AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Draft Genome Sequence of Two *Sphingopyxis* sp. Strains, Dominant Members of the Bacterial Community Associated with a Drinking Water Distribution System Simulator

Vicente Gomez-Alvarez, Stacy Pfaller, Randy P. Revetta

U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio, USA

We report the draft genomes of two *Sphingopyxis* sp. strains isolated from a chloraminated drinking water distribution system simulator. Both strains are ubiquitous residents and early colonizers of water distribution systems. Genomic annotation identified a class 1 integron (*intI1*) gene associated with sulfonamide (*sul1*) and puromycin (*pac*) antibiotic resistance genes.

Received 9 February 2016 **Accepted** 9 February 2016 **Published** 31 March 2016

Citation Gomez-Alvarez V, Pfaller S, Revetta RP. 2016. Draft genome sequence of two *Sphingopyxis* sp. strains, dominant members of the bacterial community associated with a drinking water distribution system simulator. Genome Announc 4(2):e00183-16. doi:10.1128/genomeA.00183-16.

Address correspondence to Vicente Gomez-Alvarez, Gomez-Alvarez.Vicente@epa.gov.

Members of the genus *Sphingopyxis* (*Alphaproteobacteria*) are strictly aerobic, chemoheterotrophic, yellow-pigmented, rod-shaped Gram-negative bacteria that contain glycosphingolipids as cell envelope components (1). *Sphingopyxis* spp. have been isolated from a variety of environments, including wetland (2), sludge (3), seawater (4), natural mineral water (5), and soil (6). Recent reports have confirmed the presence and dominance of *Sphingopyxis* spp. in drinking water distribution systems (DWDS) (7–9), and their ability as early colonizers to form biofilms (10, 11). Little information is available about their ecological role in DWDS or potential to cause public health problems (12, 13).

Two strains (H107 and H115) were isolated from a chloraminated DWDS simulator (11), by plating biofilm on R2A plates for 7 days at 27°C. Both strains were identified (100% sequence homology) as dominant members of the bulk water and biofilm community (7). Phylogenetic analysis of 16S rRNA genes indicated that strains H107 and H115 should be classified in the genus *Sphingopyxis* and were closely related to *Sphingopyxis soli* BL03 (99.5%) (14) and *Sphingopyxis chilensis* S37 (99.1%) (15), respectively.

Genomic DNA was extracted using the Ultra-Clean DNA microbial isolation kit (MoBio Laboratories, Solana Beach, CA) and sequenced by rapid mode sequencing on the HiSeq 2500 platform (Illumina Inc., San Diego, CA) using a paired-end 125 bp Nextera XT DNA library. Prior to assembly, libraries were (i) cleaned from contaminants (adapters, phiX, artifacts, and human), (ii) error corrected via Tadpole, (iii) normalized to ≤100×, (iv) removed of low (<6×) coverage reads, and (v) filtered to a minimum length read of 125 nucleotides (nt). Reads were processed using the software package BBMap v35.34 (http://sourceforge.net/projects/bbmap) and *de novo* assembly with SPAdes v3.5.0 (16). The draft genomes of strains H107 and H115 consist of 57 and 63 contigs for a total of 4,308,137 and 4,493,891 bp with a G+C content of 64.84 and 64.33%, respectively. Genome assemblies were annotated with Prokka v1.10 (17) available as an application in Illumina BaseSpace Labs. The genome sequence of strain H107 contains 4,244 genes, 4,191 coding sequences (CDSs), 3 rRNAs, and 50 tRNAs, and strain H115 contains 4,327 genes, 4,277 CDSs, 3 rRNAs, and 47 tRNAs.

The average nucleotide identity (ANI) between the two *Sphingopyxis* strains, a similarity index between two genomes (18), is 83.018% and was calculated using the online calculator available from EzGenome (http://www.ezbiocloud.net/ezgenome/ani). The proposed cut-off for species is 95% to 96% (19). Comparison against reference genomes estimated an ANI average of 82.931% with *Sphingopyxis alaskensis* RB2256 (20), 82.777% with *Sphingopyxis fribergensis* Kp5.2 (21), and 69.271% with *Sphingopyxis baekryungensis* DSM 16222 (22).

