



Complete Genome Sequence of vB_EcoM-UFV13, a New Bacteriophage Able To Disrupt *Trueperella pyogenes* Biofilm

Vinícius S. Duarte,^a Roberto S. Dias,^b Andrew M. Kropinski,^c [®]Pedro M. P. Vidigal,^d Flávia O. Sousa,^a André S. Xavier,^e Cynthia C. Silva,^a Sergio O. de Paula^b

Department of Microbiology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^a; Department of General Biology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^a; Departments of Food Science, Molecular and Cellular Biology and Pathobiology, University of Guelph, Guelph, Ontario, Canada^c; Núcleo de Análise de Biomoléculas (NuBioMol), Biological and Health Science Center, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil^e; Department of Phytopathology, Universidade Federal d

vB_EcoM-UFV13, a member of the *T4virus* genus, shows lytic activity against *Escherichia coli* and effectiveness in controlling the biofilm formed by *Trueperella pyogenes*, which qualifies it as a promising component of phage cocktails for mastitis and metritis control.

Received 22 September 2016 Accepted 20 October 2016 Published 8 December 2016

Citation Duarte VS, Dias RS, Kropinski AM, Vidigal PMP, Sousa FO, Xavier AS, Silva CC, de Paula SO. 2016. Complete genome sequence of vB_EcoM-UFV13, a new bacteriophage able to disrupt *Trueperella pyogenes* biofilm. Genome Announc 4(6):e01292-16. doi:10.1128/genomeA.01292-16.

Copyright © 2016 Duarte et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Sergio O. de Paula, depaula@ufv.br.

Metritis and mastitis are diseases in dairy cattle and can lead to economic losses of U.S.\$2 billion annually in the United States (1–3). Common to both diseases, *Escherichia coli* and *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes* [4]) have the ability to produce biofilm and with regard to metritis, there is a temporal relationship between both microorganisms found in the uterus. *E. coli* is the first pathogen that establishes in the uterus and is responsible for providing an appropriate intrauterine environment for development of *T. pyogenes* (1, 5). Thus, it is essential to find new agents that are effective in the control of both microorganisms.

vB_EcoM-UFV13 was isolated using a wild strain (*E. coli* 30) obtained from a dairy cow with clinical mastitis. The phage has a capsid with icosahedral symmetry (72 nm of length and width) and a 30 nm long contractile tail, thus indicating it as a member of the *Caudovirales* order, family *Myoviridae*. This virus showed lytic activity against *E. coli*, as well as being effective in the control of biofilm formed by *T. pyogenes*, which qualifies it as a promising component of phage cocktails for mastitis and metritis control. The bacteriophage was concentrated and purified using polyethylene glycol 8,000 and its genome extracted by proteinase K/phenol method (6).

The phage DNA was sent to Macrogen (South Korea) and the sequencing performed by Illumina Hiseq 2500 paired-end platform, with read quality evaluated by HCS (HiSeq Control Software v2.2.38) and RTA softwares (Real Time Analysis. v1.18.61.0). The sequence data was *de novo* assembled using SeqMan NGen 12 (DNAStar, Madison, WI, USA) software. The consensus sequence was opened to be collinear with *Yersinia* phage PST and the fragments were reassembled using SeqMan Pro 12, into a single contig of 165,771 bp (570-fold coverage) with a G+C content of 34.8%. 269 open reading frames (ORFs) were predicted and annotated using the MyRast program (7) with 193 ORFs encoding hypothetical proteins, 39 ORFs encoding structural proteins, and the majority (228 ORFs) transcribed from the negative strand. Notable is

the presence of ORF 130, which encodes a glycoside hydrolase (family 24), an enzyme able to act in a Gram-positive biofilm and cause lysis in a Gram-negative bacteria. This property was also observed by the authors of reference (8). A gene cluster, without introns or pseudogenes, encoding 10 tRNAs (Gln, Leu, Gly, Pro, Ser, Thr, Met, Tyr, Asn, and Arg) was identified using tRNAscan-SE (5, 9). The genome of UFV13 is related to *Escherichia, Shigella*, and *Yersinia* phages belonging to the *T4virus* genus. Their collinearity was confirmed by progressiveMauve analysis (10).

