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Short Research Paper

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 *Myricacaeae*. 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 CeI5 [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 *Hippophäe rhamnoides*. All ten of the *E, angustifolia* and *H. rhamnoides* plants tested formed nodules. Thus, *Frankia* sp. strain CcI49 had a plant-host-specificity pattern similar to *Frankia* sp. strains G2 and R43 [9, 10] from cluster 3 also isolated from *Casuarina* root nodules. *Frankia* sp. strain CcI49 genome 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 N_{50} 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%.

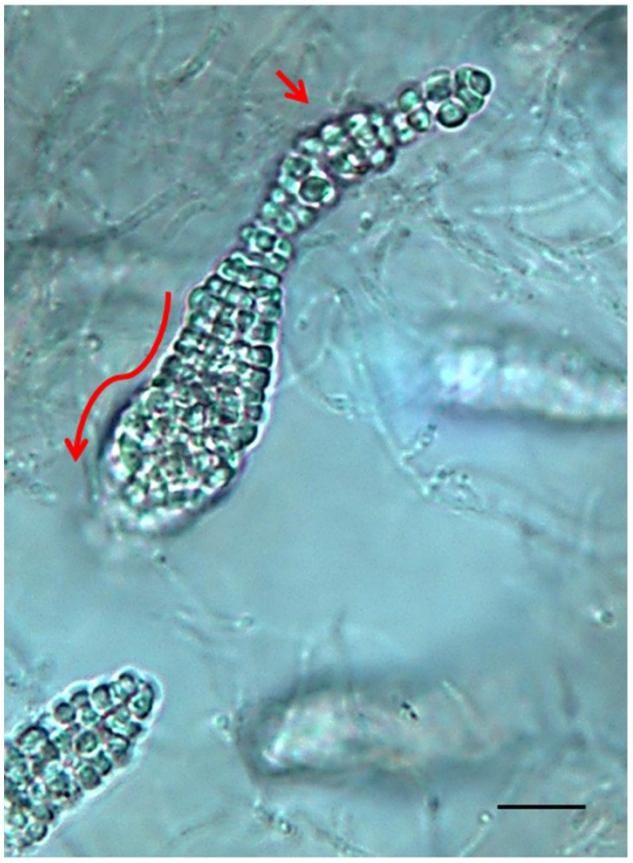

Figure 1. Photomicrograph of *Franakia* sp. strain CcI49 grown in liquid culture. The elongated arrow shows the presence of a long, narrow sessile sporangium containing differentiated mature spores at distal end (short arrow). Size bar represents 32 µm.

The assembled *Frankia* sp. strain CcI49 genome was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and resulted in 7,411 candidate protein-encoding genes, 46 tRNA and 2 rRNA regions. The genome features of *Frankia* sp. strain CcI49 fall outside the realm of the other cluster 1b genomes, but similar to other cluster 3 isolates from *Casuarina* (Table 1). Phylogenetic analysis of the 23S rDNA shows that *Frankia* sp. strain CcI49 groups with the cluster 3 strains (Figure S1). The genome size and corresponding number of CDSs were larger than the typical cluster 1b, but fit within those values reported for cluster 3 genomes [8]. The genome also contained a *nif,* 2 *hup,* and 1 *shc* operons encoding the nitrogenase and uptake hydrogenase enzymes and the hopanoid biosynthetic pathway, respectively. The operons were organized similar to those reported for *Frankia* cluster 3 genomes [8].

Table 1. Genome features of *Frankia* sp. strain CcI49 and other *Frankia* strains isolated from *Casuarina* root nodules.

Strain	Source	Location[1]	Size (Mb)	No. of Contigs	*Frankia* cluster	No. of CDS	Host Plants[2]
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*
CcI3	[24]	USA	5.43	1	1b	4,598	*Casuarinaceae*
CeD	[25]	Senegal	5.00	120	1b	4,403	*Casuarinaceae*
Allo2	[26]	Uruguay	5.33	110	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.

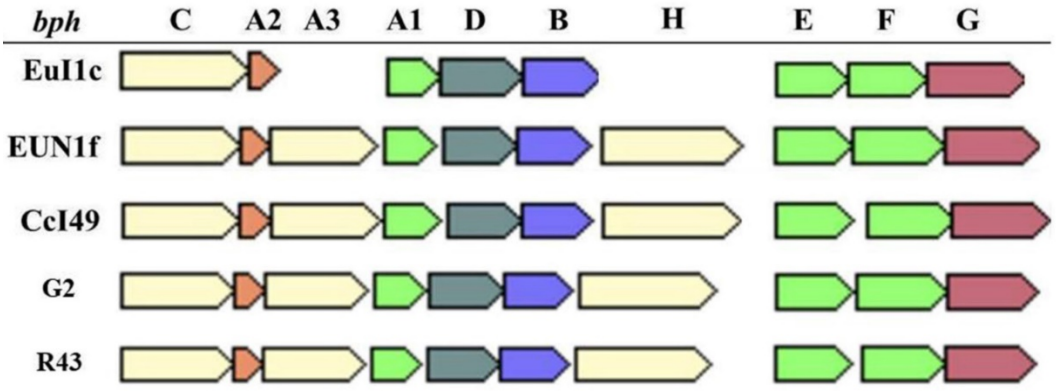

Figure 2. The *bph* operon is present in *Frankia* strains CcI49, EuI1c, G2, R43, and EUN1f. *bph* genes encode the following : BphA, A2 and A3, Biphenyl 2,3-dioxygenase; BphB, *cis* -2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase; BphC, 2,3-dihydroxybiphenyl 1,2-dioxygenase; BphD, 2-hydroxy-6-phenyl-6-oxohexa-2,4-dieneoate (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 [1]	NRPS [2]	PKS [3]	Terpene	Siderophore	Bacteriocin	Lantipeptide
CcI49	3	42	6	17	3	1	2	5
R43	3	38	4	14	3	1	2	4
G2	3	35	8	13	3	1	2	2
KB5	1b	34	4	9	6	1	1	4
CcI3	1b	29	3	5	4	1	3	6
CeD	1b	30	7	7	4	1	1	4
Allo2	1b	32	7	9	4	1	3	5
Thr	1b	33	6	7	4	1	1	6
BMG5.23	1b	31	8	6	4	1	2	4
CcI6	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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