to be an underutilized tool in holistic remediation approaches.

3. S- triazines degradation

3.1. Overview

Triazines are a class of herbicides composed of a heterocyclic six-membered ring with alternating carbon and nitrogen atoms joined by double bonds. These herbicides have been used extensively for control of broadleaf and grassy weeds in corn, sorghum, and sugarcane cultivation. Atrazine and simazine are the most ubiquitous members of the s-triazine family. Biodegradation of atrazine is a complex process and depends on the nature and amount of atrazine in soil or water [39-41]. There are four major steps in atrazine degradation: hydrolysis, dealkylation, deamination, and ring cleavage. For the hydrolysis step, an amidohydrolase enzyme (AtzA) cleaves the carbon-chlorine (C-Cl) bond and thus dechlorinates atrazine to hydroxylatrazine. This intermediate is dealkylated and deaminated at the ethyl and isopropyl groups by the amidohydrolase enzymes, AtzB and AtzC, to produce cyanuric acid. This product is converted to ammonia and carbon dioxide by the AtzD, AtzE, and AtzF enzymes [42-44].

3.2. S-triazine degradation pathway in *Frankia*

In *Frankia*, the first two steps in atrazine degradation have been identified as well as the regulation of their gene expression [17]. The mineralization of atrazine to ammonia and carbon dioxide is generally initiated by hydrolytic dechlorination, catalyzed by the enzyme atrazine chlorohydrolase (AtzA). Alternatively, this reaction is catalyzed by another atrazine chlorohydrolase (TrzN), which is also able to use atrazine derivatives including desethyl-desisopropylatrazine as substrates. Analysis of the *Frankia* genomes identified candidate genes for the atrazine degradation pathway (Figure 1). The *trzN* gene was identified in *Frankia alni* ACN14a (FRAAL1474) and *Frankia* sp EuI1c (FraEuI1c_5874) genomes and its amidohydrolase gene product is predicted to remove chlorine from s-triazine compounds to produce hydroxyatrazine or ammeline from atrazine and desethyl desisopropyl atrazine, respectively. Furthermore, a putative *atzB* gene was also identified in both *Frankia* genomes (FRAAL1473 and FraEuI1c_5875) whose predicted gene product, adenosine aminohydrolase 3, is involved in the dealkylation reaction of the *N*-ethyl group from hydroxyatrazine transforming it into *N*-isopropylammelide. Physiological studies showed that *Frankia* ACN14a and EuI1c cultures are able to break down atrazine and desethyl-desisopropylatrazine producing the end products hydroxyatrazine and *N*-isopropylammelide. Although the enzymes were not purified, these data clearly showed metabolism of atrazine. Analysis of gene expression in *Frankia* ACN14a found that the two genes, *trzN* (FRAAL1474) and *atzB* (FRAAL1473) are under control of the *atzR* (FRAAL1471) gene, which encodes a predicted LysR-type transcriptional regulator.

Bioinformatics analysis of the *Frankia* genomes revealed a potential full pathway for atrazine degradation in the *Frankia* sp EuI1c genome (Figure 2). The *atzC* (FraEuI1c_4724) gene, which encodes a putative amidohydrolase enzyme, was identified and is predicted to be involved in the dealkylation of the *N*-isopropyl group from atrazine to produce cyanuric acid. With other bacterial systems, cyanuric acid is hydrolyzed to ammonium and carbon dioxide via the *atzDEF* operon [43, 45]. In *Frankia* EuI1c, the *atzD* (FraEuI1c_3137) gene product is predicted

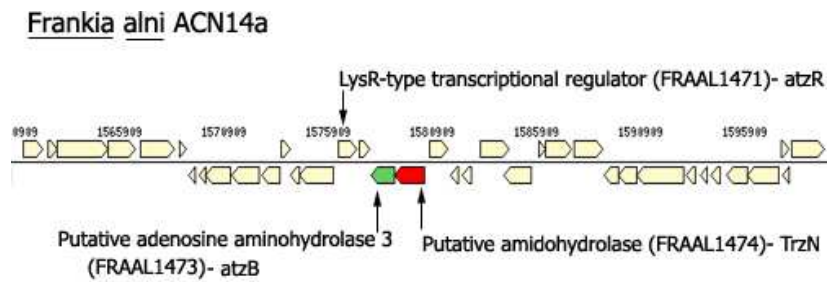


Figure 1. Gene cluster organization in *Frankia alni* ACN14a for atrazine degradation. The cluster contains a putative *trzN* (FRAAL1474), putative *atzB* (FRAAL1473), and putative LysR-family transcriptional (*atzR*).

to transform cyanuric acid into carboxybiuret, which spontaneously decarboxylates to biuret. Putative *atzE* (FraEu1c_1007 and 1008), and *atzF* (FraEu1c_3831) genes were also identified in the *Frankia* Eu1c genome and their gene products expected to complete *s*-triazine mineralization by converting biuret to allophanate and ammonia plus carbon dioxide. A *trzR* (FraEu1c_3136) gene, which encodes a GntR family transcriptional regulator, is found before the *atzD* gene and is involved in the expression of that gene (Rehan unpublished).

