AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of *Buttiauxella* spp. Isolates from Water and Gastropods with Putative β-D-Glucuronidase Activity

Carolin Leister,[a] Michael Hügler[a]

[a]TZW: DVGW-Technologiezentrum Wasser, Karlsruhe, Germany

ABSTRACT We report the draft genome sequences of *Buttiauxella* spp. strains that were isolated from water and gastropods. Three isolates show fluorescence in the Colilert system, indicating unusual β-D-glucuronidase activity, and phylogenetic analyses suggest that they represent a novel species. Another strain, without β-D-glucuronidase activity, was assigned to the species *Buttiauxella ferragutiae*.

Microbial water quality is examined using fecal indicator bacteria such as coliform bacteria and *Escherichia coli*. For their detection, β-D-galactosidase and β-D-glucuronidase activities are tested with membrane-filtration-based water quality tests or most probable number methods like the Colilert system (1, 2). *E. coli* is the most important indicator of fecal water contamination, and the presence of β-D-glucuronidase activity is considered indicative of *E. coli* (2, 3). However, other members of the *Enterobacteriaceae* family, such as certain strains of *Salmonella*, *Klebsiella*, *Citrobacter*, *Shigella*, and *Yersinia*, also possess this enzyme, resulting in false-positive *E. coli* results (3–5). Here, we report draft genome sequences of *Buttiauxella* isolates that showed false-positive *E. coli* results in water analyses.

Buttiauxella spp. strains were isolated from a drinking water sample from a small village near Bruchsal, Germany, and from feces from the gastropods *Arion vulgaris* and *Helix pomatia*, collected near Bruchsal (Table 1), using the Colilert-18/Quanti-Tray (IDEXX Laboratories, USA) according to ISO 9308-2:2012 (6). To obtain single colonies, liquid from the wells of the Colilert-18/Quanti-Tray was transferred onto heterotrophic plate count (HPC) agar plates (Merck KGaA, Darmstadt, Germany) (7), as recommended by German regulations (Deutsches Einheitsverfahren), and incubated for 24 h at 36°C. Bacterial isolates were picked, transferred to fresh HPC agar plates, and again incubated for 24 h at 36°C. Genomic DNA of pure cultures grown on these agar plates was extracted using the FastDNA SPIN kit for soil (MP Biomedicals, USA) and quantified using a Qubit fluorometer (Invitrogen, USA) according to the manufacturer's instructions.

Genome sequencing was performed as described previously (8). Preparation of sequencing libraries was performed using a DNA preparation kit (Illumina). Draft genomes were sequenced by 150-bp paired-end sequencing on an Illumina NextSeq 1000 system using NovaGene (Illumina). Reads were trimmed using Cutadapt v1.16.6 (9) and quality controlled using FastQC v0.72 (https://github.com/s-andrews/FastQC). High-quality sequence reads were assembled *de novo* using Unicycler v0.4.6.0 (10), which includes SPAdes v3.12.0 (11). Annotation was carried out using RASTtk v2.0 (12, 13) and NCBI PGAP v5.0 (14, 15). Phylogeny was determined with the codon-tree pipeline in PATRIC v3.6.9 (16, 17), which uses single-copy cross-genus protein families and analyzes aligned proteins and coding DNA from single-copy genes using RAxML v8.0.0 (18). To confirm species, the average nucleotide identity (ANI) was calculated using OrthoANI (19). Default parameters were used for all software unless otherwise noted. Genome sizes and additional information are presented in Table 1.

Phylogenetic analyses and ANI values suggest that three of the isolates (S04-F03, W03-F01, and A2-C1_F) represent a new species of the genus *Buttiauxella*, while the fourth strain

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Address correspondence to Michael Hügler, michael.huegler@tzw.de.

The authors declare no conflict of interest.

Received 25 January 2022
Accepted 16 February 2022
Published 2 March 2022

TABLE 1 Characteristics and accession numbers of genomes from *Buttiauxella* spp.

