AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Genome Sequences of 11 Human Vaginal *Actinobacteria* Strains

Amanda L. Lewis,[a,b,d] Grace E. Deitzler,[a,d] Maria J. Ruiz,[a] Cory Weimer,[a,d] SoEun Park,[a,d] Lloyd S. Robinson,[a,d] Kymberlie Hallsworth-Pepin,[c,e] Aye Wollam,[c,e] Makedonka Mitreva,[c,e] Warren G. Lewis[c,d]

Department of Molecular Microbiology,[a] Department of Obstetrics and Gynecology,[b] Department of Medicine,[c] Center for Women's Infectious Disease Research,[d] and McDonnell Genome Institute,[e] Washington University School of Medicine, St. Louis, Missouri, USA

For a commentary on this article, see doi:10.1128/genomeA.00890-16.

The composition of the vaginal microbiota is an important health determinant. Several members of the phylum *Actinobacteria* have been implicated in bacterial vaginosis, a condition associated with many negative health outcomes. Here, we present 11 strains of vaginal *Actinobacteria* (now available through BEI Resources) along with draft genome sequences.

Received 14 July 2016 **Accepted** 28 July 2016 **Published** 29 September 2016

Citation Lewis AL, Deitzler GE, Ruiz MJ, Weimer C, Park S, Robinson LS, Hallsworth-Pepin K, Wollam A, Mitreva M, Lewis WG. 2016. Genome sequences of 11 human vaginal *Actinobacteria* strains. Genome Announc 4(5):e00887-16. doi:10.1128/genomeA.00887-16.

Address correspondence to Amanda L. Lewis, allewis@wustl.edu, or Warren G. Lewis, WarrenGLewisPhD@gmail.com.

Bacterial vaginosis (BV) is a vaginal dysbiosis associated with serious health complications (1–6). It is characterized by the absence of *Lactobacillus* species in the vagina and overgrowth of a polymicrobial community often containing members of the phylum *Actinobacteria*, including *Gardnerella vaginalis, Atopobium* spp., and others. In fact, *G. vaginalis* was recently shown to elicit several features of BV in a mouse vaginal infection model (7, 8). Bifidobacteria are also commonly isolated from the vagina, although members of this genus are rarely found in pathological contexts. Here, we isolated 11 vaginal bacteria from the phylum *Actinobacteria*. Vaginal swabs were collected from nonpregnant and pregnant women according to Washington University institutional review board (IRB)-approved protocols (201108155 and 20110382). Organisms isolated from vaginal swabs were cultured anaerobically, and identification was performed by 16S rRNA gene sequencing. Genomic DNA was obtained using the Wizard genomic DNA purification kit (Promega). Methodological details on isolation and clinical information will be described elsewhere.

Genomes were assembled *de novo* using the One Button Velvet assembly pipeline (version 1.1.06) (9) with hash sizes of 31, 33, and 35 after downsizing the sample input data to 100× coverage. An internal core gene screen on the assembly tested for completeness of the genome. After assembly, the minimum length for contigs was set to 200 bp, and an internal core gene screen was performed as defined by the Human Microbiome Project (HMP) (10). Then, adapters were removed, and low-quality regions were trimmed. Finally, a screen for contamination was performed. The process of gene annotation included generating both *ab initio* and evidence-based (BLAST) predictions. Functional predictions of coding sequences were made using GeneMark and Glimmer3 (11, 12). Loci were then defined by clustering predictions with the same reading frame. We evaluated predictions using the nonredundant (NR) and Pfam databases and resolved overlaps between adjacent coding genes. Intergenic regions not spanned by GeneMark and Glimmer3 were subject to a BLAST search against NCBI's NR database and predictions generated based on protein alignments. tRNA genes were determined using tRNAscan-SE (13) and noncoding RNA genes by RNAmmer (14) and Rfam (15). Metabolic pathways and subcellular localization were predicted using KEGG and PSORTb, respectively (16, 17), and functional domains were evaluated using InterProScan (18).

Accession number(s). These whole-genome shotgun projects have been deposited in GenBank under the accession numbers listed in Table 1. We have also made the strains available to the

TABLE 1 Identifiers and nucleotide sequences for sequenced strains of vaginal *Actinobacteria*

Genus/species	Strain	BEI catalog no.	Nucleotide accession no.
Actinomyces neuii	MJR8396A	HMS-1266	LRPJ00000000
Alloscardovia omnicolens	CMW7705A	HMS-1282	LRPK00000000
Atopobium vaginae	CMW7778A	HMS-1300	LSOA00000000
Bifidobacterium bifidum	MJR8628B	HMS-1264	LRPO00000000
Bifidobacterium breve	GED8481	HMS-1261	LRPP00000000
Bifidobacterium longum	CMW7750	HMS-1299	LRPQ00000000
Corynebacterium sp.	CMW7794	HMS-1295	LSRB00000000
Gardnerella vaginalis	GED7275B	HMS-1272	LRPZ00000000
Gardnerella vaginalis	GED7760B	HMS-1284	LRQA00000000
Gardnerella vaginalis	CMW7778B	HMS-1298	LSRC00000000
Propionibacterium avidum	MJR7694	HMS-1291	LRVD00000000

research community by depositing them with the Biodefense and Emerging Infections (BEI) Research Resource Repository (see BEI numbers in Table 1).

