

Draft Genome Sequence of *Trueperella pyogenes*, Isolated from the Infected Uterus of a Postpartum Cow with Metritis

Robert J. Goldstone,^a Matt Amos,^b Richard Talbot,^c Hans-Joachim Schubert,^d Olivier Sandra,^{e,f} I. Martin Sheldon,^b David G. E. Smith^{a,g}

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom^a; Institute of Life Science, School of Medicine, Swansea University, Singleton Park, Swansea, United Kingdom^b; Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, Scotland, United Kingdom^c; Immunology Unit, University of Veterinary Medicine, Hannover, Germany^d; INRA, UMR1198 Biologie du Développement et Reproduction, Jouy-en-Josas, France^e; ENVA, Maisons Alfort, France^f; Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Midlothian, United Kingdom^g

***Trueperella pyogenes* is a common commensal bacterium and an opportunistic pathogen associated with chronic purulent disease, particularly in ruminants. We report here the genome sequence of a *T. pyogenes* isolate from a severe case of bovine metritis. This is the first full record of a *T. pyogenes* genome.**

Received 24 February 2014 Accepted 11 April 2014 Published 24 April 2014

Citation Goldstone RJ, Amos M, Talbot R, Schubert H-J, Sandra O, Sheldon IM, Smith DGE. 2014. Draft genome sequence of *Trueperella pyogenes*, isolated from the infected uterus of a postpartum cow with metritis. *Genome Announc*. 2(2):e00194-14. doi:10.1128/genomeA.00194-14.

Copyright © 2014 Goldstone et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to David G. E. Smith, David.G.Smith@glasgow.ac.uk.

Recently, the genus *Trueperella* was proposed to encompass 5 species previously classified as belonging to *Arcanobacterium* (1). Among these reclassified species is *T. pyogenes*, previously known as *Arcanobacterium pyogenes*, *Actinomyces pyogenes*, and *Corynebacterium pyogenes*. *T. pyogenes* has been long recognized as a mucosal membrane resident in many animal species and as an opportunistic pathogen (2). *T. pyogenes* is particularly associated with suppurative infections, such as mastitis, septic arthritis, liver abscessation, pneumonia, endometritis, and metritis. *T. pyogenes* is recognized as a key etiological agent in bovine endometritis and metritis, frequently in conjunction with other bacteria, including *Escherichia coli*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, and *Prevotella* spp. (3), although *T. pyogenes* correlates with most severe disease presentation and pathology (4). Despite the bacterium's being known as an opportunistic pathogen for many decades, the understanding of *T. pyogenes* remains rudimentary. No genome sequences for *T. pyogenes* had been available in the public domain; therefore, we carried out the sequencing of this genome.

T. pyogenes MS249 was isolated from the uterus of an animal with a severe case of metritis in which disease persisted and progressed to endometritis (5). Genomic DNA was prepared using the DNeasy blood and tissue kit (Qiagen), according to the manufacturer's instructions. Sequencing was carried out using 454 GS-FLX and Illumina GA IIx 130-bp paired-end sequencing. The reads were assembled using Velvet (6) and the Celera Assembler with the Best Overlap Graph (CABOG) (7), and gaps were closed using unmapped 454 and Illumina reads. The prediction of open reading frames (ORFs) was achieved using FgenesB (8) via the SoftBerry web interface (<http://linux1.softberry.com/berry.phtml>), rRNA prediction was carried out via the HMMER Web server (9), and tRNA prediction was done using tRNAscan-SE via the WebMGA interface (10). The assembled 248 contigs totaled 2,236,677 bp, with a G+C content of 59.8%. The genome

was predicted to contain 4 rRNA operons, 47 tRNAs, and 2,095 protein-coding sequences (CDSs).

The main recognized virulence factor of *T. pyogenes* is pyolysin (PLO), an exported, cholesterol-dependent, pore-forming cytotoxin (CDC) (11–13), and the PLO gene sequence was identical to that published for other strains of *T. pyogenes*. Additional virulence-associated factors in MS249 included fimbriae/pili (Fim/Pil), collagen-binding protein (CbpA) (14), and neuraminidases (NanH and NanP) (2). Multiple *fim* genes have been reported, the presences of which are variable among strains (14, 15), and in strain MS249, *fimA* and *fimC* were identified, along with an additional *fimG* locus.

Resistance to antibiotics often used in veterinary practice is common in *T. pyogenes*. A gene encoding resistance to tetracycline (*tetW*) was identified in the genome of strain MS249. Although resistance to macrolides, chloramphenicol, and β -lactam antibiotics among *T. pyogenes* strains has been reported (16–20), no specific resistance elements were identified in strain MS249, consistent with its sensitivity to these antibiotics.

This is the first reported genome sequence for *T. pyogenes*. This sequence will be used to extend the understanding of this novel bacterial opportunistic pathogen and form the basis for systematic studies of its pathogenicity and physiology, as well as to perform a comparative genomic analysis among *Actinobacteria*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JALQ00000000. The version described in this paper is version JALQ01000000.

ACKNOWLEDGMENTS

This work is a component of the project “Integrated systems approach for preventing uterine disease in dairy cattle (iPUD),” funded by BBSRC through the EMIDA ERA-NET initiative, project reference BB/1017240/1 and BB/BB/1017283/1.

