

# Twenty-One Genome Sequences from *Pseudomonas* Species and 19 Genome Sequences from Diverse Bacteria Isolated from the Rhizosphere and Endosphere of *Populus deltoides*

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To aid in the investigation of the *Populus deltoides* microbiome, we generated draft genome sequences for 21 *Pseudomonas* strains and 19 other diverse bacteria isolated from *Populus deltoides* roots. Genome sequences for isolates similar to *Acidovorax*, *Bradyrhizobium*, *Brevibacillus*, *Caulobacter*, *Chryseobacterium*, *Flavobacterium*, *Herbaspirillum*, *Novosphingobium*, *Pantoea*, *Phyllobacterium*, *Polaromonas*, *Rhizobium*, *Sphingobium*, and *Variovorax* were generated.

Plant-microbe interactions within the rhizosphere have been shown to have important roles in plant health and productivity (1, 4, 8, 20). As part of an ongoing effort to better understand the microbial communities associated with native *Populus deltoides* (Eastern cottonwood), we have undertaken both cultivation-independent and cultivation-dependent assessments of microbial communities from the *P. deltoides* rhizosphere and endosphere (3, 5, 25). Our goal is to understand the diversity of the *Populus* microbiome and to elucidate the metabolic and molecular mechanisms responsible for shaping *Populus*-microbe root interactions.

We sampled *P. deltoides* at sites along the Caney Fork River in central Tennessee and at the Yadkin River in North Carolina. These sites represent ecotypes and soil conditions that are common to this region. Approximately 1,100 diverse bacterial strains were collected to investigate phylogenetic and functional diversity. The isolates comprise 7 classes and 85 genera of bacteria, including *Actinobacterium* (14%), *Bacillus* (17%), *Flavobacterium* (6%), *Sphingobacterium* (3%), *Alphaproteobacteria* (22%), *Betaproteobacteria* (16%), and *Gammaproteobacteria* (22%). A number of our isolates were *Pseudomonas* species, which have considerable genetic and phenotypic variability and include pathogenic, biocontrol, and plant growth-promoting bacteria (14, 21). *Pseudomonads* were also found to be one of the most common operational taxonomic units (OTUs) using direct rRNA gene sequencing of *P. deltoides* endosphere and rhizosphere samples (5). While representatives were chosen for genome sequencing based on overall phenotypic traits and phylogeny, a special emphasis was placed on sequencing *P. fluorescens*-like organisms. In all, 23 strains of *Gammaproteobacteria*, 5 of *Betaproteobacteria*, 8 of *Alphaproteobacteria*, 2 of *Bacteroidetes*, and 2 of *Firmicutes* were sequenced.

Paired-end DNA libraries with an average insert size of 500 bp were created and draft genome data were generated using

Illumina (2) HiSeq2000 technology. CLC Genomics Workbench (version 4.7.1) and FASTQC (13) were applied to trim reads for quality sequence data. Trimmed Illumina data were assembled mostly using the CLC Genomics Workbench (version 4.9), although better assemblies for isolates AP49, BC25, CF314, GM16, and GM49 were generated with Velvet (version 1.2) (26). Draft genome sequences were annotated using the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) integrated microbial genomes database and comparative analysis system (IMG) (15), and contigs of 500 bp or greater were deposited at the National Center for Biotechnology Information GenBank database. The median value for the largest contig in each genome was 249,964 bp, and key attributes for the genome sequences are summarized in Table 1.

Recently, a number of genome sequences for plant growth-promoting and biocontrol bacteria have been reported (6, 7, 9–12, 16–19, 22–24, and 27). The genome sequences reported in this study will facilitate comprehensive comparisons of bacteria involved in plant-microbe interactions.

**Nucleotide sequence accession numbers.** The nucleotide sequences have been deposited in DDBJ/EMBL/GenBank under the accession numbers provided in Table 1, and the versions described in this paper are the first versions. The entire DNA sequence data set has been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession number SRA050017).

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TABLE 1 Summary of genome sequences for 40 rhizosphere and endosphere bacteria