The next steps of this work will be focused on the interaction between phage UFV13 and *Trueperella pyogenes*, using proteomic and transcriptomic approaches.

Accession number(s). The whole-genome sequence generated has been deposited at GenBank under the accession no. KU867876.

ACKNOWLEDGMENTS

We are grateful to the Núcleo de Análise de Biomoléculas and Núcleo de Microscopia e Microanálise of the Universidade Federal de Viçosa for providing the facilities for the conduction of the experiments.

We also acknowledge the financial support by the following Brazilian agencies: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (Finep), Sistema Nacional de Laboratórios em Nanotecnologias (SisNANO)/Ministério da ciência, tecnologia e Informação (MCTI).

We are also grateful to Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Gado de Leite Farm, Juiz de Fora, Minas Gerais, Brazil, that kindly provided us *Escherichia coli* 30.

FUNDING INFORMATION

This work, including the efforts of Vinícius S. Duarte, was funded by MCTI | Conselho Nacional de Desenvolvimento Científico e Tecnológico

(CNPq). This work, including the efforts of Roberto S. Dias, was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This work, including the efforts of Vinícius S. Duarte, was funded by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

REFERENCES

- Bicalho ML, Lima FS, Machado VS, Meira EB, Jr, Ganda EK, Foditsch C, Bicalho RC, Gilbert RO. 2016. Associations among *Trueperella pyogenes*, endometritis diagnosis, and pregnancy outcomes in dairy cows. Theriogenology 85:267–274. http://dx.doi.org/10.1016/ j.theriogenology.2015.09.043.
- Federman C, Joo J, Almario JA, Salaheen S, Biswas D. 2016. Citrusderived oil inhibits *Staphylococcus aureus* growth and alters its interactions with bovine mammary cells. J Dairy Sci 99:3667–3674. http://dx.doi.org/ 10.3168/jds.2015-10538.
- Shaheen M, Tantary H, Nabi S. 2016. A treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy. Adv Dairy Res 4:150. http://dx.doi.org/10.4172/2329-888X.1000150.
- 4. Yassin AF, Hupfer H, Siering C, Schumann P. 2011. Comparative chemotaxonomic and phylogenetic studies on the genus *Arcanobacterium* Collins *et al.* 1982 emend. Lehnen *et al.* 2006: proposal for *Trueperella* gen. nov. and emended description of the genus *Arcanobacte-*

rium. Int J Syst Evol Microbiol **61:**1265–1274. http://dx.doi.org/10.1099/ ijs.0.020032-0.

- Machado VS, Bicalho MLS, Meira Junior EBS, Rossi R, Ribeiro BL, Lima S, Santos T, Kussler A, Foditsch C, Ganda EK, Oikonomou G, Cheong SH, Gilbert RO, Bicalho RC. 2014. Subcutaneous immunization with inactivated bacterial components and purified protein of *Escherichia coli, Fusobacterium necrophorum* and *Trueperella pyogenes* prevents puerperal metritis in Holstein dairy cows. PLoS One 9:e91734. http:// dx.doi.org/10.1371/journal.pone.0091734.
- 6. Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42: D206-D214. http://dx.doi.org/10.1093/nar/gkt1226.
- Abouhmad A, Mamo G, Dishisha T, Amin MA, Hatti-Kaul R. 2016. T4 lysozyme fused with cellulose-binding module for antimicrobial cellulosic wound dressing materials. J Appl Microbiol 121:115–125. http:// dx.doi.org/10.1111/jam.13146.
- 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.
- Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. http://dx.doi.org/10.1371/journal.pone.0011147.