

# Chapter 2

## Ecology and Physiology of Non-*Frankia* Actinobacteria from Actinorhizal Plants

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### 2.1 Introduction

Actinorhizal plants embody a broad group of divergent dicotyledonous plants representing over 200 different plant species in 25 genera and eight families and are identified by their ability to form a symbiotic association with actinobacteria from the genus *Frankia* (Benson and Silvester 1993; Benson and Dawson 2007; Chaia et al. 2010). This association results in the formation of root nodule structures containing these nitrogen-fixing bacteria which aids the ability of these plants to colonize nutrient-poor soils. Actinorhizal plants are ecologically important as pioneer community plants, distributed worldwide in a broad range of ecological conditions, and have economic significance in land reclamation, reforestation, soil stabilization, landscaping, and fuel wood. The symbiosis allows actinorhizal plants to colonize harsh environmental terrains. The actinorhizal symbiosis has been studied at many levels including the pursuit of new *Frankia* isolates. As a by-product of these isolation attempts, large numbers of other actinobacteria were collected from these actinorhizal nodules, occupying the same microniche as *Frankia*. Despite their perpetual appearance, only a few of them have been studied in detail. Most of these isolates were ignored or discarded as being irrelevant to the plants. However, these non-*Frankia* actinobacteria have consistently been isolated from several

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actinorhizal plants including *Casuarina* (Guillén et al. 1993; Niner et al. 1996; Valdés et al. 2005; Ghodhbane-Gtari et al. 2010), *Coriaria* (Trujillo et al. 2005), *Discaria* (Solans and Vobis 2003), *Alnus* (Valdés et al. 2006; Ghodhbane-Gtari et al. 2010), and *Elaeagnus* (Gtari et al. 2007; Ghodhbane-Gtari et al. 2010) and have been hypothesized to play a beneficial role in the health and ecology of these plants. The aim of this chapter is to describe what is known about these non-*Frankia* actinobacteria with a focus on their versatility to fulfill a variety of potential ecological and functional roles.

## 2.2 Physiology and Diversity of Non-*Frankia* Actinobacterial Isolates

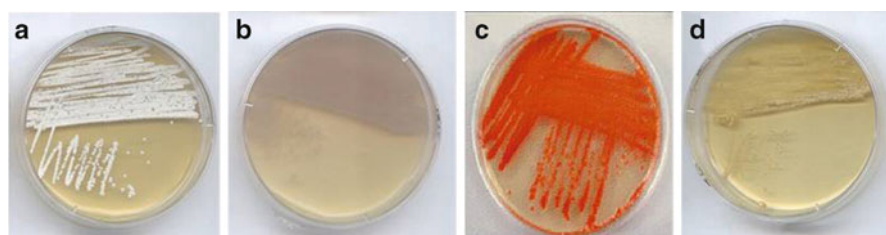
Several different genera of actinobacteria have been isolated from actinorhizal plants. Table 2.1 illustrates the diversity of actinobacterial genera isolated from actinorhizal plants (nodules and rhizosphere). Several of these isolates have been characterized to the genus level, while others have not been categorized beyond recognition as actinobacteria. These actinobacteria have been isolated from representatives for five of the eight plant families associated with the actinorhizal designation. Most of these bacterial isolates were assigned to the genera *Streptomyces*, *Nocardia*, *Micromonospora*, and *Actinoplanes*. These non-*Frankia* actinobacteria are filamentous, and mostly fall into the Actinomycetales category. All of them produce aerial hyphae and highly branched filaments when grown on the appropriate agar media. These isolates do not produce vesicle structures, similar to those found with *Frankia* isolates. For *Frankia*, vesicles are the site of nitrogen fixation and function to provide protection against oxygen inactivation to the *Frankia* strains (Murry et al. 1984; Huss-Danell 1997). Colonies and cultures of the non-*Frankia* isolates showed a wide span of different colors and variability. These pigmentations ranged in color from white, orange, yellow to maroon (Ghodhbane-Gtari et al. unpublished data), pink to brownish red (Liu et al. 2009), intense orange (Trujillo et al. 2006), and yellow-white (Trujillo et al. 2005; Valdés et al. 2005) (Fig. 2.1).

## 2.3 Potential Physiological and Ecological Roles of Non-*Frankia* Actinobacteria in Association with Actinorhizal Plants

Little is known about the potential ecophysiological roles of the non-*Frankia* actinobacteria and about their association with actinorhizal plants. Figure 2.2 shows several proposed functions for these bacteria in their association with actinorhizal plants. Aspects of these predicted roles and their relationship to the non-*Frankia* actinobacteria genome plasticity will be discussed below.

