Genome Sequences of Acholeplasma laidlawii Strains with Increased Resistance to Tetracycline and Melittin

Natalia B. Baranova,a,b Tatyana Y. Malygina,a Elena S. Medvedeva,a,b Eugenia A. Boulygina,b Maria N. Siniagina,b Mohamed Amine Dramchini,b Reshad Akbor Prottoy,b Alexey A. Mouzykantov,a,b Marina N. Davydova,a Olga A. Chernova,a,b Vladislav M. Chernova,a,b

aKazan Institute of Biochemistry and Biophysics, Kazan Scientific Centre of the Russian Academy of Sciences, Kazan, Russia
bInstitute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia

ABSTRACT Acholeplasma laidlawii is a well-suited model for studying the molecular basis for adapting mollicutes to environmental conditions. Here, we present the whole-genome sequences of two strains of A. laidlawii with increased resistance to tetracycline and melittin.

The recommended approach to suppress and eliminate bacteria belonging to the class Mollicutes—the parasites of plants, animals, and humans, as well as the main contaminants of cell cultures (1, 2)—is antibiotic therapy associated with the use of fluoroquinolones, tetracyclines, and macrolides (3, 4). An alternative method is related to antimicrobial peptides, including melittin, a peptide from honey bee venom (5, 6). Acholeplasma laidlawii, a ubiquitous mollicute, is a well-suited model for studying the molecular basis for adapting mollicutes to environmental conditions, including the development of antibiotic resistance in vitro (7–10). Previously, we presented the whole-genome sequences of A. laidlawii strains with different sensitivity to ciprofloxacin (11). The genomes of two strains of A. laidlawii with increased resistance to tetracycline (PG8RTet) and melittin (PG8RMel), which are derivatives of the PG8B strain (GenBank accession number LVCP00000000), were sequenced in this study.

DNA from cells of the A. laidlawii strains was extracted using the phenol extraction method (12). The DNA concentration was determined using a Qubit version 2.0 fluorometer (Invitrogen). The fragmentation was carried out using a Covaris S220 ultrasonic disintegrator (Thermo Fisher Scientific). After sonication, the samples were cleaned with AMPure magnetic particle beads (Beckman Coulter, Inc.). The libraries were prepared with an NEBNext Ultra II kit (New England Biolabs) according to the manufacturer’s instructions. The quality analysis of the DNA libraries was performed on a 2100 Bioanalyzer instrument (Agilent). The whole-genome sequencing of the obtained libraries was performed on the MiSeq platform (Illumina, USA) using 150-bp paired-end reads. The de novo assembly of the received reads was performed using the SPAdes version 3.7.0 genome assembler (13). Alignment to the reference genome of A. laidlawii PG-8A (GenBank accession number CP000896) and annotation of single nucleotide polymorphisms (SNPs) were performed using Bowtie2 software (14), SAMtools (15), and SnpEff version 3.3 (16). Gene prediction and annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline (17).

Mutations in the genes associated with the development of tetracycline resistance in different microorganisms were not found in the genome of A. laidlawii PG8RTet. SNPs in genes coding membrane and efflux proteins as well as proteases were found in the genome of A. laidlawii PG8RMel. It is assumed that these proteins are associated with the development of resistance to antimicrobial peptides in different microorganisms (18). In addition, SNPs in PG8RMel as well as in PG8RTet were found in many genes, and their
involvement in antibiotic resistance remains to be elucidated. Some SNPs found in PG8Rtet and PG8Rmel were also detected in A. laidlawii strain PG8R10, which has increased resistance to ciprofloxacin (GenBank accession number LXYB01000000).

The whole-genome sequences of A. laidlawii strains PG8Rtet, PG8Rtel, and PG8R10 with differential sensitivity to tetracycline, melittin, and ciprofloxacin, can be used further to determine the molecular basis for adapting mollicutes to antimicrobial agents.

**Accession number(s).** The whole-genome shotgun projects of PG8Rtet and PG8Rtel have been deposited in DDBJ/ENA/GenBank under the accession numbers NELO00000000 and NELN00000000, respectively. The versions described in this paper are the second versions, NELO02000000 and NELN02000000.

**ACKNOWLEDGMENTS**

This work was supported by grants from the Russian Foundation for Basic Research (15-44-02594 and 16-34-00660) and by a grant from the President of the Russian Federation (MK-1099.2017.4).