Organism	Origin <sup>a</sup>	Coverage (fold)	No. of contigs	% GC	$N_{50}$	No. of CDS	Genome size (bp)	Accession no.
<i>Acidovorax</i> sp. strain CF316	E	310	315	67	37,104	7,206	7,086,227	<a href="#">AKJX00000000</a>
<i>Bradyrhizobium</i> sp. strain YR681	E	250	351	64	37,719	8,302	7,831,714	<a href="#">AKIY00000000</a>
<i>Brevibacillus</i> sp. strain CF112	E	560	172	54	76,367	5,272	5,271,824	<a href="#">AKKB00000000</a>
<i>Brevibacillus</i> sp. strain BC25	E	650	140	47	68,877	6,021	6,279,079	<a href="#">AKIX00000000</a>
<i>Caulobacter</i> sp. strain AP07	R	390	326	69	30,563	5,373	5,615,958	<a href="#">AKKF00000000</a>
<i>Chryseobacterium</i> sp. strain CF314	E	1,200	119	37	80,113	4,125	4,484,672	<a href="#">AKJY00000000</a>
<i>Flavobacterium</i> sp. strain CF136	E	2,721	113	34	89,762	4,366	5,102,016	<a href="#">AKJZ00000000</a>
<i>Herbaspirillum</i> sp. strain CF444	E	520	125	60	82,125	5,004	5,594,732	<a href="#">AKJW00000000</a>
<i>Herbaspirillum</i> sp. strain YR522	E	530	168	63	54,530	4,949	5,113,042	<a href="#">AKJA00000000</a>
<i>Novosphingobium</i> sp. strain AP12	R	430	187	66	54,713	5,532	5,611,617	<a href="#">AKKE00000000</a>
<i>Pantoea</i> sp. strain GM01	R	680	102	54	91,591	4,998	5,320,548	<a href="#">AKIU00000000</a>
<i>Pantoea</i> sp. strain YR343	R	700	128	55	94,033	4,900	5,314,049	<a href="#">AKIT00000000</a>
<i>Phyllobacterium</i> sp. strain YR531	E	990	41	53	257,533	4,901	4,997,302	<a href="#">AKIZ00000000</a>
<i>Polaromonas</i> sp. strain CF318	E	620	159	65	61,518	4,828	5,008,816	<a href="#">AKIV00000000</a>
<i>Pseudomonas</i> sp. strain GM16	E	2,300	128	59	122,673	5,888	6,550,518	<a href="#">AKJV00000000</a>
<i>Pseudomonas</i> sp. strain GM84	R	256	384	63	24,795	5,295	5,818,772	<a href="#">AKJC00000000</a>
<i>Pseudomonas</i> sp. strain GM24	E	3,200	399	59	32,656	5,885	6,517,063	<a href="#">AKJR00000000</a>
<i>Pseudomonas</i> sp. strain GM102	E	440	159	59	88,165	6,039	6,657,346	<a href="#">AKJB00000000</a>
<i>Pseudomonas</i> sp. strain GM33	E	440	205	60	61,913	6,102	6,727,223	<a href="#">AKJO00000000</a>
<i>Pseudomonas</i> sp. strain GM78	E	469	235	60	57,174	6,724	7,287,561	<a href="#">AKJF00000000</a>
<i>Pseudomonas</i> sp. strain GM80	E	474	282	59	39,805	6,245	6,786,162	<a href="#">AKJD00000000</a>
<i>Pseudomonas</i> sp. strain GM17	E	480	280	63	44,590	6,105	6,786,964	<a href="#">AKJU00000000</a>
<i>Pseudomonas</i> sp. strain GM79	E	486	126	59	96,213	6,062	6,707,010	<a href="#">AKJE00000000</a>
<i>Pseudomonas</i> sp. strain GM67	E	509	183	60	68,050	6,003	6,502,113	<a href="#">AKJH00000000</a>
<i>Pseudomonas</i> sp. strain GM74	R	512	180	60	75,201	5,562	6,104,277	<a href="#">AKJG00000000</a>
<i>Pseudomonas</i> sp. strain GM30	E	520	180	60	59,627	5,611	6,140,967	<a href="#">AKJP00000000</a>
<i>Pseudomonas</i> sp. strain GM41	E	520	164	59	75,073	6,066	6,615,479	<a href="#">AKJN00000000</a>
<i>Pseudomonas</i> sp. strain GM50	E	520	155	59	68,220	6,068	6,692,143	<a href="#">AKJK00000000</a>
<i>Pseudomonas</i> sp. strain GM25	R	530	91	61	137,130	5,758	6,350,607	<a href="#">AKJQ00000000</a>
<i>Pseudomonas</i> sp. strain GM49	R	530	345	60	31,212	6,253	6,589,890	<a href="#">AKJL00000000</a>
<i>Pseudomonas</i> sp. strain GM60	E	540	181	60	62,804	5,911	6,424,244	<a href="#">AKJI00000000</a>
<i>Pseudomonas</i> sp. strain GM21	E	570	210	58	57,752	6,072	6,609,272	<a href="#">AKJS00000000</a>
<i>Pseudomonas</i> sp. strain GM55	E	574	163	60	77,637	5,985	6,489,331	<a href="#">AKJJ00000000</a>
<i>Pseudomonas</i> sp. strain GM18	E	580	140	60	106,048	5,711	6,297,618	<a href="#">AKJT00000000</a>
<i>Pseudomonas</i> sp. strain GM48	R	580	200	59	59,542	5,902	6,443,787	<a href="#">AKJM00000000</a>
<i>Rhizobium</i> sp. strain CF142	E	445	150	60	85,172	7,240	7,457,089	<a href="#">AJWE00000000</a>
<i>Rhizobium</i> sp. strain CF122	E	543	130	60	117,778	6,090	6,142,299	<a href="#">AKKA00000000</a>
<i>Rhizobium</i> sp. strain AP16	E	555	96	60	123,519	6,143	6,497,619	<a href="#">AJVM00000000</a>
<i>Sphingobium</i> sp. strain AP49	E	665	99	63	89,526	4,585	4,479,274	<a href="#">AVL00000000</a>
<i>Variovorax</i> sp. strain CF313	E	2,200	173	67	65,214	5,674	6,029,174	<a href="#">AKIW00000000</a>
Median		530	166	60	68,549	5,895	6,387,426	

<sup>a</sup> E, endosphere; R, rhizosphere.