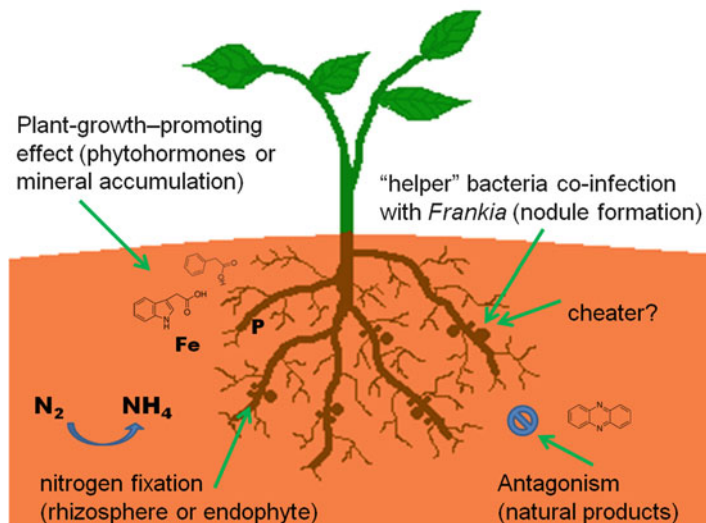
**Table 2.1** List of actinobacteria isolated from actinorhizal plants

| Host plant <sup>a</sup>            | Description of isolates <sup>b</sup>   | References   |
|------------------------------------|--|--|
| <i>Rhamnaceae</i>                  |  |  |
| <i>Discaria trinervis</i>          | <i>Streptomyces</i> , <i>Actinoplanes</i> ,<br><i>Micromonospora</i> , <i>Actinomadura</i> ,<br><i>Pilimelia</i> , <i>Streptosporangium</i><br>nocardioform isolates | Solans and Vobis (2003),<br>Solans et al. (2011)                       |
| <i>Ceanothus caeruleus</i>         | Actinobacteria   | Ramirez-Saad et al. (1998)   |
| <i>Ceanothus velutinus</i>         | <i>Streptomyces</i> sp.  | Wollum et al. (1966)   |
| <i>Elaeagnaceae</i>                |  |  |
| <i>Elaeagnus angustifolia</i>      | <i>Micromonospora</i> , <i>Nocardia</i> ,<br><i>Streptomyces</i>   | Gtari et al. (2004),<br>Ghodhbane-Gtari<br>et al. (2010)               |
| <i>Betulaceae</i>                  |  |  |
| <i>Alnus</i> spp.                  | <i>Nocardia autotrophica</i>   | Dobritsa and Sharaya<br>(1986)   |
| <i>Alnus glutinosa</i>             | <i>Micromonospora</i> , <i>Nocardia</i> ,<br><i>Streptomyces</i>   | Ghodhbane-Gtari et al.<br>(2010)                                       |
| <i>Alnus</i>                       | Non- <i>Frankia</i> actinomycete   | Valdés et al. (2006)   |
| <i>Casuarinaceae</i>               |  |  |
| <i>Casuarina glauca</i>            | <i>Micromonospora</i> , <i>Nocardia</i> ,<br><i>Streptomyces</i>   | Ghodhbane-Gtari et al.<br>(2010)                                       |
| <i>Casuarina<br/>equisetifolia</i> | <i>Micromonospora</i> ,<br><i>Thermomonospora</i> ,<br>filamentous actinobacteria  | Guillén et al. (1993),<br>Niner et al. (1996),<br>Valdés et al. (2005) |
| <i>Coriariaceae</i>                |  |  |
| <i>Coriaria myrtifolia</i>         | <i>Micromonospora coriariae</i>  | Trujillo et al. (2006)   |

<sup>a</sup>Host plant genus or species grouped by plant families<sup>b</sup>Many of the isolates are identified to the genus or species level, but several are only described superficially**Fig. 2.1** Colony formation and pigmentation by *Nocardia* strains isolated from root nodules of *C. glauca*. (a) BMG51112, (b) BMG51102, (c) BMG111207, and (d) BMG111205

### 2.3.1 Nitrogen Fixation Hypothesis

Under aerobic conditions, *Frankia* fixes atmospheric nitrogen and is able to grow in N-free media. As mentioned above, the *Frankia* nitrogenase is localized within the vesicle structure and protected from oxygen inactivation by an envelope containing



**Fig. 2.2** Potential functional roles for non-*Frankia* actinobacteria and their relationship with actinorhizal plants

a high content of bacteriohopane lipids (Berry et al. 1993; Huss-Danell 1997). Although the non-*Frankia* actinobacteria do not produce vesicle structures, there are several lines of evidence suggesting that some of these filamentous bacteria are capable of nitrogen fixation (Guillén et al. 1993; Valdés et al. 2005). First, physiological experiments with  $^{15}N_2$  isotope dilution analysis and acetylene reduction assays support this hypothesis (Valdés et al. 2005, 2006). PCR amplification of the *nifH* gene, coding for one of the structural components of nitrogenase, generated a sequence that is highly similar to the *Frankia nifH* gene. Gtari et al. (2007) isolated a *Micromonospora* strain which was able to grow in N-free media and reduced acetylene. Molecular analysis of this *Micromonospora* isolate showed the presence of a *nifH* gene with sequence similarity to the *Frankia nifH* gene. Furthermore, recent *Nocardia* isolates obtained from root nodules of *Casuarina glauca* were shown to grow in N-free defined propionate medium and exhibited acetylene reduction activity (Ghodhbane-Gtari et al. unpublished data). These results confirm their ability to fix the nitrogen and their contribution to the symbiosis process.