ACKNOWLEDGMENTS

We acknowledge Justin Perry, Deborah Frank, and the Women's and Infant's Health Specimen Consortium (especially Michele Landeau). We also thank Jeff Peipert, Jenifer Allsworth, and Jennifer Bick of the Contraceptive CHOICE project for providing specimens from nonpregnant women.

FUNDING INFORMATION

This work, including the efforts of Makedonka Mitreva, was funded by HHS | National Institutes of Health (NIH) (5U54HG00496804). This work, including the efforts of Amanda L. Lewis and Warren G. Lewis, was funded by HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID) (R01 AI114635). This work, including the efforts of Amanda L. Lewis, was funded by HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (P50 DK064540-11). This work, including the efforts of Grace E. Deitzler, was funded by HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (P20DK097798). This work, including the efforts of Amanda L. Lewis, was funded by Burroughs Wellcome Fund (BWF).

This work was funded by the Burroughs-Wellcome Fund Preterm Birth Initiative (A.L.L.). Summer stipends were provided by the Planning Center for Research in Benign Urology Summer Research Program (P20DK097798 to G.E.D.) and the Washington University in St. Louis Summer Research Program in Global Health with the Center for Global Health & Infectious Disease (CGHID)/Institute for Public Health (3054-93396B to M.J.R.).

REFERENCES

1. **Wiesenfeld HC, Hillier SL, Krohn MA, Landers DV, Sweet RL.** 2003. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. Clin Infect Dis **36:**663–668. http://dx.doi.org/10.1086/367658.
2. **Marrazzo JM, Martin DH, Watts DH, Schulte J, Sobel JD, Hillier SL, Deal C, Fredricks DN.** 2010. Bacterial vaginosis: identifying research gaps proceedings of a workshop sponsored by DHHS/NIH/NIAID. Sex Transm Dis **37:**732–744. http://dx.doi.org/10.1097/OLQ.0b013e3181fbbc95.
3. **Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG, 2nd, Rao AV, McNellis D, Regan JA, Carey C, Klebanoff MA.** 1995. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. N Engl J Med **333:**1737–1742. http://dx.doi.org/10.1056/NEJM199512283332604.
4. **Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, Horvath LB, Kuzevska I, Fairley CK.** 2006. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis **193:**1478–1486. http://dx.doi.org/10.1086/503780.
5. **Wilson J.** 2004. Managing recurrent bacterial vaginosis. Sex Transm Infect **80:**8–11. http://dx.doi.org/10.1136/sti.2002.002733.
6. **Allsworth JE, Peipert JF.** 2007. Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data. Obstet Gynecol **109:**114–120. http://dx.doi.org/10.1097/01.AOG.0000247627.84791.91.
7. **Gilbert NM, O'Brien VP, Hultgren S, Macones G, Lewis WG, Lewis AL.** 2013. Urinary tract infection as a preventable cause of pregnancy complications: opportunities, challenges, and a global call to action. Global Adv Health Med **2:**59–69. http://dx.doi.org/10.7453/gahmj.2013.061.
8. **Lewis WG, Robinson LS, Gilbert NM, Perry JC, Lewis AL.** 2013. Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted actinobacterium *Gardnerella vaginalis*. J Biol Chem **288:**12067–12079. http://dx.doi.org/10.1074/jbc.M113.453654.
9. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res **18:**821–829. http://dx.doi.org/10.1101/gr.074492.107.
10. **Nelson KE, Weinstock GM, Highlander SK, Worley KC, Creasy HH, Wortman JR, Rusch DB, Mitreva M, Sodergren E, Chinwalla AT, Feldgarden M, Gevers D, Haas BJ, Madupu R, Ward DV, Birren BW, Gibbs RA, Methe B, Petrosino JF, Strausberg RL, Sutton GG, White OR, Wilson RK, Durkin S, Giglio MG, Gujja S, Howarth C, Kodira CD, Kyrpides N, Mehta T, Muzny DM, Pearson M, Pepin K, Pati A, Qin X, Yandava C, Zeng Q, Zhang L, Berlin AM, Chen L, Hepburn TA, Johnson J, McCorrison J, Miller J, Minx P, Nusbaum C, Russ C, Sykes SM, Tomlinson CM, Young S, et al.** 2010. A catalog of reference genomes from the human microbiome. Science **328:**994–999. http://dx.doi.org/10.1126/science.1183605.
11. **Borodovsky M, Mills R, Besemer J, Lomsadze A.** 2003. Prokaryotic gene prediction using GeneMark and GeneMark.Hmm. Curr Protoc Bioinformatics Chapter 4:Unit 4.5. http://dx.doi.org/10.1002/0471250953.bi0405s01.
12. **Delcher AL, Harmon D, Kasif S, White O, Salzberg SL.** 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res **27:**4636–4641. http://dx.doi.org/10.1093/nar/27.23.4636.
13. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res **25:**955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
14. **Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res **35:**3100–3108. http://dx.doi.org/10.1093/nar/gkm160.
15. **Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A.** 2005. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res **33:**D121–D124. http://dx.doi.org/10.1093/nar/gki081.
16. **Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M.** 2004. The KEGG resource for deciphering the genome. Nucleic Acids Res **32:**D277–D280. http://dx.doi.org/10.1093/nar/gkh063.
17. **Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R, Dao P, Sahinalp SC, Ester M, Foster LJ, Brinkman FS.** 2010. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics **26:**1608–1615. http://dx.doi.org/10.1093/bioinformatics/btq249.
18. **Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R.** 2005. InterProScan: protein domains identifier. Nucleic Acids Res **33:**W116–W120. http://dx.doi.org/10.1093/nar/gki442.