REFERENCES

1. 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 *Arcanobacterium*. *Int. J. Syst. Evol. Microbiol.* 61:1265–1274. <http://dx.doi.org/10.1099/ijs.0.020032-0>.
2. Jost BH, Billington SJ. 2005. *Arcanobacterium pyogenes*: molecular pathogenesis of an animal opportunist. *Antonie Van Leeuwenhoek* 88:87–102. <http://dx.doi.org/10.1007/s10482-005-2316-5>.
3. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biol. Reprod.* 81:1025–1032. <http://dx.doi.org/10.1095/biolreprod.109.077370>.
4. Bonnett BN, Martin SW, Gannon VP, Miller RB, Etherington WG. 1991. Endometrial biopsy in Holstein-Friesian dairy cows. III. Bacteriological analysis and correlations with histological findings. *Can. J. Vet. Res.* 55:168–173.
5. Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction* 123:837–842. <http://dx.doi.org/10.1530/rep.0.1230837>.
6. 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>.
7. Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428:37–43. <http://dx.doi.org/10.1038/nature02340>.
8. Miller JR, Delcher AL, Koren S, Venter E, Walenz BP, Brownley A, Johnson J, Li K, Mobarry C, Sutton G. 2008. Aggressive assembly of pyrosequencing reads with mates. *Bioinformatics* 24:2818–2824. <http://dx.doi.org/10.1093/bioinformatics/btn548>.
9. Finn RD, Clements J, Eddy SR. 2011. HMMER Web server: interactive sequence similarity searching. *Nucleic Acids Res.* 39:W29–W37. <http://dx.doi.org/10.1093/nar/gkr367>.
10. Wu S, Zhu Z, Fu L, Niu B, Li W. 2011. WebMGA: a customizable Web server for fast metagenomic sequence analysis. *BMC Genomics* 12:444. <http://dx.doi.org/10.1186/1471-2164-12-444>.
11. Billington SJ, Jost BH, Cuevas WA, Bright KR, Songer JG. 1997. The *Arcanobacterium* (*Actinomyces*) *pyogenes* hemolysin, pyolysin, is a novel member of the thiol-activated cytolysin family. *J. Bacteriol.* 179:6100–6106.
12. Billington SJ, Songer JG, Jost BH. 2001. Molecular characterization of the pore-forming toxin, pyolysin, a major virulence determinant of *Arcanobacterium pyogenes*. *Vet. Microbiol.* 82:261–274. [http://dx.doi.org/10.1016/S0378-1135\(01\)00373-X](http://dx.doi.org/10.1016/S0378-1135(01)00373-X).
13. Jost BH, Songer JG, Billington SJ. 1999. An *Arcanobacterium* (*Actinomyces*) *pyogenes* mutant deficient in production of the pore-forming cytolysin pyolysin has reduced virulence. *Infect. Immun.* 67:1723–1728.
14. Esmay PA, Billington SJ, Link MA, Songer JG, Jost BH. 2003. The *Arcanobacterium pyogenes* collagen-binding protein, CbpA, promotes adhesion to host cells. *Infect. Immun.* 71:4368–4374. <http://dx.doi.org/10.1128/IAI.71.8.4368-4374.2003>.
15. Silva E, Gaivão M, Leitão S, Jost BH, Carneiro C, Vilela CL, Lopes da Costa L, Mateus L. 2008. Genomic characterization of *Arcanobacterium pyogenes* isolates recovered from the uterus of dairy cows with normal puerperium or clinical metritis. *Vet. Microbiol.* 132:111–118. <http://dx.doi.org/10.1016/j.vetmic.2008.04.033>.
16. Billington SJ, Jost BH. 2006. Multiple genetic elements carry the tetracycline resistance gene tet(W) in the animal pathogen *Arcanobacterium pyogenes*. *Antimicrob. Agents Chemother.* 50:3580–3587. <http://dx.doi.org/10.1128/AAC.00562-06>.
17. Liu MC, Wu CM, Liu YC, Zhao JC, Yang YL, Shen JZ. 2009. Identification, susceptibility, and detection of integron-gene cassettes of *Arcanobacterium pyogenes* in bovine endometritis. *J. Dairy Sci.* 92:3659–3666. <http://dx.doi.org/10.3168/jds.2008-1756>.
18. Santos TM, Caixeta LS, Machado VS, Rauf AK, Gilbert RO, Bicalho RC. 2010. Antimicrobial resistance and presence of virulence factor genes in *Arcanobacterium pyogenes* isolated from the uterus of postpartum dairy cows. *Vet. Microbiol.* 145:84–89. <http://dx.doi.org/10.1016/j.vetmic.2010.03.001>.
19. Sheldon IM, Bushnell M, Montgomery J, Rycroft AN. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. *Vet. Rec.* 155:383–387. <http://dx.doi.org/10.1136/vr.155.13.383>.
20. Trinh HT, Billington SJ, Field AC, Songer JG, Jost BH. 2002. Susceptibility of *Arcanobacterium pyogenes* from different sources to tetracycline, macrolide and lincosamide antimicrobial agents. *Vet. Microbiol.* 85:353–359. [http://dx.doi.org/10.1016/S0378-1135\(01\)00524-7](http://dx.doi.org/10.1016/S0378-1135(01)00524-7).