The research was performed using the equipment of the Interdisciplinary Center for Collective Use of Kazan Federal University as part of the Russian Government Program for the Competitive Growth of Kazan Federal University.

**REFERENCES**

1. Blanchard A, Bébéar CM. 2002. Mycoplasmas of humans, p 45–71. In Razin Sh, Hermann R (ed), Molecular biology and pathogenicity of mycoplasmas. Kluwer Academic Publishers, New York, NY.

2. Razin S, Hayflick L. 2010. Highlights of mycoplasma research—an historical perspective. Biologicals 38:183–190. https://doi.org/10.1016/j.biologicals.2009.11.008.

3. Uphoff CC, Drexler HG. 2014. Eradication of mycoplasma contaminations from cell cultures. Curr Protoc Mol Biol 106:28.5.1–28.5.12. https://doi.org/10.1002/0471142727.mb2805s106.

4. Waite KB, Lysynska I, Bebear CM. 2014. Emerging antimicrobial resistance in mycoplasmas of humans and animals, p 289–322. In Browning GF, Citti C (ed), Mollicutes: molecular biology and pathogenesis. Caister Academic Press, Norfolk, United Kingdom.

5. Béven L, Castano S, Dufourcq J, Wieslander A, Wróblewski H. 2003. The antibiotic activity of cationic linear amphipathic peptides: lessons from the action of leucine/lysine copolymers on bacteria of the class Mollicutes. Eur J Biochem 270:2207–2217. https://doi.org/10.1046/j.1432-1033.2003.03587.x.

6. Lazarev VN, Stipkovits L, Biro J, Miklodi D, Shkarupeta MM, Titova GA, Akopian TA, Govorun VM. 2004. Induced expression of the antimicrobial peptide melittin inhibits experimental infection by Mycoplasma gallisepticum in chickens. Microbes Infect 6:536–541. https://doi.org/10.1016/j.micinf.2004.02.006.

7. Tully JG. 1979. Special features of the acholoplasmas, p 431–449. In Barile MF, Razin Sh (ed), The mycoplasmas, vol 1. Academic Press, New York, NY.

8. Lazarev VN, Levitskii SA, Basovskii YI, Chukin MM, Akopian TA, Vereshchagin VV, Kostrjukova ES, Kovaleva GY, Kazanov MD, Malko DB, Vitreschak AG, Sernova NV, Gelfand MS, Demina IA, Serebyakova MV, Galyamina MA, Vtyurin NN, Rogov SI, Alexeev DG, Ladygina VG, Govorun VM. 2011. Complete genome and proteome of Acholeplasma laidlawii. J Bacteriol 193:4943–4953. https://doi.org/10.1128/JB.05059-11.

9. Medvedeva ES, Bankevich A, Antipov D, Gurevich AA, Korobeinikov A, Lapidus AA, Polyanovsky M, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.

10. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.

11. Medvedeva ES, Davydova MN, Mouzykantov AA, Baranova NB, Grigoreva TY, Siniagina MN, Biro J, Stipkovits L, Miklodi D, Shkarupeta MM, Titova GA, Akopian TA, Govorun VM. 2016. Genomic and proteomic profiles of Acholeplasma laidlawii strains differing in sensitivity to ciprofloxacin. Dokl Biochim Biophys 466:23–27. https://doi.org/10.1134/S1607679216010075.

12. Medvedeva ES, Siniagina MN, Malanin SY, Biro J, Shkarupeta MM, Titova GA, Akopian TA, Govorun VM. 2017. Genome sequences of Acholeplasma laidlawii strains differing in sensitivity to ciprofloxacin. Genome Announc 5(44):e01189-17. https://doi.org/10.1128/genomeA.01189-17.

13. Barile MF, Razin Sh (ed), The mycoplasmas, vol 1. Academic Press, New York, NY.

14. Barile MF, Razin Sh (ed), The mycoplasmas, vol 2. Academic Press, New York, NY.

15. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

16. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 6:80–92. https://doi.org/10.4161/fly.10.4161/fly.19695.

17. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciuko S, Li W. 2013. Prokaryotic genome annotation pipeline. In The NCBI handbook, 2nd ed. National Center for Biotechnology Information, Bethesda, MD.

18. Joo HS, Fu CI, Otto M. 2016. Bacterial strategies of resistance to antimicrobial peptides. Philos Trans R Soc Lond B Biol Sci 371:2015029. https://doi.org/10.1098/rstb.2015.0292.