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## REFERENCES

1. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57:233–266.
2. Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438.
3. Brown SD, et al. 2012. Draft genome sequence of *Rhizobium* sp. strain PDO1-076, a bacterium isolated from *Populus deltoides*. *J. Bacteriol.* 194: 2383–2384.
4. Compant S, Clement C, Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* 42:669–678.
5. Gottel NR, et al. 2011. Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. *Appl. Environ. Microbiol.* 77:5934–5944.
6. Hao K, et al. 2012. The genome of plant growth-promoting *Bacillus amyloliquefaciens* subsp. *plantarum* strain YAU B9601-Y2 contains a gene cluster for mersacidin synthesis. *J. Bacteriol.* 194:3264–3265.
7. Hao X, et al. 2012. Draft genome sequence of plant growth-promoting rhizobium *Mesorhizobium amorphae*, isolated from zinc-lead mine tailings. *J. Bacteriol.* 194:736–737.
8. Hodge A, Berta G, Doussan C, Merchan F, Crespi M. 2009. Plant root growth, architecture and function. *Plant Soil* 321:153–187.
9. Hu D, et al. 2012. Genome sequence of *Streptomyces* sp. strain TOR3209, a rhizosphere microecology regulator isolated from tomato rhizosphere. *J. Bacteriol.* 194:1627.
10. Jeong H, et al. 2011. Draft genome sequence of the *Paenibacillus polymyxa* type strain (ATCC 842<sup>T</sup>), a plant growth-promoting bacterium. *J. Bacteriol.* 193:5026–5027.

11. Kim HJ, et al. 2012. Draft genome sequence of *Pantoea ananatis* B1-9, a nonpathogenic plant growth-promoting bacterium. *J. Bacteriol.* 194:729.
12. Kim HJ, et al. 2011. Draft genome sequence of the biocontrol bacterium *Chromobacterium* sp. strain C-61. *J. Bacteriol.* 193:6803–6804.
13. Kircher M, Heyn P, Kelso J. 2011. Addressing challenges in the production and analysis of Illumina sequencing data. *BMC Genomics* 12:382.
14. Lugtenberg BJJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol.* 39:461–490.
15. Markowitz VM, et al. 2012. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res.* 40:D115–D122.
16. Mathimaran N, Srivastava R, Wiemken A, Sharma AK, Boller T. 2012. Genome sequences of two plant growth-promoting fluorescent *Pseudomonas* strains, R62 and R81. *J. Bacteriol.* 194:3272–3273.
17. Niu B, Rueckert C, Blom J, Wang Q, Borris R. 2011. The genome of the plant growth-promoting rhizobacterium *Paenibacillus polymyxa* M-1 contains nine sites dedicated to nonribosomal synthesis of lipopeptides and polyketides. *J. Bacteriol.* 193:5862–5863.
18. Park JY, et al. 2011. Draft genome sequence of the biocontrol bacterium *Pseudomonas putida* B001, an oligotrophic bacterium that induces systemic resistance to plant diseases. *J. Bacteriol.* 193:6795–6796.
19. Redondo-Nieto M, et al. 2012. Genome sequence of the biocontrol strain *Pseudomonas fluorescens* F113. *J. Bacteriol.* 194:1273–1274.
20. Reinhold-Hurek B, Hurek T. 2011. Living inside plants: bacterial endophytes. *Curr. Opin. Plant Biol.* 14:435–443.
21. Rojo F. 2010. Carbon catabolite repression in *Pseudomonas*: optimizing metabolic versatility and interactions with the environment. *FEMS Microbiol. Rev.* 34:658–684.
22. Rong X, Baysal Gurel F, Meulia T, McSpadden Gardener BB. 2012. Draft genome sequences of the *Pseudomonas fluorescens* biocontrol strains Wayne1R and Wood1R. *J. Bacteriol.* 194:724–725.
23. Rong X, Gurel F-B, Meulia T, McSpadden Gardener BB. 2012. Draft genome sequences of the biocontrol bacterium *Mitsuaria* sp. strain H24L5A. *J. Bacteriol.* 194:734–735.
24. Song JY, et al. 2012. Genome sequence of the plant growth-promoting rhizobacterium *Bacillus* sp. strain JS. *J. Bacteriol.* 194:3760–3761.
25. Weston DJ, et al. 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant responses in defense networks, primary metabolism, photosynthesis, and fitness. *Mol. Plant Microbe Interact.* 25: 765–778.
26. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829.
27. Zhu B, et al. 2012. Genome sequence of *Stenotrophomonas maltophilia* RR-10, isolated as an endophyte from rice root. *J. Bacteriol.* 194:1280–1281.