### 2.3.2 *Helper Bacteria Hypothesis and Plant Phytohormone Production by Non-Frankia Actinobacteria*

Another potential role for these actinobacteria is to act as “helper bacteria” that aid the infection and nodulation process by *Frankia* into its host plant. *Alnus rubra* seedlings showed increased nodulation under conditions of co-inoculation with *Frankia* and the “helper” bacteria, *Burkholderia cepacia* (previously, *Pseudomonas*

*cepacia*) (Knowlton et al. 1980; Knowlton and Dawson 1983). The presence of the “helper” bacteria alone caused root hair deformation, an early step in the infection, suggesting a preconditioning of the plant for the nodulation process. Thus, the presence of these other bacteria, especially the actinobacteria, may play an important role in the establishment of the root invasion and the nodule formation. Solans (2007) showed that several isolates of rhizospheric actinomycetes belonging to the genera *Micromonospora*, *Streptomyces*, and *Actinoplanes* that were isolated from the rhizosphere of *Discaria trinervis* are able to enhance plant growth and to increase nodulation when co-inoculated with *Frankia*. The *Nocardia* isolates from *C. glauca* helped to promote root length and increased growth of the aerial parts of their host plant (Ghodhbane-Gtari et al. unpublished data). Infection of *C. glauca* plants with these non-*Frankia* actinobacteria also showed a root hair deformation.

Many of these actinobacteria isolates produce plant growth hormones such as auxins (Ghodhbane-Gtari et al. 2010; Solans et al. 2011). One study focused on three isolates (*Streptomyces*, *Actinoplanes*, and *Micromonospora*) from a pool of 122 that were obtained from the rhizosphere of *D. trinervis* (*Ochetophila trinervis*) (Solans et al. 2011). These saprophytic rhizoactinomycetes produced three different phytohormones: indole-3-acetic acid (IAA), gibberellic acid (GA<sub>3</sub>), and zeatin at higher levels than those produced by the symbiotic *Frankia* strain BCU110501. Co-inoculation experiments showed significant increases in plant shoot and root dry weight. Furthermore, triple-inoculation with *Frankia*, *Streptomyces*, and *Actinoplanes* resulted in the most significant increases in plant shoot and root dry weight and showed greatest effect on nodulation. Although the normal infection process for *Discaria* proceeds via the intercellular route without root hair deformation, root hair deformation was observed under these co-inoculation conditions (Solans et al. 2011).

These actinobacteria might contribute to the growth of the plants either directly or indirectly. Many of them produce an impressive array of secondary metabolites (or natural products) exhibiting a wide variety of biological activity including antibiotics, antitumor and anti-infection agents, plant growth promoters, and enzymes (Ghodhbane-Gtari et al. 2010; Qin et al. 2011). These agents could have a direct effect on the plant. On the other hand, these bacteria could facilitate the incorporation of nutrients indirectly through nitrogen fixation or phosphorous solubilization (Solans et al. 2011). The beneficial effects of these non-*Frankia* actinobacteria could be provided by the presence of the bacteria in the rhizosphere or inside the plants. Although these bacteria have been isolated from actinorhizal nodules, their location within the plant roots has not been confirmed by follow-up studies. The exact location of these actinobacteria within the plant including their distribution and abundance needs to be clarified through further work.

### 2.3.3 Antagonism Model

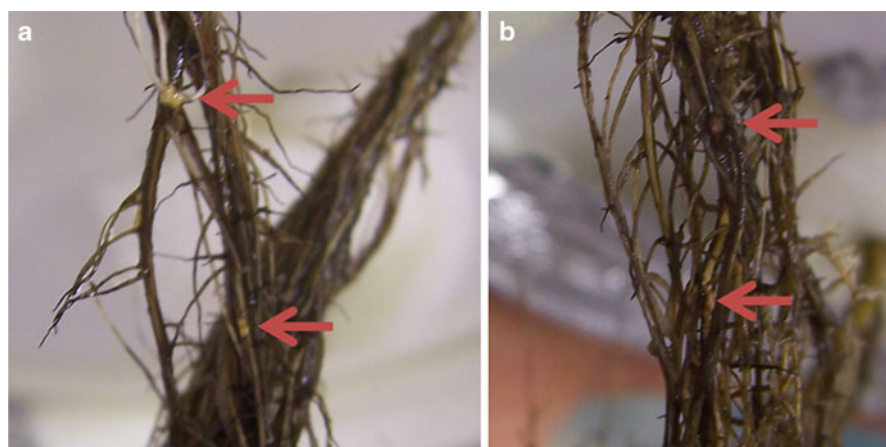
The ability to combat potential plant pathogens would provide an excellent plant-growth-promoting activity. Actinobacteria isolated from surface-sterilized root

nodules of *Alnus glutinosa*, *C. glauca* and *Elaeagnus angustifolia* exhibited cellulase, chitinase, and antifungal activities (Ghodhbane-Gtari et al. 2010). These isolates showed antagonism activities against fungal plant pathogens, *Fusarium* sp. and *Trichoderma* sp. Cellulase and chitinase activities were detected which could also provide defense actions against other fungal and insect predators. The potential of these non-*Frankia* actinobacteria to produce novel natural products has only begun to be revealed and the range of activities and targets (i.e., bacteria, fungi, and metazoans) has not yet been explored. These microbes may prove to be a valuable new source of novel products and merit further investigation.