Genomic annotation of the environmental H115 strain confirmed the presence of the class 1 integrase (*intI1*) gene associated with the dihydropteroate synthase (*sul1*) gene, encoding resistance to sulfonamide (23). In addition, *sul1* gene was found linked to the puromycin-N-acetyltransferase (*pac*) gene. The *pac* gene encodes resistance to puromycin, an aminonucleoside antibiotic whose mode of action is distinct from the dihydropteroate synthase (24). Class 1 integrons are often embedded in plasmids and transposons, facilitating the lateral transfer of antibiotic resistance genes among bacteria (25).

Nucleotide sequence accession numbers. The whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers LNSJ00000000 and LNSA00000000. The versions described in this paper are the first versions, LNSJ01000000 and LNSA01000000.

ACKNOWLEDGMENTS

We thank Jonathan Pressman and David Wahman for valuable discussions and assistance for this project.

The opinions expressed are those of the authors, and do not necessarily reflect the official positions and policies of the U.S. EPA. Any mention of product or trade names does not constitute recommendation for use by the U.S. EPA.

FUNDING INFORMATION

The U.S. EPA, through the Office of Research and Development, funded and managed this research.

REFERENCES

1. **Takeuchi M, Hamana K, Hiraishi A**. 2001. Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. Int J Syst Evol Microbiol **51:**1405–1417. http://dx.doi.org/10.1099/00207713-51-4-1405.
2. **Baik KS, Choe HN, Park SC, Hwang YM, Kim EM, Park C, Seong CN**. 2013. *Sphingopyxis rigui* sp. nov. and *Sphingopyxis wooponensis* sp. nov., isolated from wetland freshwater, and emended description of the genus *Sphingopyxis*. Int J Syst Evol Microbiol **63:**1297–1303. http://dx.doi.org/10.1099/ijs.0.044057-0.
3. **Kämpfer P, Witzenberger R, Denner EB, Busse HJ, Neef A**. 2002. *Sphingopyxis witflariensis* sp. nov., isolated from activated sludge. Int J Syst Evol Microbiol **52:**2029–2034. http://dx.doi.org/10.1099/00207713-52-6-2029.
4. **Kim BS, Lim YW, Chun J**. 2008. *Sphingopyxis marina* sp. nov. and *Sphingopyxis litoris* sp. nov., isolated from seawater. Int J Syst Evol Microbiol **58:**2415–2419. http://dx.doi.org/10.1099/ijs.0.65614-0.
5. **Lee JS, Shin YK, Yoon JH, Takeuchi M, Pyun YR, Park YH**. 2001. *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov., and *Sphingomonas taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. Int J Syst Evol Microbiol **51:**1491–1498. http://dx.doi.org/10.1099/00207713-51-4-1491.
6. **Lee HW, Ten IL, Jung HM, Liu QM, Im WT, Lee ST**. 2008. *Sphingopyxis panaciterrae* sp. nov., isolated from soil from ginseng field. J Microbiol Biotechnol **18:**1011–1015.
7. **Gomez-Alvarez V, Pfaller S, Revetta RP**. 2015. Comparative genomic analysis of nontuberculous mycobacteria (NTM) and environmental isolates associated with a simulated chloraminated drinking water distribution system subjected to episodes of nitrification. *In* Final Program of the 115th General Meeting of the American Society for Microbiology. Washington, DC.
8. **Homonnay ZG, Török G, Makk J, Brumbauer A, Major E, Márialigeti K, Tóth E**. 2014. Bacterial communities in the collection and chlorinated distribution sections of a drinking water system in Budapest, Hungary. J Basic Microbiol **54:**729–738. http://dx.doi.org/10.1002/jobm.201300960.
9. **Li D, Li Z, Yu J, Cao N, Liu R, Yang M**. 2010. Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water. Appl Environ Microbiol **76:**7171–7180. http://dx.doi.org/10.1128/AEM.00832-10.
