Permanent Draft Genome sequence for *Frankia* sp. strain CcI49, a Nitrogen-Fixing Bacterium Isolated from *Casuarina cunninghamiana* that Infects *Elaeagnaceae*

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**Abstract**

*Frankia* sp. strain CcI49 was isolated from *Casuarina cunninghamiana* nodules. However the strain was unable to re-infect *Casuarina*, but was able to infect other actinorhizal plants including *Elaeagnaceae*. Here, we report the 9.8-Mbp draft genome sequence of *Frankia* sp. strain CcI49 with a G+C content of 70.5 % and 7,441 candidate protein-encoding genes. Analysis of the genome revealed the presence of a *bph* operon involved in the degradation of biphenyls and polychlorinated biphenyls.

Key words: Actinorhizal symbiosis, bioremediation, nitrogen fixation, natural products, host microbe interactions, genomes.

Soil dwelling actinobacteria of the genus *Frankia* form an endophytic symbiosis with actinorhizal plants, which are comprised of over 200 species from 8 angiosperm families [1, 2]. Actinorhizal plants in symbiosis with *Frankia* play important ecological roles as pioneer species and are used in agroforestry, land reclamation, crop protection, and soil stabilization projects [3]. Molecular phylogenetic approaches have identified four major clusters of *Frankia* that also follow host plant specificity groups [4-7]. Members of cluster 1 are divided into sub-cluster 1a that are infective on *Alnus* and *Myricaceae* and sub-cluster 1b strains which are infective on *Allocasuarina*, *Casuarina* and *Myricaceae*. Cluster 2 represents strains infective on *Coriariaceae*, *Datiscaceae*, *Dryadoideae* and *Ceanothus*, while cluster 3 comprises strains that are infective on *Colletieae*, *Elaeagnaceae*, *Gymnostoma* and *Myricaceae*. Finally, cluster 4 groups *Frankia* strains isolated from actinorhizal nodules that are unable to undertake the nitrogen-fixation process (Fix-) and/or re-infect their host plant causing nodulation (Nod-) and are classified as “atypical *Frankia*”. Genomes for representatives from each cluster have been sequenced [8]. The availability of these *Frankia* genome databases has opened up the use of “omics” approaches. Analysis of *Frankia* genomes has revealed new potential in respect to metabolic diversity, natural product biosynthesis, and stress tolerance, which may aid the cosmopolitan nature of the actinorhizal symbiosis.

Several *Frankia* strains isolated from *Casuarina* nodules are unable to re-infect it, but are able to infect other actinorhizal plant genera like *Elaeagnus* [9, 10]. Although isolated from *Casuarina* nodules, these *Frankia* strains are classified as members of cluster 3 based on molecular phylogeny and genomes for two members of this group have been sequenced [11, 12]. *Frankia* sp. strain CcI49 was isolated from root nodules.
of *Casuarina cunninghamiana* grown on the edge of a cultivated field on side of the highway in Ismailia-Port Said, Egypt. The fresh nodules were washed, dissected into individual lobes, and surface-sterilized as described previously [13]. Each lobe was checked for sterility in sterile nutrient-rich medium. Nodules that were free from contamination were selected, dissected and homogenized, the homogenates were transferred to 100-ml screw caped bottle containing modified BAP medium for outgrowth. Hyphal outgrowth was homogenized and plated onto solid medium. After 3-4 weeks, colonies picked from the plates were homogenized and incubated in liquid medium. Surprisingly, *Frankia* sp. strain CcI49 produced reddish colonies, while other *Frankia* isolates from *Casuarina* do not. *Frankia* sp. strain CcI49 produced sporangia and spores that were smaller and narrower than normal *Frankia* sporangia and spores (Figure 1). Spores from *Frankia* sp. strain CcI49 had a high germination rate similar to *Frankia* strain Cel5 [14, 15]. We tested the ability of *Frankia* sp. strain CcI49 to re-infect actinorhizal plants. Four different actinorhizal plant species were tested to assay the plant host range and ten plants of each species were inoculated. *Frankia* sp. strain CcI49 was unable to infect *C. cunninghamiana* and *Alnus glutinosa*, but formed nodules on *Elaeagnus angustifolia* and *Hippophae rhamnoides*. All ten of the *E. angustifolia* and *H. rhamnoides* plants tested formed nodules. Thus, *Frankia* sp. strain CcI49 was chosen to be sequenced for several reasons including an interesting physiology including the production of a reddish pigment, development of smaller sporangia and spores than are typical found with *Frankia*, and providing more information on this *Frankia* subcluster.

Sequencing of the draft genome of *Frankia* sp. strain CcI49 was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH) using Illumina technology techniques [16]. A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq2500 platform, which generated 7,939,466 reads (260-bp insert size) totaling 1,921 MBp. The Illumina sequence data were trimmed by Trimmonatic version 0.32 [17], assembled using Spades version 3.5 [18], and ALLPaths-LG version r52488 [19]. The final draft assembly for *Frankia* sp. strain CcI49 consisted of 78 contigs with an N₅₀ contig size of 282.1 kb and 167X coverage of the genome. The final assembled genome contained a total sequence length of 9,758,130 bp with a G+C content of 70.5%.