### 2.3.4 Plant Colonization Hypothesis or Cheater Model

It is not clear how these non-*Frankia* actinobacteria colonize root nodules and where they are specifically located. These strains may reside in the internal tissues of the nodules or may inhabit their outer layer. It is possible that they may have more rhizospheric origin. The root nodule has many pockets in its cortex that could provide a microniche for many bacteria including the actinobacteria. The cortical layer of *Casuarina* nodules is capable of hosting mixed bacterial populations including both symbiotic and atypical *Frankia* strains (Nazaret et al. 1989). Atypical *Frankia* strains, which form the fourth *Frankia* lineage, are unable to reinfect their host plant. One hypothesis, we propose, is that these bacteria have adopted a cheater strategy to colonize the plant and coinfect with an infective strain. Thus, these non-*Frankia* actinobacteria could also be using this strategy for infection.

Actinorhizal-like nodule structures have been observed on *E. angustifolia* roots infected with *Agrobacterium rhizogenes* (Berg et al. 1992) and on roots of *A. glutinosa* and *Alnus incana* infected with the fungus, *Penicillium nodositatum* (Capellano et al. 1987; Sequerra et al. 1994; Wolters et al. 1999). For the non-*Frankia*-actinobacteria, there are no published reports on their infectivity on the actinorhizal plants. These experiments are crucial and need to be performed for many of these isolates. Initial preliminary studies (Ghodhbane-Gtari et al. unpublished data) indicate that *Nocardia* sp. strain BMG111209 was able to induce a nodule-like structure on the roots of *C. glauca* (Fig. 2.3). *Nocardia* sp. strain BMG111209 was isolated from *Casuarina* plants. After the inoculation, these plants were grown hydroponically and observed over 4 months. A “nodule-like” structure formed that was very similar to a typical *C. glauca* nodule (Fig. 2.3). Co-inoculation of *Nocardia* sp. strain BMG111209 with *Frankia* sp. strain CcI3 leads to early onset of nodulation and to a fourfold increase in the number of nodules formed per plant compared to *Frankia* inoculation alone. The presence of *Nocardia* sp. strain BMG111209 within these nodules needs to be confirmed by molecular methods and microscopic techniques. However, these very preliminary results suggest a new avenue of study on these non-*Frankia* actinobacteria.



**Fig. 2.3** Plant infection by *Nocardia* BMG11209 results in the formation of an actinorhizal-like nodule structure. Panel (a) shows root of *C. glauca* infected with *Frankia* sp. Cc13. Panel (b) shows roots of *C. glauca* infected with *Nocardia* BMG11209. Arrows point out root nodule structures

## 2.4 Genomics and Other Molecular Genetic Aspects of Non-*Frankia* Actinobacteria

Our understanding of *Frankia* has been greatly enhanced by the availability of several *Frankia* sequenced genomes (Normand et al. 2007a, b; Persson et al. 2011; Sen et al. 2013; Ghodbhane-Gtari et al. 2013). The *Frankia* genomes maintain a rich natural product biosynthetic potential comparable to that in *Streptomyces* and many of these *Frankia* compounds are potential signaling molecules involved in plant-microbe interactions (Udwary et al. 2011). Bioinformatic analysis of the genome-based *Frankia* secretomes indicated that the predicted secretomes are reduced in size compared to those of other soil bacteria (Mastrorunzio et al. 2008), suggesting that the microsymbiont *Frankia* has a low plant cell wall degrading capacity. Thus, the “helper” actinobacteria may aid the infection process through this mechanism.

The generation of a genome database for these non-*Frankia* actinobacteria would greatly facilitate our understanding of their plasticity and versatility. Those sequencing efforts have just been initiated recently. At present, only three genomes for these non-*Frankia* actinobacteria have been sequenced and all of them at the Joint Genome Institute (JGI) as part of their Community Sequencing Program. First, the genome of *Micromonospora* sp. strain L5, isolated from *Coriaria myrtifolia* (Trujillo et al. 2006), was sequenced. Detailed information about genome annotation and other genome properties are available at <http://img.jgi.doe.gov> (Markowitz et al. 2006). A second project at JGI centered on sequencing two *Nocardia* strains (BMG111209 and BMG51109) that have been isolated from the nodules of *C. glauca*. Although sequencing has been completed for both genomes, only one BMG111209 (formally called Cas13) has been annotated at the time of writing and will be available soon on the above website.



To provide insight of the plasticity of these bacteria, we compared genome characteristics of the sequenced non-*Frankia* actinobacteria to three sequenced *Frankia* genomes (Tables 2.2 and 2.3). The *Micromonospora* sp. strain L5 genome is smaller in size (7 megabase pairs, or Mb) compared to the *Nocardia* sp. strain BMG111209 (9.1 Mb). While the *Micromonospora* sp. strain L5 genome was sequenced completely and consists of a single circular chromosome, the *Nocardia* sp. strain BMG111209 genome represents a permanent draft sequence consisting of five scaffolds. It is not clear if these scaffolds may represent more than one replicon. One indicator of bacterial genome plasticity is the presence of mobile elements including genes for transposases and integrases, horizontally transferred genes (HTG), prophages, and phage remnants. The *Frankia* genomes exhibit signs of both genome expansion and contraction which have been correlated with their biogeographic distribution and plant-host specificity (Normand et al. 2007a). This genome plasticity is partly driven by their insertion sequence (IS) elements content (Bickhart et al. 2009). While IS elements content is lower in the *Nocardia* sp. strain BMG111209 genome compared to the *Frankia* genomes, it contains similar levels of HTG, phage genes, and clustered regularly interspaced short palindromic repeats (CRISPR) content suggesting these elements may drive genome plasticity. The *Micromonospora* sp. strain L5 genome contains reduced numbers of these elements indicating a more stable, less plastic genome.