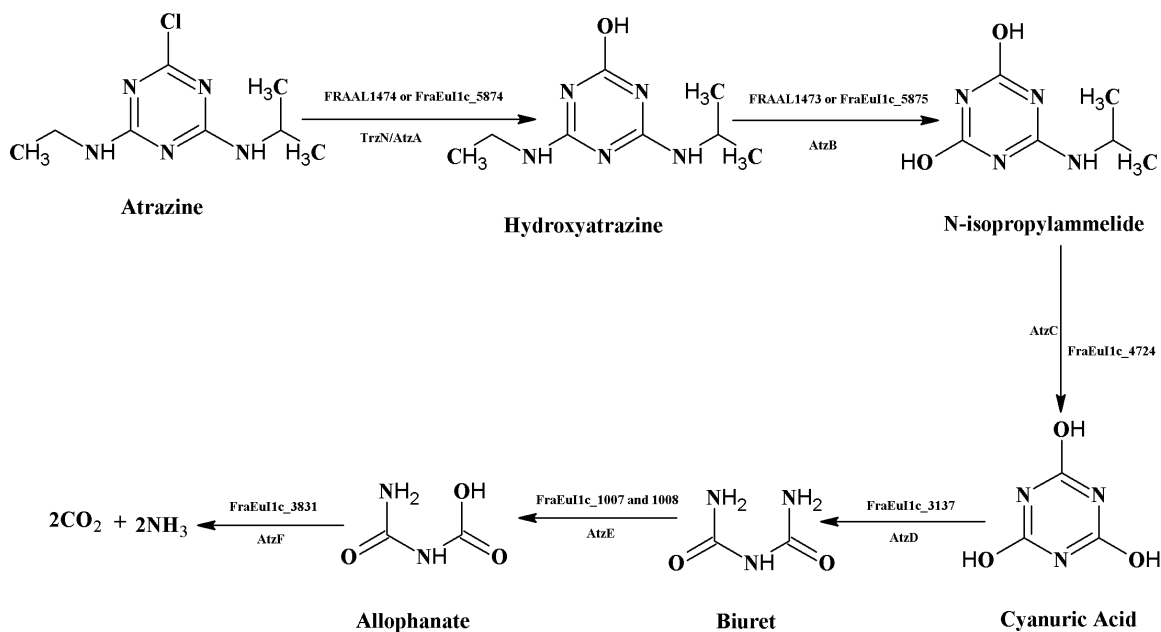


Figure 2. The atrazine degradation steps in *Frankia* strains Eu1c and ACN14a include atrazine dechlorination and dealkylation and ring cleavage by TrzN, *atzB*, and *atzD* enzymes.

4. Aromatic compounds degradation

4.1. Biphenyl and polychlorinated biphenyl

Biphenyls and polychlorinated biphenyls (PCBs) are some of the most recalcitrant xenobiotics found in the environment. The degree of chlorination differs greatly among the PCBs, ranging

from 1 to 10, as does their position on the carbon atoms. Since the mid-1980s, the use of PCBs has been phased out in many countries. However, due to their toxicity, persistence in the environment, and potential carcinogenicity, they are still a major global environmental problem [46-48].

Bacteria degrade biphenyl and PCBs via the *meta*-cleavage pathway, which is encoded by the *bph* operon, and produces tricarboxylic acid and chlorobenzoate (CBA) as intermediates [47-50]. The first enzyme in this pathway is biphenyl dioxygenase, which is a multimeric complex consisting of the large α and small β subunits, and the ferredoxine and ferredoxine reductase subunits. The degradation process is initiated by biphenyl dioxygenase which incorporates two oxygen atoms at the 2 and 3 carbon positions of the aromatic ring (called 2,3-dioxygenation) to generate hydroxyl groups. For PCBs degradation, biphenyl dioxygenase catalyzes the initial 2,3-dioxygenation, and dihydrodiol dehydrogenase converts the product into 2,3-dihydroxybiphenyl. The enzyme, 2,3 dihydroxybiphenyl dioxygenase, cleaves the dihydroxylated ring to produce (chlorinated) 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA). A hydrolase enzyme then hydrolyzes HOPDA to (chlorinated) benzoic acid and 2-hydroxypent a-2,4-dienoate.