![Figure 1](http://www.jgenomics.com)
Table 1. Genome features of Frankia sp. strain CcI49 and other Frankia strains isolated from Casuarina root nodules.

| Strain | Source | Location¹ | Size (Mb) | No. of Contigs | Frankia cluster | No. of CDS | Host Plants² |
|--------|--------|-----------|----------|---------------|----------------|-----------|--------------|
| CcI49  | This study | Egypt      | 9.76     | 78            | 3              | 7,441     | Elaeagnaceae |
| R43    | [12]   | USA        | 10.45    | 46            | 3              | 7,644     | Elaeagnaceae |
| G2     | [11]   | Guadeloupe | 9.54     | 90            | 3              | 7,790     | Elaeagnaceae |
| KB5    | [23]   | Australia  | 5.46     | 420           | 1b             | 4,958     | Casuarinaceae |
| CcE3   | [24]   | USA        | 5.43     | 420           | 1b             | 4,958     | Casuarinaceae |
| CeD    | [25]   | Senegal    | 5.00     | 120           | 1b             | 4,403     | Casuarinaceae |
| Alio2  | [26]   | Uruguay    | 5.33     | 120           | 1b             | 4,838     | Casuarinaceae |
| Thr    | [27]   | Egypt      | 5.31     | 171           | 1b             | 4,805     | Casuarinaceae |
| BMG5.23| [28]   | Tunisia    | 5.27     | 167           | 1b             | 4,747     | Casuarinaceae |
| CcI6   | [29]   | Egypt      | 5.39     | 138           | 1b             | 4,902     | Casuarinaceae |
| BR     | [30]   | Brazil     | 5.23     | 180           | 1b             | 4,777     | Casuarinaceae |

¹ The source of the isolate
² Re-infection plant host range

Analysis of the Frankia sp. strain CcI49 revealed the presence of the bph operon coding for a potential metabolic pathway involved in the degradation of biphenyl and polychlorinated biphenyls (Figure 2). The bph operon is also present in Frankia sp. strains EuI1c and EUN1f genomes [20] and was also found in the genomes of Frankia sp. strains G2 and R43 [11, 12], cluster 3 strains isolated from Casuarina root nodules. Both Frankia sp. strains CcI49 and EUN1f contained the entire bph operon, while two genes (bphA3 and bphH) are missing in Frankia sp. EuI1c (Figure 2). The presence of the complete bph operon suggests that Frankia sp. strain CcI49 may be capable of degrading these recalcitrant xenobiotics.

Bioinformatic analysis of this genomes by the use of the AntiSMASH program [21] revealed the presence of high numbers of secondary metabolic biosynthetic gene clusters, which is consistent with previous results with other Frankia genomes including cluster 3 [8, 22]. Table 2 shows a comparison of the various profiles of different Frankia strains isolated from Casuarina for these secondary metabolic biosynthetic gene clusters. The profile of Frankia sp. strain CcI49 differed from those shown by Frankia strains that are able re-infect Casuarina and was similar to the pattern exhibited by the other two cluster 3 strains (R43 and G2) isolated from Casuarina nodules. These cluster 3 genomes contained more polyketide synthase (PKS) biosynthetic clusters than the cluster 1b genomes. The Frankia sp. strain CcI49 genome contained several unique clusters that had homologues in other bacteria or were completely novel.

In summary, the Frankia sp. strain CcI49 genome has revealed an interesting potential metabolic pathways and natural product profile, and serves as a representative of Frankia cluster 3. Further analysis of this genome and experimental evidence will be needed to support the predicted natural product profile and metabolic potential of Frankia sp. strain CcI49.

Figure 2. The bph operon is present in Frankia strains CcI49, EuI1c, G2, R43, and EUN1f. bph genes encode the following: BphA, A2 and A3, Biphenyl 2,3-dioxygenase; BphB, cis-2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase; BphC, 2,3-dihydroxybiphenyl 1,2-dioxygenase; BphD, 2-hydroxy-6-phenyl-6-oxohexa-2,4-dieneoate (HOPDA) hydrolase; BphE, 2-hydroxypenta-2,4-dienoate hydratase; BphF, acylating acetaldehyde dehydrogenase; and BphG, 4-hydroxy-2-oxovalerate aldolase.
Table 2. Biosynthetic gene clusters for natural products found in the genomes from *Casuarina Frankia* strains.

| Strain | *Frankia* Cluster | No. of Biosynthetic gene clusters | NRPS | PKS | Terpene | Siderophore | Bacteriocin | Lantipeptide |
|--------|-------------------|----------------------------------|------|-----|---------|------------|------------|-------------|
| Gc49   | 3                 | 42                               | 6    | 17  | 3       | 1          | 2          | 5           |
| R43    | 3                 | 38                               | 4    | 14  | 3       | 1          | 2          | 4           |
| G2     | 3                 | 35                               | 8    | 13  | 3       | 1          | 1          | 6           |
| Kb85   | 1b                | 34                               | 4    | 9   | 6       | 1          | 1          | 4           |
| Cc13   | 1b                | 29                               | 3    | 5   | 4       | 1          | 3          | 6           |
| CoD    | 1b                | 30                               | 7    | 7   | 4       | 1          | 1          | 4           |
| All2o  | 1b                | 32                               | 7    | 9   | 4       | 1          | 3          | 5           |
| Thr    | 1b                | 33                               | 6    | 7   | 4       | 1          | 1          | 6           |
| BMGS23 | 1b               | 31                               | 8    | 6   | 4       | 1          | 2          | 4           |
| Cc16   | 1b                | 33                               | 8    | 8   | 4       | 1          | 3          | 5           |
| BR     | 1b                | 29                               | 5    | 5   | 4       | 1          | 2          | 5           |

1 Biosynthetic gene clusters were identified by the use of the AntiSMASH software [21]
2 NRPS: Nonribosomal peptide synthase
3 PKS: polyketide synthase including Type I, II, III, Trans-AT, and other types

Nucleotide sequence accession numbers

This whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under the accession number MOWP00000000.1. The version described in this paper is the first version, MOWP01000000.

Supplementary Material

Figure S1. http://www.jgenomics.com/v05p0119s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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