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GENOME SEQUENCES

Complete Genome Sequences of *Brenneria rubrifaciens* Strain 6D370 and *Brenneria nigrifluens* Strain ATCC 13028, Causative Agents of Bark Cankers in Walnut

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ABSTRACT *Brenneria rubrifaciens* and *Brenneria nigrifluens*, respectively, cause deep and shallow bark canker disease in walnut. *B. rubrifaciens* exhibits quorum sensing-controlled virulence and rubrifacine pigment production. The complete genome sequences of these species will be useful for studying the role of genes regulated by quorum sensing, including pathways mediating pathogenesis.

Brenneria rubrifaciens and *Brenneria nigrifluens*, respectively, cause deep and shallow bark cankers in English walnut (1, 2). While these cankers do not kill the trees, they weaken trunks and branches over time and, combined with other stresses, reduce walnut production. These bacterial species can coinfect walnut and often live as quiescent endophytes that become pathogenic when trees experience environmental stress (3, 4). Here, we report the genome sequences of *B. rubrifaciens* strain 6D370, isolated by Azad and Kado (5), and *B. nigrifluens* strain ATCC 13028, isolated by Wilson et al. (2). Both species were isolated from symptomatic walnut trees in the Central Valley of California (2, 5).

Brenneria rubrifaciens strain 6D370 is available upon request. *B. nigrifluens* strain ATCC 13028 was obtained from the American Type Culture Collection. For both strains, a single colony was inoculated into tryptic soy broth medium and incubated for 24 hours at 28°C and 210 rpm. Genomic DNA was isolated using the MasterPure complete DNA purification kit (Epicentre, Madison, WI) and submitted to MR DNA (Shallowater, TX) for library preparation and 2 × 250-bp paired-end sequencing on an Illumina MiSeq instrument. This generated ~4.7 million (*B. rubrifaciens*) and 5.7 million (*B. nigrifluens*) paired-end reads. We also sequenced both genomes at the Michigan State University Research Technology Support Facility (RTSF) Genomics Core on a GridION instrument using one flow cell and the RBK004 rapid barcoding kit (Oxford Nanopore Technologies, San Francisco, CA) and obtained 2 Gbp (*B. rubrifaciens*) and 0.42 Gbp (*B. nigrifluens*) with average lengths of 10,925 and 10,783 bases, respectively. Raw sequences from both platforms were quality trimmed, filtered, and used for hybrid assembly via Unicycler v0.4.4 under default parameters (6). This yielded complete circular chromosomes of 4.03 Mbp (G+C content, 52.6%) for *B. rubrifaciens* strain 6D370 and 4.89 Mbp (G+C content, 55.9%) for *B. nigrifluens* strain ATCC 13028.

Both genomes were annotated via the NCBI Prokaryotic Genome Annotation Pipeline (7). The *B. rubrifaciens* genome contained 3,613 predicted genes, including 3,354 protein-coding sequences, 7 complete rRNA gene operons, 73 tRNAs, 5 noncoding RNAs (ncRNAs), and 159 pseudogenes. The *B. nigrifluens* genome consisted of 4,600 predicted genes, including 4,366 protein-coding sequences, 7 complete rRNA gene operons, 72 tRNAs, 7 ncRNAs, and 163 pseudogenes. We previously identified quorum-sensing (QS) genes encoding an acyl homoserine lactone (AHL) synthase (*bruI*) and an AHL-responsive transcription factor (*bruR*) in *B. rubrifaciens* that control production of

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the red pigment rubrifacine and virulence (4, 8, 9). *B. rubrifaciens* with a disrupted *brul* is rubrifacine negative, but growth of the mutant in the presence of *B. nigrifluens*, which produces similar QS signal molecules, restores pigment production (4). Homologs of *brul* and *bruR* were present in *B. nigrifluens* and are candidates for future studies to assess the role of QS in pathogenicity on walnut. Both genomes also encoded type III secretion systems (10). The *B. rubrifaciens* genome harbored two nearly identical gene clusters encoding a type IV secretion system (11). These secretion systems are important for virulence in plant-pathogenic bacteria and may be involved in enabling the development of the bark cankers caused by these *Brenneria* species. The complete genome sequences of both species also will facilitate our understanding of how environmental and physiological factors affect development of bark canker diseases in walnut.

Data availability. The complete genome sequences are available in the NCBI database under BioProject number PRJNA478423 and GenBank accession numbers CP034035 (*B. rubrifaciens* strain 6D370) and CP034036 (*B. nigrifluens* strain ATCC 13028). The raw sequence reads are available under the Sequence Read Archive accession number SRP199005. The versions described in this paper are the first versions.

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REFERENCES

1. Wilson EE, Zeitoun FM, Frederickson DL. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618–621.
2. Wilson EE, Starr MP, Berger JA. 1957. Bark canker, a bacterial disease of the Persian walnut tree. Phytopathology 47:669–673.
3. Morone C, Janse JD, Scortichini M. 1998. Bark canker of Persian walnut (*Juglans regia*) trees incited by *Erwinia nigrifluens* in Italy. J Phytopathol 146:637–639. https://doi.org/10.1111/j.1439-0434.1998.tb04766.x.
4. McClean AE, Kluepfel DA. 2009. Genetic loci involved in rubrifacine production the walnut pathogen *Brenneria rubrifaciens*. Phytopathology 99:145–151. https://doi.org/10.1094/PHYTO-99-2-0145.
5. Azad HR, Kado CI. 1980. Numerical and DNA: DNA reassociation analyses of *Erwinia rubrifaciens* and other members of the *Enterobacteriaceae*. J Gen Microbiol 120:117–129. https://doi.org/10.1099/00221287-120-1-117.
6. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
7. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt K, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
8. Feistner G, Budzikiewicz H. 1985. On the structure of rubrifacine. Can J Chem 63:495–499. https://doi.org/10.1139/v85-080.
9. McClean AE, Duerkop BA, Greenberg EP, Kluepfel DA. 2012. AHL signals induce rubrifacine production in a *brul* mutant of *Brenneria rubrifaciens*. Phytopathology 102:195–203. https://doi.org/10.1094/PHYTO-04-11-0111.
10. Büttner D, He SY. 2009. Type III protein secretion in plant pathogenic bacteria. Plant Physiol 150:1656–1664. https://doi.org/10.1104/pp.109.139089.
11. Wallden K, Rivera-Calzada A, Waksman G. 2010. Type IV secretion systems: versatility and diversity in function. Cell Microbiol 12:1203–1212. https://doi.org/10.1111/j.1462-5822.2010.01499.x.