Analysis of both genomes revealed the absence of any known nitrogenase genes suggesting alternative mechanism for nitrogen fixation (Gtari et al. 2012). The *Nocardia* genome contained a *hup* operon (Fig. 2.4b) indicating the potential for hydrogenase activity, while the *Micromonospora* genome lacked these genes. The gene neighborhood of the *hup* operon is similar to that of the two *Frankia* genomes, which is very similar among all of the *Frankia* genomes. Although the *Nocardia* BMG111209 contains all of the *hup* genes, their organization is different showing gene rearrangements including inverted direction for two genes (*hypA* and *hypB*). This pattern suggests potential genome rearrangements and provides further insight on the genome plasticity. Among the *Frankia* genomes, the *hup* operon organization is similar and showing a high degree of synteny for the region.

Further analyses of these genomes reveal another area of interest. Compared to the other genomes, the *Nocardia* BMG111209 genome is rich with genes in COG group K (transcription) and Q (secondary metabolism) (Table 2.3). Among the five analyzed genomes, the *Nocardia* BMG111209 genome contained the highest percentages of these two COGs. Among the COG Q genes found in this genome, there were a variety of genes for non-ribosomal peptide synthetases (NRPS) and multiple types of polyketide synthases (PKS). The five genomes were analyzed for the presence of biosynthetic clusters by the use of the antiSMASH program (Medema et al. 2011). *Nocardia* BMG111209 genome has similar numbers of predicted biosynthetic gene clusters (Table 2.2) and the potential to produce several novel natural products or secondary metabolites (Fig. 2.5). Analysis of the *Micromonospora* L5 genome revealed the presence of fewer clusters than were found with the other four genomes. Among the 29 clusters found within the *Nocardia* BMG111209 genome, several regions were predicted to produce interesting natural products. One PKS



**Table 2.2** General genome properties for non-Frankia actinobacteria and selected Frankia strains from actinorhizal plants

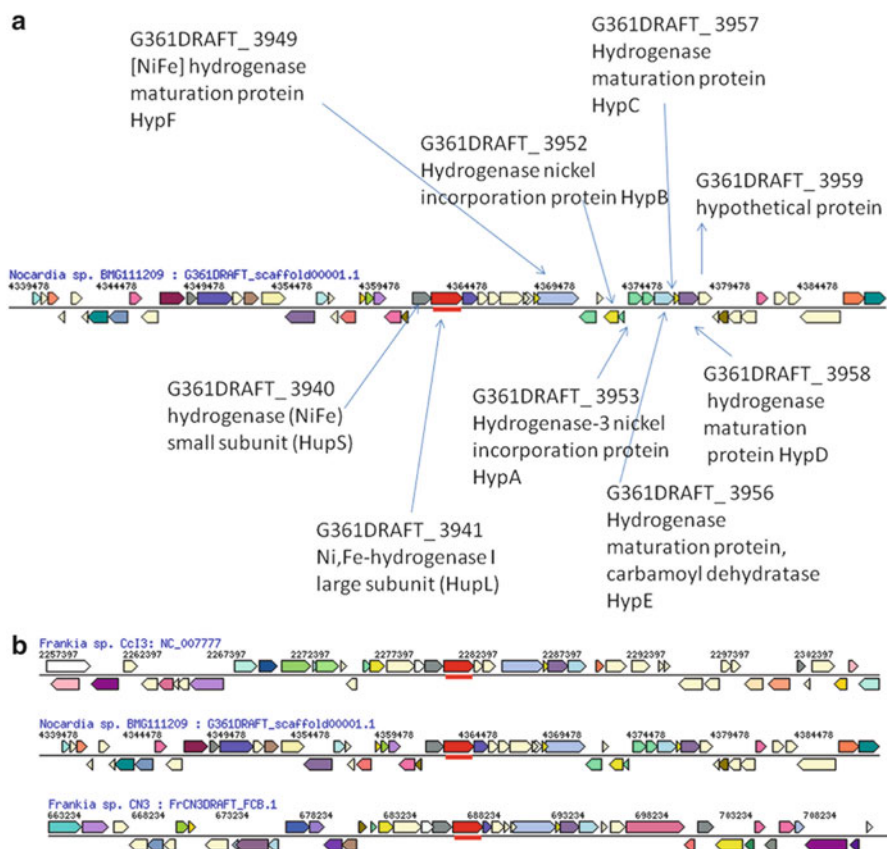
| Genome property | Genome of actinobacterium |                                  |                |                        |                             | Frankia ACN14a | Frankia CcI3 | Micromonospora L5 | Nocardia BMG111209                           | Frankia EAN1pec |
|-----------------|---------------------------|----------------------------------|----------------|------------------------|-----------------------------|----------------|--------------|-------------------|--|-----------------|
|                 | Size (Mb)                 | Number and topology of replicons | GC content (%) | No. of predicted genes | Transposons and IS elements | Phage genes    | CRISPR       | HTG <sup>a</sup>  | Predicted biosynthetic clusters <sup>b</sup> | Frankia EAN1pec |
|                 | 9.14                      | (5 scaffolds)                    | 69             | 8,197                  | 28 (0.3 %)                  | 10             | 2            | 278 (2.8 %)       | 29   | 8.98            |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 1 circular      |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 71              |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 7,250           |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 183 (2.2 %)     |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 11              |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 6               |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 262 (3.61 %)    |
|                 |                           |                                  |                |                        |                             |                |              |                   |  | 28 (20)         |

