Metagenome-Assembled Draft Genome Sequence of a Novel Microbial Stenotrophomonas maltophilia Strain Isolated from Caenorhabditis remanei Tissue

Janna L. Fierst, a Duncan A. Murdock, a Chamali Thanthiriwatte, a John H. Willis, b Patrick C. Phillips b

Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama, USA a; Institute for Ecology and Evolution and Department of Biology, The University of Oregon, Eugene, Oregon, USA b

ABSTRACT Stenotrophomonas maltophilia is a Gram-negative aerobic bacterium and emerging nosocomial pathogen. Here, we present a draft genome sequence for an S. maltophilia strain assembled from a metagenomic DNA extract isolated from a laboratory stock of the nematode worm Caenorhabditis remanei. S. maltophilia is a ubiquitous aerobe found in clinical samples and soil environments (1). It is the most frequent Gram-negative microbe found in hospitals after Acinetobacter sp. and Pseudomonas sp. (2) and a source of dangerous nosocomial infections (3), due to its genomic repertoire of drug-resistance systems (4) and ability to adhere to plastics and form biofilms (5). S. maltophilia has been found with natural isolates of the nematode Pristionchus (6) but is a lethal pathogen of C. elegans in the laboratory (7). Lethality is strain-specific, and S. maltophilia soil isolates result in high C. elegans mortality, while the clinical type sample K279a causes low mortality similar to the standard laboratory C. elegans food source Escherichia coli OP50 (8). C. remanei strain PX356 is an inbred population derived from the Caenorhabditis Genetic Center strain EM464 and has been maintained in the laboratory for >50 generations. The S. maltophilia-nematode association is an intriguing system for studying host–pathogen interactions and coevolution in a clinically important bacterium.

Sequencing libraries were prepared as described previously (9). Briefly, genomic DNA was isolated from starved L1 C. remanei larvae and mixed stage populations with the DNeasy blood and tissue kit (Qiagen) following the C. elegans supplementary protocol. Paired-end libraries were constructed with the Nextera kit (Illumina) and size-selected on 2% agarose gels for an average genomic insert size of 180 bp. Mate-pair libraries were constructed by shearing genomic DNA using a Bioruptor sonicator (Diagenode) and purifying with the desalting and concentrating DNA section for the QIAEX-II kit (Qiagen). End repair was performed with the End-it kit (Epicenter). Genomic DNA was biotin-labeled with 1 mM dNTP (4% biotin), and 3-, 5-, and 7-kb fragments were isolated and purified with the QIAEX II kit. Libraries were circularized overnight using T3 ligase (Enzymatics) and T4 ligase buffer. DNA was sheared to 400 bp, and biotin-labeled fragments were isolated with Dynabeads M-280 strepavidin (Invitrogen). All libraries were sequenced as 2 × 101-nucleotide paired-end reads with an Illumina HiSeq instrument.

Assembly of the sequenced libraries with ALLPATHS-LG (10) produced ~18 Mb of sequence data, identified with BLAST (11) to be of nonnematode origin. The Blobology protocol (12) was used to assemble the S. maltophilia genome sequence. Briefly, short contiguous sequences were assembled with ABYSS (13) and assigned taxonomic origin.
with BLAST (11). Sequence reads were assigned to 32 species of Alpha-, Beta-, and Gammaproteobacteria with >80% of the microbial sequences originating from Xanthomonadales spp.; 14,267,624 Xanthomonadales sequence reads were isolated and assembled with ALLPATHS-LG. The resulting genome sequence was 4,602,647 bp (310 × coverage; 66.23% GC) contained in two scaffolds.

Functional annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (14) and the RAST annotation server (15). The genome contained 4,142 genes and 4,068 coding sequences. Functional annotation identified six rRNAs, 64 tRNAs, four noncoding RNAs, and 34 pseudogenes. Phylogenetic analysis of the 16S ribosomal sequence indicated that this strain of _S. maltophilia_ is novel and closely related to _S. maltophilia_ ZZ7, isolated from marigold soils (16).

**Accession number(s)**

The genome sequence is available from the NCBI GenBank database under BioProject PRJNA248909, BioSample SAMN06040735, and accession number **MP5I00000000** (_S. maltophilia_ strain SIDR01).