4.1.1. Biphenyl degradation pathway in Frankia

At least four *Frankia* strains (ACN14a, CcI3, EUN1f, and EuI1c) are resistant to biphenyl and polychlorinated biphenyl (PCB) at concentrations up to 5mM [51, Swanson unpublished results]. Data mining for known organisms capable of biphenyl degradation [46, 52] and the availability of a *Frankia* genome database enabled the identification of genes potentially involved in biphenyl degradation in several of the *Frankia* strains listed above. Five genes were identified that encode enzymes involved in biphenyl degradation: the alpha and beta subunits of the aromatic-ring-hydroxylating dioxygenase, a Rieske (2Fe-2S) iron-sulfur domain protein, an alpha/beta hydrolase fold protein, and a short-chain dehydrogenase/reductase (SDR). These enzymes are putatively capable of oxidizing and hydroxylating benzene rings, and are also known as the upper meta-cleavage pathway. A lower pathway of aromatic ring degradation consisting of three genes (encoding the 2-hydroxypenta-2,4-dienoate hydratase; acylating acetaldehyde dehydrogenase; and 4-hydroxy-2-oxovalerate aldolase) is located downstream of this operon [53, Swanson and Tisa unpublished data]. Figure [3] shows the gene neighborhood of the Biphenyl degradation genes. These genes were also found in *Frankia* strain EUN1f and Dg1 genomes (Swanson and Tisa unpublished). Both the meta-cleavage upper and the lower pathways are commonly referred to as the *bph* operon in several other PCB-degrading bacteria. *Rhodococcus* RAH1, a species closely related to *Frankia*, utilizes *bph* genes homologous to those found in *Frankia* to metabolize PCBs as a sole carbon and energy source [54]. Since at least two genes (Aromatic-ring-hydroxylating dioxygenase, subunit alpha-like protein (FraEuI1c_4097) and short-chain dehydrogenase/reductase (FraEuI1c_4101) in the *bph* operon in *Frankia* are upregulated in the presence of biphenyl, it is likely that *Frankia* also uses the *bph* operon to metabolize biphenyl and PCBs (Rehan and Tisa unpublished)

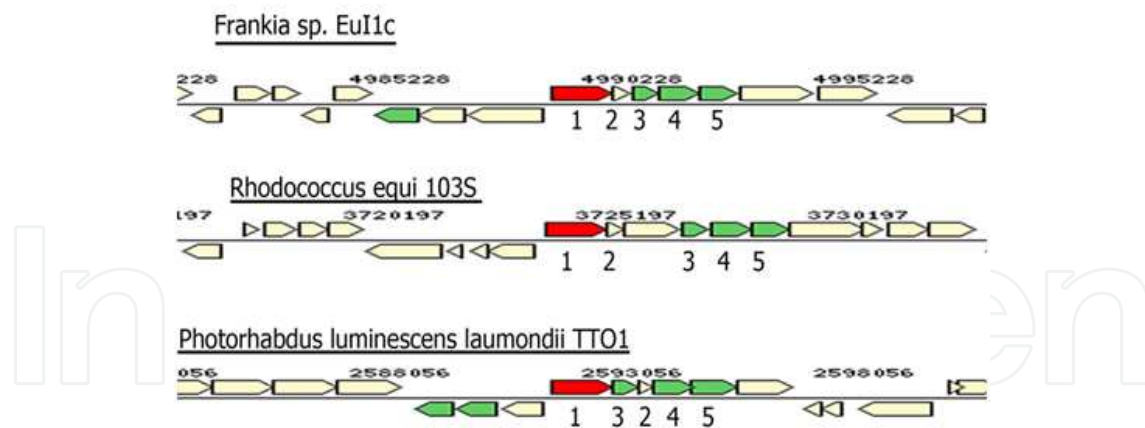


Figure 3. The gene neighborhood of *bph* operon in *Frankia* Eu11c in comparison to *Rhodococcus equi* 103S and *Photorhabdus luminescens laumondii* TTO1 operon. (1) Aromatic-ring-hydroxylating dioxygenase, subunit alpha. (2) Rieske (2Fe-2S) iron-sulfur domain protein. (3) Aromatic-ring-hydroxylating dioxygenase, subunit beta. (4) Alpha/beta hydrolase fold protein. (5) Short-chain dehydrogenase/reductase SDR.