<sup>a</sup>Horizontally transferred genes  
<sup>b</sup>Biosynthetic clusters for secondary metabolites were identified by the use of the antiSMASH program (Medema et al. 2011). Frankia data in brackets are from Udawary et al. (2011)

**Table 2.3** *Frankia* and non-*Frankia* actinobacteria coding sequences classified into clusters of orthologous genes (COG)

| Genome of actinobacterium                 |  |                              |                          |                     |                          |                           |
|---|--|------------------------------|--------------------------|---------------------|--------------------------|---------------------------|
| COG code                                  | COG functional category                                      | Genome of actinobacterium    |                          |                     |                          |                           |
|   |  | <i>Nocardia</i><br>BMG111209 | <i>Micromonospora</i> L5 | <i>Frankia</i> Cc13 | <i>Frankia</i><br>ACN14a | <i>Frankia</i><br>EAN1pec |
| <i>Cellular processes and signaling</i>   |  |                              |                          |                     |                          |                           |
| D   | Cell cycle control, cell division, chromosome partitioning   | 51 (0.74 %)                  | 49 (0.89 %)              | 42 (1.27 %)         | 41 (0.86 %)              | 46 (0.79 %)               |
| M   | Cell wall/membrane/envelope biogenesis                       | 231 (3.37 %)                 | 252 (4.93 %)             | 177 (5.35 %)        | 209 (4.40 %)             | 243 (4.29 %)              |
| N   | Cell motility  | 16 (0.23 %)                  | 8 (0.16 %)               | 4 (0.12 %)          | 2 (0.04 %)               | 4 (0.07 %)                |
| O   | Posttranslational modification, protein turnover, chaperones | 163 (2.38 %)                 | 161 (2.54 %)             | 113 (3.42 %)        | 144 (3.03)               | 145 (2.56 %)              |
| T   | Signal transduction mechanisms                               | 289 (4.22 %)                 | 279 (5.45 %)             | 210 (6.35 %)        | 295 (6.20)               | 364 (6.43 %)              |
| U   | Intracellular trafficking and secretion                      | 41 (0.60 %)                  | 47 (0.92 %)              | 40 (1.21 %)         | 29 (0.61 %)              | 48 (0.75 %)               |
| V   | Defense mechanisms   | 82 (1.20 %)                  | 112 (2.19 %)             | 49 (1.48 %)         | 70 (1.47)                | 89 (1.57 %)               |
| Z   | Cytoskeleton   | 1 (0.01 %)                   | 2 (0.04 %)               | 0                   | 3 (0.06 %)               | 0                         |
| <i>Information storage and processing</i> |  |                              |                          |                     |                          |                           |
| A   | RNA processing and modification                              | 14 (0.20 %)                  | 12 (0.23 %)              | 1 (0.03 %)          | 1 (0.02 %)               | 2 (0.04 %)                |
| B   | Chromatin structure and dynamics                             | 2 (0.03 %)                   | 1 (0.02 %)               | 1 (0.03 %)          | 1 (0.02 %)               | 1 (0.02 %)                |
| J   | Translation, ribosomal structure and biogenesis              | 194 (8.83 %)                 | 193 (3.77 %)             | 161 (4.87 %)        | 167 (3.51 %)             | 165 (2.91 %)              |
| K   | Transcription  | 913 (13.32 %)                | 528 (10.32 %)            | 277 (8.38 %)        | 435 (9.15 %)             | 546 (9.64 %)              |
| L   | Replication, recombination and repair                        | 211 (3.08 %)                 | 210 (4.11 %)             | 273 (8.26 %)        | 206 (4.33 %)             | 387 (6.84 %)              |

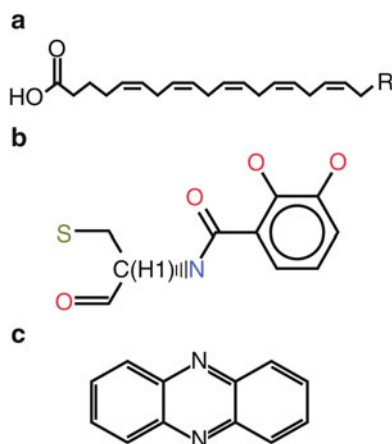




**Fig. 2.4** Hydrogenase gene clusters in *Nocardia* BMG11209 and *Frankia* genomes. Panel (a) shows the *hup* genes cluster found in the *Nocardia* BMG11209 genome and identifies the genes involved in hydrogenase biosynthesis. Panel (b) shows a comparison of the *Nocardia* BMG11209 *hup* gene neighborhood to those found in two *Frankia* genomes. The red gene represents *hupL* in these three genomes

cluster has similarity to *Frankia* clusters FA04, FC15a, and FE17 (Udwary et al. 2011) and is predicted to produce a polyunsaturated fatty acid (PUFA). The predicted chemical structure produced by this cluster polyketide synthase is shown in Fig. 2.5a. Another BMG11209 NRPS cluster is similar to *Frankia* cluster FE07 (Udwary et al. 2011) and is predicted to produce a hexapeptide siderophore (Fig. 2.5b). Three potential antagonism-related biosynthetic clusters were identified in the BMG11209 genome. Two of these clusters are predicted to produce bacteriocin and phenazine molecules (Fig. 2.5c). The compounds could function in the antagonism model described above (Sect. 2.3.3).