Bacterial species	Strain	Sample	Sampling date (yr-mo-day)	*uidA*	No. of reads	Genome coverage (×)	Genome size (bp)	No. of contigs	N_{50} (bp)	G+C content (%)	No. of coding sequences	GenBank accession no.	SRA accession no.
Buttiauxella sp.	S04-F03	*Arion vulgaris*	2017-06-06	+	592,495	36.3	4,799,994	36	310,867	50.1	4,365	JAJSOS000000000	SRR17259675
Buttiauxella sp.	W03-F01	*Helix pomatia*	2017-06-06	+	556,906	34.1	4,785,902	25	398,126	50.0	4,350	JAJSOT000000000	SRR17259674
Buttiauxella sp.	A2-C1_F	Water	2016-11-10	+	504,641	30.9	4,830,451	36	290,519	50.1	4,406	JAJSOU000000000	SRR17259673
Buttiauxella ferragutiae	A2-C2_NF	Water	2016-11-10	−	549,687	33.7	5,218,071	61	324,474	50.6	4,747	JAJSOV000000000	SRR17259672

(A2-C2_NF) could be assigned to the species *Buttiauxella ferragutiae*. The analyses further confirmed the presence of the *uidA* gene, coding for β-D-glucuronidase, in the three isolates with fluorescence in the Colilert-18/Quanti-Tray test (S04-F03, W03-F01, and A2-C1_F) and its absence in the fourth isolate (A2-C2_NF). Glucuronidase activity has also been demonstrated in *Buttiauxella noackiae* MCE (formerly *Buttiauxella agrestis*), which was isolated from surface water (2). Thus, certain *Buttiauxella* isolates from environmental sources that harbor β-D-glucuronidase can lead to false-positive *E. coli* results in drinking water testing.

Data availability. The whole-genome shotgun projects and the raw sequence reads have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1. They belong to the BioProject PRJNA789423. For all sequences, the first versions of the accession numbers are described in this paper.

ACKNOWLEDGMENTS

This research was funded by the German Federal Ministry of Education and Research (BMBF grant 161L0285B, project MultiKulti) and the German Technical and Scientific Association (DVGW grant W 201823).

REFERENCES

1. Kilian M, Bülo P. 2009. Rapid diagnosis of *Enterobacteriaceae*. I. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand 84B:245–251. https://doi.org/10.1111/j.1699-0463.1976.tb01933.x.
2. Jothikumar N, Kahler A, Strockbine N, Gladney L, Hill VR. 2014. Draft genome sequence of *Buttiauxella agrestis*, isolated from surface water. Genome Announc 2:e01060-14. https://doi.org/10.1128/genomeA.01060-14.
3. Molina F, López-Acedo E, Tabla R, Roa I, Gómez A, Rebollo JE. 2015. Improved detection of *Escherichia coli* and coliform bacteria by multiplex PCR. BMC Biotechnol 15:48. https://doi.org/10.1186/s12896-015-0168-2.
4. Feng PC, Hartman PA. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl Environ Microbiol 43:1320–1329. https://doi.org/10.1128/aem.43.6.1320-1329.1982.
5. Little MS, Pellock SJ, Walton WG, Tripathy A, Redinbo MR. 2018. Structural basis for the regulation of β-glucuronidase expression by human gut *Enterobacteriaceae*. Proc Natl Acad Sci U S A 115:E152–E161. https://doi.org/10.1073/pnas.1716241115.
6. International Organization for Standardization. 2012. Water quality: enumeration of Escherichia coli and coliform bacteria: part 2: most probable number method. ISO 9308-2:2012. https://www.iso.org/standard/52246.html.
7. German standard methodes for the examination of water, waste water und sludge; microbiological methods (group K); determination of Escherichia coli and coliform organisms (K6). DIN 38411-6: 1991-06. https://doi.org/10.31030/2421267.
8. Reitter C, Neuhaus K, Hügler M. 2021. Draft genome sequences of *Enterobacter* spp., *Lelliottia* spp., and *Serratia* spp., coliform bacteria from drinking water reservoirs and lakes. Microbiol Resour Announc 10:e0062221. https://doi.org/10.1128/MRA.00622-21.
9. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10. https://doi.org/10.14806/ej.17.1.200.
10. 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.
11. 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. https://doi.org/10.1089/cmb.2012.0021.
12. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
13. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma 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.
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. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/nar/gkaa1105.
16. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. Nucleic Acids Res 48:D606–D612. https://doi.org/10.1093/nar/gkz943.
17. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:D581–D591. https://doi.org/10.1093/nar/gkt1099.
18. Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. https://doi.org/10.1093/bioinformatics/btl446.
19. Lee I, Ouk Kim Y, Park S-C, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760.