4.2. Phenol degradation

4.2.1. Overview

Phenol (or hydroxybenzene) consists of a benzene ring substituted with a hydroxyl group. Derivatives of this molecule are colloquially known as phenolic compounds. Phenolic compounds are ubiquitous chemicals with diverse properties and uses. The simplest phenolic compound, phenol, is widely used in oil and coal processing, tinctorial and metallurgic industries, and many other industrial applications. Phenol also enters the environment via vehicle exhaust and as the product of natural metabolic processes, and chlorophenols are widely used as biocides in agricultural applications [for a review see 55]. While anthropogenic phenolics are often hazardous, natural phenolic compounds are mostly harmless in the concentrations that are found in foods such as coffee and tea, and some are used as antibiotics [56, 57]. However, the toxicity of some phenolics, particularly phenol and chlorinated phenols, has prompted considerable research activity devoted to phenol remediation. Acute and chronic exposure to phenol and chlorophenol has serious health effects. Phenol and chlorophenol cause lipid peroxidation which ultimately leads to tissue necrosis, and liver and kidney damage [58]. Additionally, chlorophenol exposure is associated with elevated risks of cancer, immune deficiencies, and teratogenic effects [59-61].

4.2.2. General phenol degradation pathway

One of the most promising techniques for removing anthropogenic phenolics from the environment is bioremediation. As was the case for many compounds, the degradation pathway for phenol was first elucidated in a *Pseudomonas* strain [62]. Most bacteria degrade phenolics using catechol catabolic enzymes, most importantly catechol-2,3-dioxygenase. Phenols are first hydroxylated to form catechol, and then catechol-2,3-dioxygenase cleaves the

benzene ring at the meta position [62]. Therefore, the degradation pathway that begins with catechol-2,3-dioxygenase is called the meta pathway (Figure 4). While the meta pathway is most prevalent, degradation can also begin with cleavage at the para or ortho position using catechol-1,2-oxygenase [63-65]. After ring cleavage, 2-hydroxymuconic semialdehyde hydrolase catalyzes a decarboxylation reaction yielding 4-oxalocrotonate. 4-oxalocrotonate is hydrated by 2-oxopent-4-enoate hydratase to form 4-hydroxy-2-oxovalerate. 4-hydroxy-2-oxovalerate aldolase then splits 4-hydroxy-2-oxovalerate into pyruvate and acetaldehyde, which can then be incorporated into the central metabolic pathways [62].

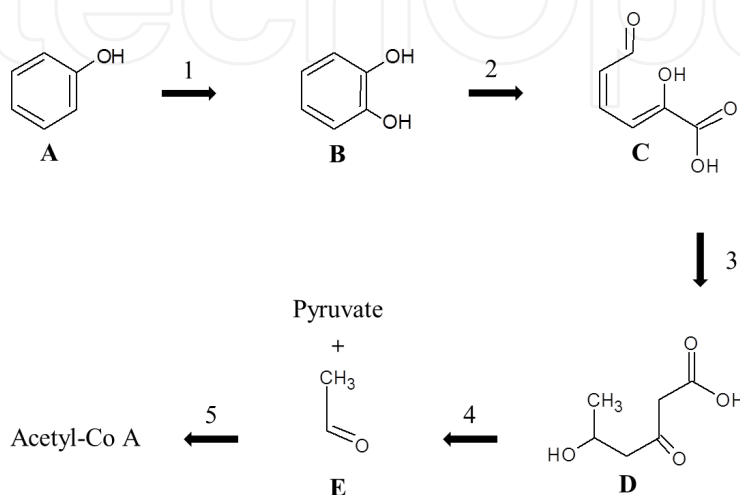


Figure 4. General phenol degradation pathway.

