







High-Quality Draft Genome Sequences of Eight Bacteria Isolated from Fungus Gardens Grown by *Trachymyrmex septentrionalis* Ants

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ABSTRACT For their food source, *Trachymyrmex septentrionalis* ants raise symbiotic fungus gardens that contain bacteria whose functions are poorly understood. Here, we report the genome sequences of eight bacteria isolated from these fungus gardens to better describe the ecology of these strains and their potential to produce secondary metabolites in this niche.

Fungus-growing ants (tribe *Attini*) form symbioses with a cultivar fungus belonging to the genus *Leucoagaricus*, which 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 inter-specific interactions in fungus gardens, although this remains poorly understood (6).

Trachymyrmex septentrionalis is the northernmost fungus-growing ant and occurs throughout the eastern United States (7). Its colonies are relatively small (~1,000 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 (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 1.8 mM KH₂PO₄), 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 genes were 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 nonredundant 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 manufacturer's protocol and sequenced using a PacBio RS instrument. The resulting reads were assembled using the HGAP pipeline version 2.3.0_p5. Genes were predicted using Prodigal (13) and GenePRIMP (14) and annotated using the UniProt (15), TIGRFAMs (16), Pfam (17), KEGG (18), COG (19), InterPro (20), and IMG nonredundant (21) databases. Noncoding RNAs were annotated using tRNAScanSE (22), INFERNAL (23), and the IMG's

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TABLE 1 GenBank accession numbers and metadata for the strains sequenced in this study

Strain name	Collection location	Collection date (day/mo/yr)	Genome size (Mb)	Coverage (×)	No. of contigs	GenBank accession no.
<i>Serratia</i> sp. JKS000199	Wekiwa Springs State Park, FL, USA	20/5/13	5.12	178	1	LT907843
<i>Enterobacteriaceae</i> sp. JKS000233	Wekiwa Springs State Park, FL, USA	20/5/13	5.46	117	6	PEES00000000
<i>Enterobacteriaceae</i> sp. JKS000234	Wekiwa Springs State Park, FL, USA	20/5/13	5.45	70	5	OCMY00000000
<i>Pantoea</i> sp. JKS000250	Wekiwa Springs State Park, FL, USA	20/5/13	4.87	90	3	QICZ00000000
<i>Serratia</i> sp. JKS000296	Wekiwa Springs State Park, FL, USA	20/5/13	5.15	93	4	OCMX00000000
<i>Burkholderia</i> sp. JKS000303	Wharton State Forest, NJ, USA	25/6/14	8.16	99	6	PDBZ00000000
<i>Bacillus</i> sp. JKS001846	Singletary Lake State Park, NC, USA	10/6/15	5.96	217	2	FWYG00000000
<i>Micrococcaceae</i> sp. JKS001869	Paynes Creek Historic State Park, FL, USA	16/11/14	2.59	239	2	PDBY00000000

rRNA gene models (15). Additional gene prediction and annotation were performed using the IMG's ER platform (24).

The sequenced bacteria belong to the genera *Bacillus*, *Burkholderia*, *Pantoea*, and *Serratia* and poorly resolved taxa within the families *Enterobacteriaceae* and *Micrococcaceae* (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 to 6 contigs and are therefore high-quality drafts. These bacteria likely include both persistent and transient colonists of *T. septentrionalis* fungus gardens. The genome sequences of these strains will inform future studies of their ecology in *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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