10. **Douterelo I, Sharpe R, Boxall J**. 2014. Bacterial community dynamics during the early stages of biofilm formation in a chlorinated experimental drinking water distribution system: implications for drinking water discolouration. J Appl Microbiol **117:**286–301. http://dx.doi.org/10.1111/jam.12516.
11. **Revetta RP, Gomez-Alvarez V, Gerke TL, Curioso C, Santo Domingo JW, Ashbolt NJ**. 2013. Establishment and early succession of bacterial communities in monochloramine-treated drinking water biofilms. FEMS Microbiol Ecol **86:**404–414. http://dx.doi.org/10.1111/1574-6941.12170.
12. **Lee J, Lee CS, Hugunin KM, Maute CJ, Dysko RC**. 2010. Bacteria from drinking water supply and their fate in gastrointestinal tracts of germ-free mice: a phylogenetic comparison study. Water Res **44:**5050–5058. http://dx.doi.org/10.1016/j.watres.2010.07.027.
13. **Vaz-Moreira I, Nunes OC, Manaia CM**. 2011. Diversity and antibiotic resistance patterns of *Sphingomonadaceae* isolates from drinking water. Appl Environ Microbiol **77:**5697–5706. http://dx.doi.org/10.1128/AEM.00579-11.
14. **Choi JH, Kim MS, Jung MJ, Roh SW, Shin KS, Bae JW**. 2010. *Sphingopyxis soli* sp. nov., isolated from landfill soil. Int J Syst Evol Microbiol **60:**1682–1686. http://dx.doi.org/10.1099/ijs.0.013128-0.
15. **Godoy F, Vancanneyt M, Martínez M, Steinbüchel A, Swings J, Rehm BH**. 2003. *Sphingopyxis chilensis* sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of *Sphingomonas alaskensis* to *Sphingopyxis alaskensis* comb. nov. Int J Syst Evol Microbiol **53:**473–477. http://dx.doi.org/10.1099/ijs.0.02375-0.
16. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA**. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **19:**455–477. http://dx.doi.org/10.1089/cmb.2012.0021.
17. **Seemann T**. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30:**2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.
18. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM**. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol **57:**81–91. http://dx.doi.org/10.1099/ijs.0.64483-0.
19. **Richter M, Rosselló-Mora R**. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA **106:**19126–19131. http://dx.doi.org/10.1073/pnas.0906412106.
20. **Lauro FM, McDougald D, Thomas T, Williams TJ, Egan S, Rice S, DeMaere MZ, Ting L, Ertan H, Johnson J, Ferriera S, Lapidus A, Anderson I, Kyrpides N, Munk AC, Detter C, Han CS, Brown MV, Robb FT, Kjelleberg S, Cavicchioli R**. 2009. The genomic basis of trophic strategy in marine bacteria. Proc Natl Acad Sci USA **106:**15527–15533. http://dx.doi.org/10.1073/pnas.0903507106.
21. **Oelschlägel M, Rückert C, Kalinowski J, Schmidt G, Schlömann M, Tischler D**. 2015. *Sphingopyxis fribergensis* sp. nov., a soil bacterium with the ability to degrade styrene and phenylacetic acid. Int J Syst Evol Microbiol **65:**3008–3015. http://dx.doi.org/10.1099/ijs.0.000371.
22. **Yoon JH, Lee CH, Yeo SH, Oh TK**. 2005. *Sphingopyxis baekryungensis* sp. nov., an orange-pigmented bacterium isolated from seawater of the Yellow Sea in Korea. Int J Syst Evol Microbiol **55:**1223–1227. http://dx.doi.org/10.1099/ijs.0.63495-0.
23. **Sköld O**. 2000. Sulfonamide resistance: mechanisms and trends. Drug Resist Updat **3:**155–160. http://dx.doi.org/10.1054/drup.2000.0146.
24. **Lacalle RA, Pulido D, Vara J, Zalacaín M, Jiménez A**. 1989. Molecular analysis of the *pac* gene encoding a puromycin N-acetyl transferase from *Streptomyces alboniger*. Gene **79:**375–380. http://dx.doi.org/10.1016/0378-1119(89)90220-5.
25. **Gillings M, Boucher Y, Labbate M, Holmes A, Krishnan S, Holley M, Stokes HW**. 2008. The evolution of class 1 integrons and the rise of antibiotic resistance. J Bacteriol **190:**5095–5100. http://dx.doi.org/10.1128/JB.00152-08.