4.2.3. Phenolic compounds and Frankia

Frankia spp. both produce and are affected by phenolic compounds. However, it is unclear whether *Frankia* may degrade phenol and other phenolic compounds. The response of *Frankia* to phenolics was first studied in the context of plant-microbe interactions. Despite apparent functional and morphological similarities between *Frankia* nodules and leguminous nodules, the molecular and physiological mechanisms that control nodulation are distinct. Therefore, the unique process of nodulation by *Frankia* is still an area of intense research. *Alnus* spp. (Alders) plants are a major host plant for *Frankia*, and also have unusually high levels of phenolics in their root exudates, which affect the growth of *Frankia*. Most *Alnus* phenolics tested inhibit *Frankia* growth to varying degrees [66, 67]. Specifically, benzoic acids are less inhibitory than cinnamic acids such as caffeinic acid. However, one plant phenolic, o-hydroxyphenylacetic acid, promoted *Frankia* growth, and both benzoic and cinnamic acids caused increased branching of *Frankia* hyphae. Low concentration plant phenolics also mediate a global shift in *Frankia* gene expression, while higher concentrations (above 30 mg L⁻¹) simply inhibit biosynthesis [33]. Interestingly, *Frankia* also increases phenolic expression of their host plant, causing them to produce more phenol, flavonoids, and hydroxycinnamic acid [68].

Frankia may promote excretion of phenolics as a way to increase available nutrients. However, this explanation depends on *Frankia* having the ability to degrade phenolic compounds. While

no study has demonstrated that *Frankia* degrades phenolic compounds, there is genetic evidence that this bacterium may have the ability to degrade phenolics. First, some *Frankia* strains have genes coding for the production of catechol and other phenolic compounds [34]. Because bacteria often salvage the biomolecules they produce, the presence of an anabolic pathway suggests that a catabolic pathway is also present [69]. Furthermore, multiple *Frankia* strains contain catechol-2, 3-dioxygenase, the most important enzyme in the phenol degradation pathway (Swanson and Tisa unpublished data) [64]. A closely related bacterium, *Rhodococcus* spp., uses the catechol-2,3-dioxygenase pathway to grow with phenol as its sole carbon source [70]. The same species is also able to break down the more recalcitrant pentachlorophenol via the para pathway [71]. This suggests that *Frankia* may break down phenol, a trait that could be applied in bioremediation efforts. Several *Frankia* strains are able to grow on phenol, quercetin, catechol, and other phenolic compounds (Furnholm, Greenleaf, and Tisa unpublished data), but the metabolism of their breakdown has not been studied.

4.3. Naphthalene degradation

4.3.1. Overview

Naphthalene is a ubiquitous polycyclic aromatic hydrocarbon composed of two benzene rings joined at the 9 and 10 carbons (Figure 5). Naphthalene is produced by distilling and crystallizing coal tar, and also as by-product of fossil fuel combustion and cigarette smoke [72]. Naphthalene is used in a number of industrial applications including as feed stock for the production of plastics and resins, and as a component of creosote-based wood preservatives. Naphthalene is also used in tincture and leather tanning industries [72]. Unlike many organic pollutants, naphthalene does not bioaccumulate. Instead, naphthalene is metabolized and excreted in the urine of rats and humans [72, 73]. Nonetheless, naphthalene is a problematic pollutant with numerous toxic effects. Acute exposure to naphthalene causes hemolytic anemia, and liver and neurological damage [74]. Chronic naphthalene exposure is associated with elevated cancer risk [75, 76]. The toxicity of naphthalene and its prevalence as a pollutant has spurred research on remediation techniques, including bioremediation and biodegradation.

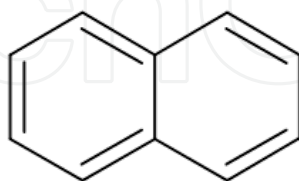


Figure 5. Structure of naphthalene.

4.3.2. Degradation pathway

The naphthalene biodegradation pathway was first studied in a strain of *Pseudomonas* which has two related naphthalene degradation pathways. The upper pathway catabolizes naph-

thalene to produce salicylate and a molecule of pyruvate [77]. The lower pathway breaks salicylate down into acetyl Co-A and pyruvate [78]. The first step of the upper pathway is catalyzed by four proteins: naphthalene dioxygenase reductase, naphthalene dioxygenase ferredoxin, and naphthalene dioxygenase Fe-S protein small and large subunits. This collection of enzymes oxidizes naphthalene to produce *cis*-naphthalene dihydrodiol, which is subsequently dehydrogenated by naphthalene *cis*-dihydrodiol dehydrogenase to form 1,2-dihydroxynaphthalene. 1,2-dihydroxynaphthalene dioxygenase then produces 2-hydroxychromene-2-carboxylate which is then cleaved by 2-hydroxychromene-2-carboxylate dehydrogenase to form *cis*-*o*-hydroxybenzylpyruvate. 1,2-dihydroxybenzylpyruvate aldolase then splits *cis*-*o*-hydroxybenzylpyruvate producing pyruvate and salicylaldehyde. Finally, salicylaldehyde dehydrogenase carboxylates salicylaldehyde to form salicylate [77, 78].