**ACKNOWLEDGMENTS**

This work was supported by NIH grant R01 GM102511 to P.C.P. J.L.F., D.A.M., and C.T. were supported by University of Alabama (UA) Startup Funds and a UA RGC Level 2 grant.

**REFERENCES**

1. Minkwitz A, Berg G. 2001. Comparison of antifungal activities and 16S ribosomal sequences of clinical and environmental isolates of _Stenotrophomonas maltophilia_. J Clin Microbiol 39:139–145. https://doi.org/10.1128/JCM.39.1.139-145.2001.

2. Sader HS, Jones RN. 2005. Antimicrobial susceptibility of uncommonly isolated non-entering Gram-negative bacilli. Int J Antimicrob Agents 25:95–109. https://doi.org/10.1016/j.ijantimicag.2004.10.002.

3. Lockhart SR, Abramson MA, Beekman SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV. 2007. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. _J Clin Microbiol_ 45: 3352–3359. https://doi.org/10.1128/JCM.01284-07.

4. Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebahia M, Saunders D, Arrowsmith C, Carver T, Peters N, Adlem E, Kerhornou A, Lord A, Murphy L, Seeger K, Squares R, Rutter S, Quail MA, Rajandream MA, Harris D, Churcher C, Bentley SD, Parkhill J, Thomson NR, Avison MB. 2008. The complete genome, comparative and functional analysis of _Stenotrophomonas maltophilia_ reveals an organism heavily shielded by drug resistance determinants. Genome Biol 9:R74. https://doi.org/10.1186/gb-2008-9-4-r74.

5. De Oliveira-Garcia D, Dall’Agnol M, Rosales M, Azzuz ACGS, Alcântara N, Martinez MB, Giron JA. 2003. Fimbriae and adherence of _Stenotrophomonas maltophilia_ to epithelial cells and to abiotic surfaces. _Cell Microbiol_ 5:625–636. https://doi.org/10.1046/j.1462-5822.2003.00306.x.

6. Rae R, Riebesell M, Dinkelacker I, Wang Q, Herrmann M, Weller AM, Dieterich C, Sommer RJ. 2008. _Stenotrophomonas maltophilia_: pathogenesis model using _Caenorhabditis elegans_. _J Med Microbiol_ 62: 1777–1779. https://doi.org/10.1099/jmm.0.063230-0.

7. Thomas R, Hamat RA, Neela V. 2013. _Stenotrophomonas maltophilia_ strain evades a major _Caenorhabditis elegans_ defense pathway. _Infec Immum_ 81:524–536. https://doi.org/10.1128/IAI.00711-15.

8. White CV, Darby BJ, Breeden RJ, Herman MA. 2016. _A Stenotrophomonas maltophilia_ strain evades a major _Caenorhabditis elegans_ defense pathway. _Infec Immum_ 84:524–536. https://doi.org/10.1128/IAI.00711-15.

9. Fierst JL, Willis JH, Thomas CG, Wang W, Reynolds RM, Aheearne TE, Cutter AD, Phillips PC. 2015. Reproductive mode and the evolution of genome size and structure in _Caenorhabditis_ nematodes. _PLoS Genet_ 11:e1005323. https://doi.org/10.1371/journal.pgen.1005323.

10. Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Ginike A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. _Proc Natl Acad Sci U S A_ 108:1513–1518. https://doi.org/10.1073/pnas.1017351108.

11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. _J Mol Biol_ 215:403–410. https://doi.org/10.1016/S0022-2836(95)80360-2.

12. Kumar S, Jones M, Koutsovoulos G, Clarke M, Blaxter M. 2013. Blobology: exploring raw genome data for contaminants, symbionts and parasites using taxon-annotated GC-coverage plots. _Front Genet_ 4:237. https://doi.org/10.3389/fgene.2013.00237.

13. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. _Genome Res_ 19:1117–1123. https://doi.org/10.1101/gr.089532.108.

14. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. _Nucleic Acids Res_ 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.

15. Aziz RK, Bartels D, Best AA, DeLongh M, Disz T, Edwards RA, Formskma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. _BMC Genomics_ 9:75. https://doi.org/10.1186/1471-2164-9-75.

16. Zahir Z, Seed KD, Dennis JJ. 2006. Isolation and characterization of novel organic solvent-tolerant bacteria. _Extremophiles_ 10:129–138. https://doi.org/10.1007/s00792-005-0483-y.