



Draft Genome Sequence of *Actinomyces neuui* UMB1295, Isolated from the Female Urinary Tract

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ABSTRACT *Actinomyces neuui* is an opportunistic pathogen. Within the urogenital tract, it has been associated with bacterial vaginosis and overactive bladder symptoms. Here, we investigate a draft genome sequence of *A. neuui* UMB1295, which was isolated from a catheterized urine sample from a woman with a urinary tract infection.

Actinomyces neuui is a Gram-positive opportunistic bacterial pathogen. While *A. neuui* infections are generally regarded as rare, *A. neuui* can cause infections in multiple different organs (see reviews in references 1 and 2). *A. neuui* is frequently found within the female urogenital tract. Even though it has been found within the vaginal microbiota of healthy women (3), it is associated with bacterial vaginosis, and recent evidence suggests that it may enhance *Gardnerella vaginalis* virulence (4). Within the urinary tract, it is most frequently found in women with overactive bladder symptoms (5, 6). *A. neuui* UMB1295 was isolated from a catheterized urine sample obtained from a woman with a urinary tract infection (UTI) who was seeking clinical care at the Loyola University Medical Center Female Pelvic Medicine and Reconstructive Surgery Center (Maywood, IL, USA) as part of a prior institutional review board (IRB)-approved study (7) (Loyola University Chicago IRB approval no. 206469).

A. neuui UMB1295 was isolated using the expanded quantitative urinary culture (EQUC) protocol (5). The genus and species designations were determined by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (5). The *A. neuui* strain was streaked onto a Columbia naldixic acid (CNA) agar plate using the quadrant streaking method and was incubated at 35°C in 5% CO₂ for 48 h. LB broth was then inoculated with a single colony from this plate and grown under the same conditions. DNA was extracted using the DNeasy blood and tissue kit, following the Gram-positive protocol with the following exceptions: we used 230 μl of lysis buffer (180 μl of 20 mM Tris-Cl, 2 mM sodium EDTA, and 1.2% Triton X-100 and 50 μl of lysozyme). The DNA was quantified using a Qubit fluorometer and sent to the Microbial Genome Sequencing Center (MiGS) at the University of Pittsburgh for sequencing. There, it was first enzymatically fragmented with an Illumina tagmentation enzyme, and indices were attached using PCR. The library was sequenced using the Illumina NextSeq 550 platform, producing 1,380,900 pairs of 150-bp reads. The reads were trimmed using Sickle v1.33 (<https://github.com/najoshi/sickle>) and assembled using SPAdes v3.13.0 with the only-assembler option for k values of 55, 77, 99, and 127 (8). The genome coverage was calculated using BBMap v38.47 (<https://sourceforge.net/projects/bbmap>). The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.11 (9) was used to annotate the publicly available genome assembly. PATRIC v3.6.3 was also used to

Citation Markovic T, Miller-Ensminger T, Voukadinova A, Wolfe AJ, Putonti C. 2020. Draft genome sequence of *Actinomyces neuui* UMB1295, isolated from the female urinary tract. *Microbiol Resour Announc* 9:e00402-20. <https://doi.org/10.1128/MRA.00402-20>.

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

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Received 13 April 2020

Accepted 18 May 2020

Published 4 June 2020

annotate the genome sequence (10). The default parameters were used for each software tool.

The *A. neuii* UMB1295 draft genome is 2,174,960 bp long in 21 contigs with a GC content of 56.59%, genome coverage of 159×, and an N_{50} score of 193,074 bp. PGAP identified 1,968 coding sequences, with 1,953 coding for proteins, 48 tRNAs, 3 complete 5S rRNAs, 1 complete 16S rRNA, and 1 complete 23S rRNA. One CRISPR array with 32 spacer sequences was identified by CRISPRCasFinder (11). While PATRIC identified 24 genes associated with antibiotic resistance, further investigation of the genome assembly by ResFinder v3.2 (12) did not reveal any predicted resistances.

The *A. neuii* UMB1295 genome is just the sixth genome for this species and the third from the urinary microbiome (13). Further analysis of this genome and comparison between available genomes will greatly improve our understanding of this species, including its role in the female urogenital tract.

Data availability. This whole-genome shotgun project has been deposited in GenBank under the accession no. [JAAUWI000000000](https://doi.org/10.1093/jbcr/11.128/CMR.00100-14). The version described in this paper is the first version, JAAUWI010000000. The raw reads have been deposited in SRA under the accession no. [SRR11441016](https://doi.org/10.1093/jbcr/11.128/CMR.00100-14).

ACKNOWLEDGMENTS

This work was conducted as part of the Loyola University Chicago's Department of Biology Bacterial Genomics course. For prior patient recruitment, we acknowledge the Loyola Urinary Education and Research Collaborative and the patients who provided samples for this study.

REFERENCES

- Könönen E, Wade WG. 2015. *Actinomyces* and related organisms in human infections. *Clin Microbiol Rev* 28:419–442. <https://doi.org/10.1128/CMR.00100-14>.
- von Graevenitz A. 2011. *Actinomyces neuii*: review of an unusual infectious agent. *Infection* 39:97–100. <https://doi.org/10.1007/s15010-011-0088-6>.
- Santiago G, Cools P, Verstraelen H, Trog M, Missine G, El Aila N, Verhelst R, Tency I, Claeys G, Temmerman M, Vanechoutte M. 2011. Longitudinal study of the dynamics of vaginal microflora during two consecutive menstrual cycles. *PLoS One* 6:e28180. <https://doi.org/10.1371/journal.pone.0028180>.
- Castro J, Machado D, Cerca N. 2019. Unveiling the role of *Gardnerella vaginalis* in polymicrobial bacterial vaginosis biofilms: the impact of other vaginal pathogens living as neighbors. *ISME J* 13:1306–1317. <https://doi.org/10.1038/s41396-018-0337-0>.
- Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker L, Gai X, Wolfe AJ, Schreckenberger PC. 2014. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 52:871–876. <https://doi.org/10.1128/JCM.02876-13>.
- Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, Kliethermes S, Schreckenberger PC, Brubaker L, Gai X, Wolfe AJ. 2014. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *mBio* 5:e01283-14. <https://doi.org/10.1128/mBio.01283-14>.
- Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L, Wolfe AJ, Mueller ER, Schreckenberger PC. 2016. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. *J Clin Microbiol* 54:1216–1222. <https://doi.org/10.1128/JCM.00044-16>.
- 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. <https://doi.org/10.1089/cmb.2012.0021>.
- Tatusova T, DiCuccio M, Badretdin A, Chetverin 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>.
- 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>.
- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a Web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res* 35:W52–W57. <https://doi.org/10.1093/nar/gkm360>.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
- Thomas-White K, Forster SC, Kumar N, Van Kuiken M, Putonti C, Stares MD, Hilt EE, Price TK, Wolfe AJ, Lawley TD. 2018. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. *Nat Commun* 9:1557. <https://doi.org/10.1038/s41467-018-03968-5>.