In the lower pathway, salicylate hydroxylase hydroxylates salicylate to produce catechol. The remaining benzene ring is then cleaved by catechol-2,3-dioxygenase to produce 2-hydroxymuconic semialdehyde [78]. Hydroxymuconic semialdehyde dehydrogenase then produces 2-hydroxyhexa-2,4-diene-1,6-dioate which is subsequently isomerized by 4-oxalocrotonate isomerase to produce 2-oxohexa-3-ene-1,6-dioate. This is then transformed into 2-oxopent-4-enoate by 4-oxalocrotonate decarboxylase. 2-oxopent-4-enole hydratase produces 4-hydroxy-2-oxovalerate, which is subsequently split into acetaldehyde and pyruvate by 2-oxo-4-hydroxypentanoate aldolase. Finally, acetaldehyde dehydrogenase converts acetaldehyde into acetyl Co-A [78]. Both of these pathways are also found in *Rhodococcus* spp, a close relative of *Frankia* [79].

4.3.3. Naphthalene degradation in *Frankia*

Not surprisingly, *Frankia* also metabolizes naphthalene as a sole carbon and energy source via a related pathway [18]. Specifically, *Frankia* uses the protocatechuate pathway to convert naphthalene or a naphthalene derivative into acetyl Co-A and succinyl Co-A (Figure 6) [18]. This finding confirms the role of *Frankia* in naphthalene degradation, which was suggested by earlier field studies [8-10, 37, 38]. In symbiosis with alders, *Frankia* increases polyaromatic hydrocarbon degradation in oil-sand tailings for the first 1.5 years [8, 10, 37]. However, after 2.5 years, alders without *Frankia* symbionts demonstrated naphthalene degradation equal to the degradation of *Frankia*-inoculated alders [8]. The *Frankia*-alder symbiosis thrives in PAH-contaminated areas [15]. Interestingly, alder plants found in these PAH-contaminated areas maintained a symbiosis with *Frankia* lineage III as opposed to the normal lineage I, suggesting that this pollutant affected nodulation and/or survival of the actinorhizal plants. Taken together, these findings indicate that *Frankia* could be a useful tool in naphthalene remediation.

4.4. Protocatechuate

4.4.1. Overview

Under oxic conditions, microbial degradation of many aromatic compounds occurs through the catechol or protocatechuate branch of the β -keto adipate pathway via either *ortho* cleavage

