

1 High Quality Draft Genomes of Eight Bacteria Isolated from Fungus Gardens Grown by  
2 *Trachymyrmex septentrionalis* Ants

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10 Running title: Genomes of *T. septentrionalis* fungus garden bacteria

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14    **Abstract:**

15           *Trachymyrmex septentrionalis* ants raise symbiotic fungus gardens as their food source,  
16    which contain bacteria whose functions are poorly understood. We sequenced the genomes of  
17    eight bacteria isolated from these fungus gardens to better describe the ecology of these strains  
18    and their potential to produce secondary metabolites in this niche.

**Genome Announcement (500 words, including accession numbers):**

Fungus-growing ants (tribe Attini) form symbioses with a cultivar fungus (*Leucoagaricus*) that they grow in underground fungus gardens as their essential food source (1). Other bacteria also inhabit these fungus gardens and provide nutrients to the cultivar fungus, at least in some cases (2–5). These bacteria have the genetic potential to produce secondary metabolites that may mediate interspecific interactions in fungus gardens, although this remains poorly understood (6).

*Trachymyrmex septentrionalis* is the northernmost fungus-growing ant, occurring throughout the Eastern USA (7). Its colonies are relatively small (~1000 ants/colony) and subsist largely on caterpillar frass, oak catkins, and some fresh plant material (8). The *T. septentrionalis* fungus garden microbiome remains poorly characterized (9). We therefore isolated several bacteria from *T. septentrionalis* fungus gardens and sequenced their genomes to better understand their potential functions within this symbiotic niche.

*T. septentrionalis* fungus gardens were collected in Florida, New Jersey, and North Carolina following established protocols (10). Fungus garden fragments were resuspended in phosphate-buffered saline (137mM NaCl, 2.7mM KCl, 10mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8mM KH<sub>2</sub>PO<sub>4</sub>) and bacteria were isolated on tryptic soy agar (Difco; adjusted to pH 6) using the spread plate technique. Genomic DNA was extracted from each isolate and their 16S rRNA gene was PCR amplified as described previously (11). PCR amplicons were Sanger sequenced at the University of Connecticut DNA Biotechnology Center and the resulting sequences were compared to the NCBI nr database (12) to identify each strain.

Genomes from eight *T. septentrionalis* fungus garden bacteria were sequenced at the Department of Energy Joint Genome Institute (JGI). Pacific Biosciences (PacBio) SMRTbell libraries were constructed following the manufacture's protocols and sequenced using a PacBio RS instrument. The resulting reads were assembled using the HGAP pipeline (v2.3.0\_p5). Genes were predicted using Prodigal (13) and GenePRIMP (14), and annotated using the IMG nr (15), UniProt (16), TIGRFam (17), Pfam (18), KEGG (19), COG (20), and InterPro (21) databases. Non-coding RNAs were annotated using tRNAScanSE (22), INFERNAL (23), and the IMG's ribosomal RNA gene models (15). Additional gene prediction and annotation was performed using the JGI's IMG ER platform (24).

The sequenced bacteria belong to the genera *Bacillus*, *Burkholderia*, *Micrococcus*, *Pantoea*, and *Serratia*, and a poorly resolved taxon within the Enterobacteriaceae (Table 1). The genome of *Serratia* sp. JKS000199 was assembled into a single contig and is therefore complete. All other genomes were assembled into 2-6 contigs and are therefore high-quality drafts. These bacteria likely include both persistent and transient colonists of *T. septentrionalis* fungus gardens. Their genomes will inform future studies of these strains' ecology in the *T. septentrionalis* symbiosis and how secondary metabolites might mediate interspecific interactions within this niche.

## **Data Availability**

The whole-genome shotgun projects for strains JKS000199, JKS000233, JKS000234, JKS000250, JKS000296, JKS000303, JKS001846, and JKS001869 have been deposited in DDBJ/EMBL/GenBank under the accession numbers listed in Table 1.

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148 Table 1. Accession numbers and metadata for the strains sequenced during this study

Strain name	Collection location	Collection date (d/m/y)	Genome size (Mbp)	Coverage	Number of Contigs	GenBank accession number
<i>Serratia</i> sp. JKS000199	Wekiwa Springs State Park, FL, USA	20/05/13	5.12	178X	1	LT907843.1
Enterobacteriaceae sp. JKS000233	Wekiwa Springs State Park, FL, USA	20/05/13	5.46	117X	6	PEES01000000
Enterobacteriaceae sp. JKS000234	Wekiwa Springs State Park, FL, USA	20/05/13	5.45	70X	5	OCMY01000000
<i>Pantoea</i> sp. JKS000250	Wekiwa Springs State Park, FL, USA	20/05/13	4.87	90X	3	QICZ01000000
<i>Serratia</i> sp. JKS000296	Wekiwa Springs State Park, FL, USA	20/05/13	5.15	93X	4	OCMX01000000
<i>Burkholderia</i> sp. JKS000303	Wharton State Forest, NJ, USA	25/06/14	8.16	99X	6	PDBZ01000000
<i>Bacillus</i> sp. JKS001846	Singletary Lake State Park, NC, USA	10/06/15	5.96	217X	2	FWYG01000000
<i>Micrococcus</i> sp. JKS001869	Paynes Creek Historic State Park, FL, USA	16/11/14	2.59	239X	2	PDBY01000000

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