**Fig. 2.5** Bioinformatic analysis of *Nocardia* sp. BMG111209 biosynthetic gene clusters revealed putative chemical structures of their products: polyunsaturated fatty acid (a), hexapeptide siderophore (b), and phenazine (c)



## 2.5 Perspectives and Future Directions

As increasing numbers of non-*Frankia* actinobacteria are being isolated from actinorhizal plants, there is growing interest in their ecophysiological functions. Until recently, these bacteria were ignored or discarded. However, the increased awareness of community interactions and multiple partners in symbiotic associations has shed new light on our vision of these bacteria and raised several questions for future investigations. Are these non-*Frankia* actinobacteria able to reinfect their host plant and fulfill Koch's postulates? These experiments are vital toward our understanding of plant-microbe interaction and may provide clues of the evolutionary trajectory of the development of a symbiosis. Where are these non-*Frankia* actinobacteria located within the plant? Is there a specific region, tissue, or cellular habitat? A cytological study with molecular probes would determine the location, abundance, and diversity of these bacteria within the plant. As more genomes are sequenced for these non-*Frankia* actinobacteria, their metabolic potential will be revealed hopefully providing a new source of natural products including antimicrobial compounds.

## 2.6 Conclusion

The non-*Frankia* actinobacteria play an important ecological role in the enhancement of plant growth and may act as helper bacteria to facilitate the establishment of the *Frankia* symbiosis with the actinorhizal plant. The two completed and one upcoming genome databases for these non-*Frankia* actinobacteria have provided baseline information on the genome plasticity and metabolic versatility of these microbes. However, further genome sequencing will help clarify and extend the potential of these microbes.

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## References

- Benson DR, Dawson JO (2007) Recent advances in the biogeography and genecology of symbiotic *Frankia* and its host plants. *Physiol Plant* 130(3):318–330
- Benson DR, Silvester WB (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol Rev* 57(2):293–319
- Berg RH, Liu LX, Dawson JO et al (1992) Induction of pseudoactinorhizae by the plant pathogen *Agrobacterium rhizogenes*. *Plant Physiol* 98(2):777–779
- Berry AM, Harriott OT, Moreau RA et al (1993) Hopanoid lipids compose the *Frankia* vesicle envelope, presumptive barrier of oxygen diffusion to nitrogenase. *Proc Natl Acad Sci U S A* 90(13):6091–6094
- Bickhart DM, Gogarten JP, Lapierre P et al (2009) Insertion sequence content reflects genome plasticity in strains of the root nodule actinobacterium *Frankia*. *BMC Genomics* 10:468
- Capellano A, Dequatre B, Valla G, Moiroud A (1987) Root-nodules formation by *Penicillium* sp. on *Alnus glutinosa* and *Alnus incana*. *Plant Soil* 104(1):45–51
- Chai EE, Wall LG, Huss-Danell K (2010) Life in soil by the actinorhizal root nodule endophyte *Frankia*. A review. *Symbiosis* 51(3):201–226
- Dobritsa SV, Sharaya LS (1986) Genome identity of different *Nocardia autotrophica* isolates from *Alnus* spp. root nodules and rhizosphere. In: Szabo G, Biro S, Goodfellow M (eds) *Biological, biochemical and biomedical aspects of Actinomycetes*. Akademiai Kiado, Budapest, pp 497–506
- Ghodhbane-Gtari F, Beauchemin N, Bruce D et al (2013) Draft genome sequence of *Frankia* sp. strain CN3, an atypical, non-infective (Nod<sup>-</sup>) ineffective (Fix<sup>-</sup>) isolate from *Coriaria nepalensis*. *Genome Announc* 1(2):00085–13
- Ghodhbane-Gtari F, Essoussi I, Chattaoui M et al (2010) Isolation and characterization of non-Frankia actinobacteria from root nodules of *Alnus glutinosa*, *Casuarina glauca* and *Elaeagnus angustifolia*. *Symbiosis* 50(1–2):51–57
- Gtari M, Brusetti L, Skander G et al (2004) Isolation of *Elaeagnus*-compatible *Frankia* from soils collected in Tunisia. *FEMS Microbiol Lett* 234(2):349–355
- Gtari M, Daffonchio D, Boudabous A (2007) Assessment of the genetic diversity of *Frankia microsymbionts* of *Elaeagnus angustifolia* L. plants growing in a Tunisian date-palm oasis by analysis of PCR amplified *nifD-K* intergenic spacer. *Can J Microbiol* 53(3):440–445
- Gtari M, Ghodhbane-Gtari F, Nouioui I et al (2012) Phylogenetic perspectives of nitrogen-fixing actinobacteria. *Arch Microbiol* 194(1):3–11
- Guillén GM, Valdés M, Liao J, Hirsch AM (1993) Identificación de actinobacterias aisladas de nódulos de *Casuarina*, por técnicas tradicionales y moleculares. *Rev Lat-Am Microbiol* 35:195–200
- Huss-Danell K (1997) Tansley review no. 93. Actinorhizal symbioses and their N<sub>2</sub> fixation. *New Phytol* 136(3):375–405
- Knowlton S, Dawson JO (1983) Effects of *Pseudomonas cepacia* and cultural factors on the nodulation of *Alnus rubra* roots by *Frankia*. *Can J Bot* 61(11):2877–2882
- Knowlton S, Berry A, Torrey JG (1980) Evidence that associated soil bacteria may influence root hair infection of actinorhizal plants by *Frankia*. *Can J Microbiol* 26(8):971–977