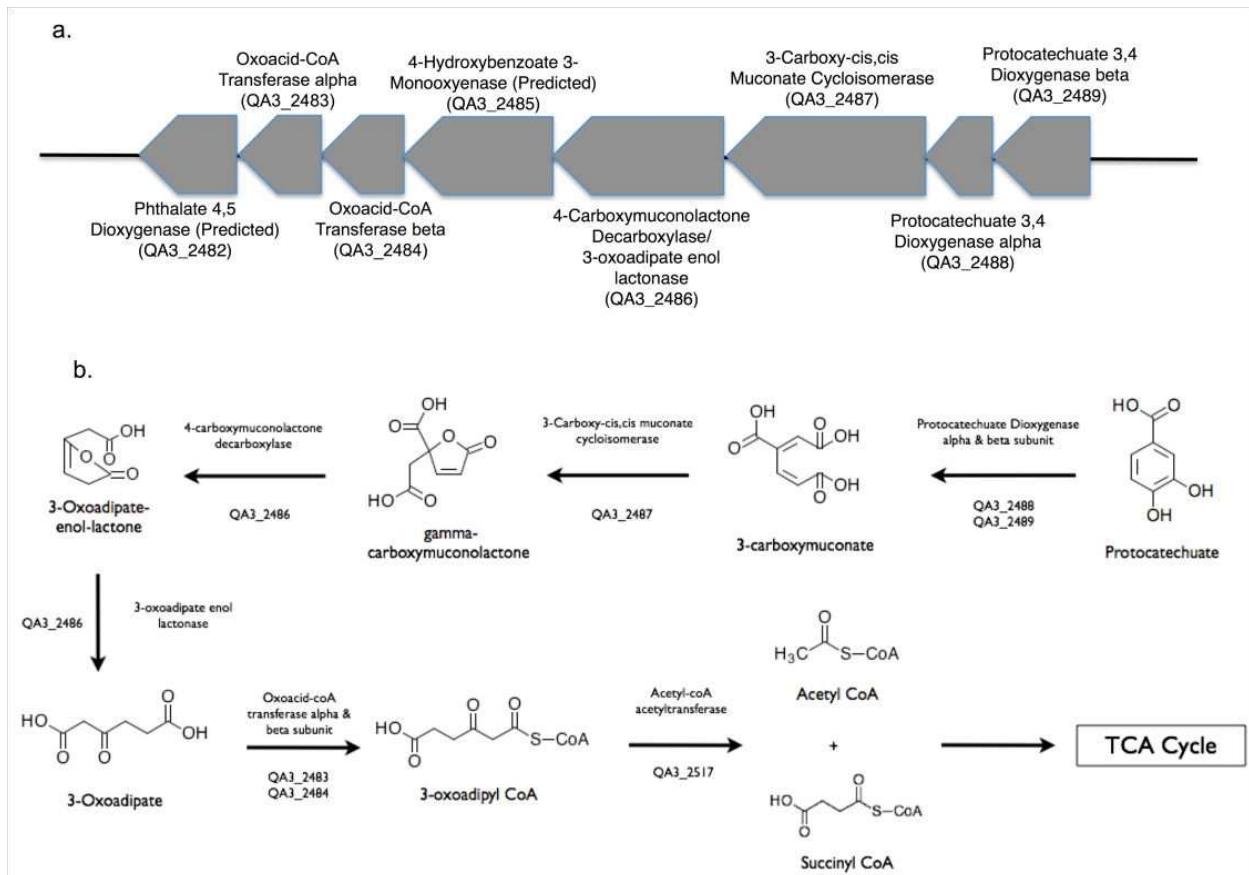


Figure 6. Putative naphthalene degradation pathway in *Frankia* [18]. (Figure is recopied with permission from *Canadian Journal of Microbiology*.)

by catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase or *meta*-cleavage by catechol-2,3-dioxygenase and protocatechuate-4,5-dioxygenase.

4.4.2. Potential protocatechuate degradation pathway in *Frankia*

Besides the protocatechuate pathway found in *Frankia* QA3 [18], several other potential protocatechuate pathways have been identified from bioinformatics analysis of the available *Frankia* genomes. In *Frankia* Eu1c, a potential operon (*FraEu1c_2560* -to- *FraEu1c_2564*) for a putative protocatechuate pathway was identified (Figure 7). This operon encodes the predicted gene products involved in the putative pathway including protocatechuate 3,4-dioxygenase alpha and beta subunits, fumarate lyase, 3-oxoadipate enol-lactonase, and 4-hydroxybenzoate 3-monooxygenase. These gene products are similar to the protocatechuate degradation pathway found in *Rhodococcus opacus* 1CP [80, 81]. These results suggest that *Frankia* may use the protocatechuate degradation pathway to degrade many aromatic ring compounds after their conversion to protocatechuate.

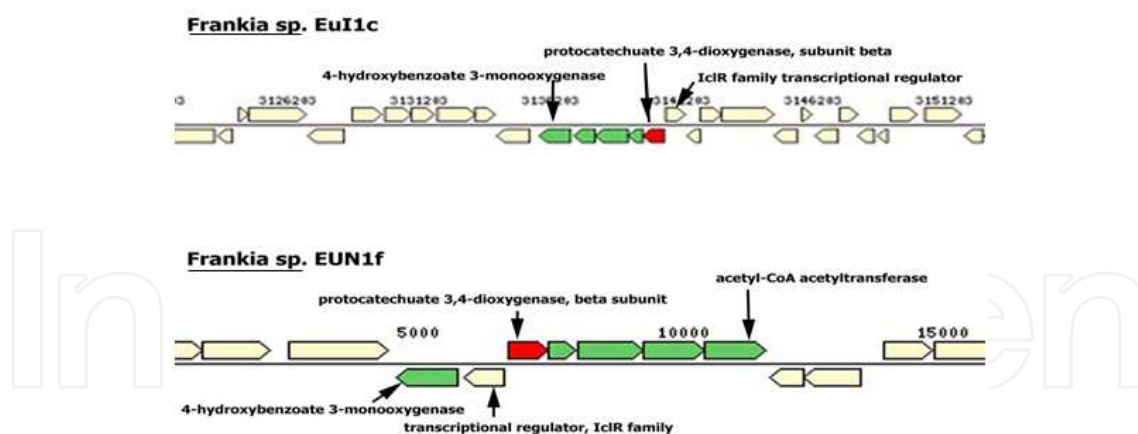


Figure 7. The proposed protocatechuate degradation pathway in *Frankia* strains Eu11c and EUN1f.

5. Hydrocarbons

5.1. Overview

Petroleum-based energy and products are used extensively around the world. The pervasiveness of petroleum inevitably leads to serious environmental pollution. Petroleum is a complex mixture of hydrocarbons, cycloalkanes, aromatic hydrocarbons, and more complex chemicals like asphaltenes. These chemicals and their derivatives, which are termed petrogenic compounds, are released into the environment as a result of oil spills and combustion of petroleum-based products [82]. Oil spills are one of the most serious sources of petroleum pollution and devastate aquatic and marine environments. Ongoing research to identify new methods for petroleum remediation is important because oil spills and other types of petroleum-derived pollution continue to pose environmental health risks.

Hydrocarbon-degrading bacteria and fungi are widely distributed in marine and freshwater environments, as well as soil habitats [83, 84]. In *Pseudomonas*, the alkane hydroxylase (monooxygenase) system consists of three components: alkane hydroxylase (AlkB), rubredoxin, and rubredoxin reductase. This system is responsible for the first oxidation step in the utilization of n-alkanes [85]. Similar alkane hydroxylase systems have been found in a variety of alkane-degrading bacteria [86, 87]. *Alcanivorax* sp. strain 2B5 will degrade C13–C30 n-alkanes and branched alkanes (pristine and phytane) from crude oil as the sole carbon source via a novel alkane hydroxylase gene (*alkB*). Other *Acinetobacter* are able to use n-alkanes with chain length C10–C40 as a sole source of carbon. In addition, the presence of multiple alkane hydroxylases in two *Rhodococcus* strains were characterized and both organisms contained at least four alkane monooxygenase gene homologs (*alkB1*, *alkB2*, *alkB3*, and *alkB4*) [76, 88].

A bioinformatics approach was used to identify these potential hydrocarbon degradation pathways among the sequenced *Frankia* strains. Functionally analyzed genes for the known hydrocarbon degradation pathways [84, 88] were used to probe the *Frankia* genome database

and identify potential pathways. Our preliminary results (Rehan unpublished data) revealed that the *F. alni* ACN14a genome possesses a putative alkane-1 monooxygenase (Alkane omega-hydroxylase) gene (*FRAAL1986*), which is one of the known enzymes involved in the breakdown of n-alkanes (Figure 8). Furthermore, a similar gene (*Franean1_2192*) was also found in the *Frankia* sp. EAN1pec genome. These bioinformatics results support the hypothesis that *Frankia* may be able to degrade oil-spill-derived hydrocarbons. However, these preliminary results need further study.

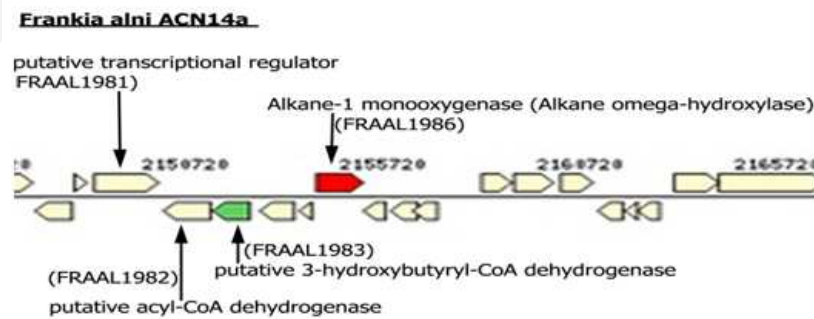


Figure 8. Potential alkane-1 monooxygenase identified in *F. alni* ACN14a.

6. Future aspects

Clearly, we have only begun to scratch the surface of the metabolism of *Frankia* and its biodegradative potential. These initial studies correlating metabolic capacity to gene function are the first step in exploiting the bacteria for their bioremediation ability. Further bioinformatics data mining are necessary to elucidate the unique metabolic potential of *Frankia*. However, these *in silico* studies require “wet lab” experiments to confirm these capabilities.

From limited field studies, actinorhizal nodule occupancy seems to be under control by environmental conditions. The presence of *Frankia* lineage III strains inside alder nodules found under PAH-stressed soils suggests that this lineage may have a greater metabolic potential. The larger genome size of this lineage compared to the other infective strains also supports this hypothesis. However, further experiments are required to confirm this postulate.

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