- Liu N, Wang HB, Liu M et al (2009) *Streptomyces alni* sp nov., a daidzein-producing endophyte isolated from a root of *Alnus nepalensis* D. Don. Int J Syst Evol Microbiol 59(2):254–258
- Markowitz V, Korzeniewski F, Palaniappan K et al (2006) The integrated microbial genomes (IMG) system. Nucleic Acids Res 34:D344–D348
- Mastrorunzio JE, Tisa LS, Normand P, Benson DR (2008) Comparative secretome analysis suggests low plant cell wall degrading capacity in *Frankia* symbionts. BMC Genomics 9:47
- Medema MH, Blin K, Cimermanic P et al (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39(suppl 2):W339–W346
- Murry MA, Fontaine MS, Tjepkema JD (1984) Oxygen protection of nitrogenase in *Frankia* sp. HFPAr13. Arch Microbiol 139(2–3):162–166
- Nazaret S, Simonet P, Normand P, Bardin R (1989) Genetic diversity among *Frankia* isolated from *Casuarina* nodules. Plant Soil 118(1–2):241–247
- Niner BM, Brandt JP, Villegas M et al (1996) Analysis of partial sequences of genes coding for 16S rRNA of actinomycetes isolated from *Casuarina equisetifolia* nodules in Mexico. Appl Environ Microbiol 62(8):3034–3036
- Normand P, Lapierre P, Tisa LS et al (2007a) Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. Genome Res 17(1):7–15
- Normand P, Queiroux C, Tisa LS et al (2007b) Exploring the genomes of *Frankia*. Physiol Plant 130(3):331–343
- Persson T, Benson DR, Normand P et al (2011) Genome sequence of “*Candidatus Frankia datiscae*” Dg1, the uncultured microsymbiont from nitrogen-fixing root nodules of the dicot *Datisca glomerata*. J Bacteriol 193(24):7017–7018
- Qin S, Xing K, Jiang JH et al (2011) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89(3):457–473
- Ramirez-Saad H, Janse JD, Akkermans ADL (1998) Root nodules of *Ceanothus caeruleus* contain both the N<sub>2</sub>-fixing *Frankia* endophyte and a phylogenetically related Nod<sup>+</sup>/Fix<sup>+</sup> actinomycete. Can J Microbiol 44(2):140–148
- Sen A, Beauchemin N, Bruce D et al (2013) Draft genome sequence of *Frankia* sp. strain QA3, a nitrogen-fixing actinobacterium isolated from the root nodule of *Alnus nitida*. Genome Announc 1(2):e00103–13
- Sequerra J, Capellano A, Faure-Raynard M, Moiroud A (1994) Root hair infection process and myconodule formation on *Alnus incana* by *Penicillium nodositatum*. Can J Bot 72(7):955–962
- Solans M (2007) *Discaria trinervis*—*Frankia* symbiosis promotion by saprophytic actinomycetes. J Basic Microbiol 47(3):243–250
- Solans M, Vobis G (2003) Actinomycetes saprofíticos asociados a la rizósfera de *Discaria trinervis*. Ecología Austral 13:97–107
- Solans M, Vobis G, Cassán F et al (2011) Production of phytohormones by root-associated saprophytic actinomycetes isolated from the actinorrhizal plant *Ochetophila trinervis*. World J Microbiol Biotechnol 27(9):2195–2202
- Trujillo ME, Willems A, Abril A et al (2005) Nodulation of *Lupinus albus* by strains of *Ochrobactrum lupini* sp. nov. Appl Environ Microbiol 71(3):1318–1327
- Trujillo ME, Kroppenstedt RM, Schumann P et al (2006) *Micromonospora coriariae* sp nov., isolated from root nodules of *Coriaria myrtifolia*. Int J Syst Evol Microbiol 56(10):2381–2385
- Udwary DW, Gontang EA, Jones AC et al (2011) Significant natural product biosynthetic potential of actinorrhizal symbionts of the genus *Frankia*, as revealed by comparative genomic and proteomic analyses. Appl Environ Microbiol 77(11):3617–3625
- Valdés M, Pérez NO, Estrada-de los Santos P (2005) Non-*Frankia* actinomycetes isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. Appl Environ Microbiol 71(1):460–466

- Valdés D, Huss-Danell K, Lavire C et al (2006) Further characterization of new symbiotic nitrogen fixing non-*Frankia* actinomycetes isolated from nodules of *Alnus acuminata*. Paper presented at the 14th International Meeting on *Frankia* and Actinorhizal Plants, Umea University, Umea, Sweden
- Wollum AG, Youngberg CT, Gilmour CM (1966) Characterization of *Streptomyces* sp. isolated from root nodules of *Ceanothus velutinus* Dougl. Soil Sci Soc Am J 30(4):463–467
- Wolters DJ, Van Dijk C, Akkermans ADL, Woldendorp JW (1999) Ineffective *Frankia* and host resistance in natural populations of *Alnus glutinosa* (L.) Gaertn. Acta Oecol 20(2):71–79

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