

Complete Genome of *Lactococcus lactis* subsp. *cremoris* UC509.9, Host for a Model Lactococcal P335 Bacteriophage

Stuart Ainsworth,^a Aldert Zomer,^b Victor de Jager,^{b,c,d} Francesca Bottacini,^f Sacha A. F. T. van Hijum,^{b,e} Jennifer Mahony,^a Douwe van Sinderen^{a,f}

Department of Microbiology, University College Cork, Cork, Ireland^a; Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University, Medical Centre, Nijmegen, the Netherlands^b; Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands^c; Netherlands Bioinformatics Centre, Nijmegen, The Netherlands^d; NIZO food research, Ede, The Netherlands^e; Alimentary Pharmabiotic Centre, Biosciences Institute, University College Cork, Cork, Ireland^f

Here, we report the complete genome of *Lactococcus lactis* subsp. *cremoris* UC509.9, an Irish dairy starter. The circular chromosome of *L. lactis* UC509.9 represents the smallest among those of the sequenced lactococcal strains, while its large complement of eight plasmids appears to be a reflection of its adaptation to the dairy environment.

Received 9 November 2012 Accepted 19 November 2012 Published 31 January 2013

Citation Ainsworth S, Zomer A, de Jager V, Bottacini F, van Hijum SAFT, Mahony J, van Sinderen D. 2013. Complete genome of *Lactococcus lactis* subsp. *cremoris* UC509.9, host for a model lactococcal P335 bacteriophage. *Genome Announc.* 1(1):e00119-12. doi:10.1128/genomeA.00119-12.

Copyright © 2013 Ainsworth et al. This is an open-access article distributed under the terms of the [Attribution 3.0 Unported Creative Commons License](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Douwe van Sinderen, d.vansinderen@ucc.ie.

Lactococcus lactis strains are used extensively worldwide for the production of fermented dairy products. Bacteriophage (phage) attack during this fermentation process can lead to slow or failed fermentations and is therefore of major economic concern (1). *L. lactis* subsp. *cremoris* UC509 is an Irish cheddar starter strain and is the lysogenic host of the model P335-type phage Tuc2009 (2–6). *L. lactis* UC509.9, whose genome sequence is presented here, is a prophage-cured Tuc2009-sensitive derivative of UC509 (7).

While lactococcal phages are subject to intensive scientific scrutiny, the specific interactions with their hosts are poorly understood. To further our understanding regarding the molecular interplay between Tuc2009 and its host, we sequenced the genome of *L. lactis* UC509.9. Sequencing was performed by Agencourt Bioscience (Beverly, MA) and Macrogen (Seoul, Republic of Korea) using a combination of 454 sequencing of a 3-kb fragment library using Roche standard procedures and of Sanger sequencing of a 36-kb insert library followed by homopolymer tract correction using Illumina sequencing. Initial sequence assembly was performed using GSAssembler (Roche). Gap closure and quality improvements were performed by Sanger sequencing of gap-closing PCR products as suggested by Projector 2 (8) with the Staden package (9). Homopolymer tract single nucleotide polymorphisms (SNPs) were detected and corrected using Robust Variant detection (ROVAR) (V. de Jager, B. Renckens, R. J. Siezen, and S. A. F. T. van Hijum, unpublished data [<https://trac.nbic.nl/rovar/>]) applied to Illumina sequencing data as described previously (10), resulting in a >200-fold coverage of the genome. Putative protein-encoding genes were identified using Prodigal version 2.0 (11). The results were inspected using Artemis (12), with manual checking and editing using BLASTP, Pfam (13), Kyoto Encyclopedia of Genes and Genomes (KEGG) (14), and Clusters of Orthologous Groups (COG) databases (15).

The complete genome of *L. lactis* UC509.9 consists of a single circular chromosome of 2,250,427 bp (35.88% G+C content)

plus eight plasmids: pCIS1 (4,263 bp), pCIS2 (5,961 bp), pCIS3 (6,159 bp), pCIS4 (7,045 bp), pCIS5 (11,676 bp), pCIS6 (40,285 bp), pCIS7 (53,051 bp), and pCIS8 (80,592 bp). The *L. lactis* UC509.9 genome is predicted to contain 2,066 protein-encoding genes, of which 168 are pseudogenes. Forty-three of these 168 pseudogenes are identical to those found in *L. lactis* subsp. *cremoris* SK11 (GenBank accession no. CP000425.1). The genome of *L. lactis* UC509.9 contains 104 transposase-encoding genes involving a total of 106,746 bp, including 42 copies of IS182 and 29 copies of IS981. The combination of the smallest lactococcal chromosome identified so far and the high number of transposons and pseudogenes suggests that the genome has undergone significant genome decay while adapting to the nutrient-rich dairy environment. A region of approximately 11 kb in size not present in other *L. lactis* genomes appears to be an integrated plasmid that includes the restriction-modification system ScrFII (16). The *L. lactis* UC509.9 plasmid complement encodes various traits for adaptation to the dairy environment, such as lactose and casein metabolism.

Nucleotide sequence accession numbers. The complete chromosome and plasmid complement of *L. lactis* subsp. *cremoris* UC509.9 were deposited in GenBank under accession no. CP003157 (chromosome), CP003165 (pCIS1), CP003164 (pCIS2), CP003163 (pCIS3), CP003162 (pCIS4), CP003161 (pCIS5), CP003160 (pCIS6), CP003159 (pCIS7), and CP003158 (pCIS8).

ACKNOWLEDGMENTS

This research was funded by a Science Foundation Ireland (SFI) Principal Investigatorship award (reference no. 08/IN.1/B1909) to D.V.S.

REFERENCES

1. Mahony J, Murphy J, van Sinderen D. 2012. Lactococcal 936-type phages and dairy fermentation problems: from detection to evolution and prevention. *Front. Microbiol.* 3:335.

2. Seegers JF, Mc Grath S, O'Connell-Motherway M, Arendt EK, van de Guchte M, Creaven M, Fitzgerald GF, van Sinderen D. 2004. Molecular and transcriptional analysis of the temperate lactococcal bacteriophage Tuc2009. *Virology* 329(1):40–52.
3. Mc Grath S, Neve H, Seegers JF, Eijlander R, Vegge CS, Brøndsted L, Heller KJ, Fitzgerald GF, Vogensen FK, van Sinderen D. 2006. Anatomy of a lactococcal phage tail. *J. Bacteriol.* 188(11):3972–3982.
4. Sciara G, Blangy S, Siponen M, Mc Grath S, van Sinderen D, Tegoni M, Cambillau C, Campanacci V. 2008. A topological model of the baseplate of lactococcal phage Tuc2009. *J. Biol. Chem.* 283(5):2716–2723.
5. Veessler D, Spinelli S, Mahony J, Lichière J, Blangy S, Bricogne G, Legrand P, Ortiz-Lombardia M, Campanacci V, van Sinderen D, Cambillau C. 2012. Structure of the phage TP901-1 1.8 MDa baseplate suggests an alternative host adhesion mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 109(23):8954–8958.
6. Vegge CS, Vogensen FK, Mc Grath S, Neve H, van Sinderen D, Brøndsted L. 2006. Identification of the lower baseplate protein as the antireceptor of the temperate lactococcal bacteriophages TP901-1 and Tuc2009. *J. Bacteriol.* 188(1):55–63.
7. Costello V. 1988. Characterization of bacteriophage-host interactions in *Streptococcus cremoris* UC503 and related lactic streptococci. Ph.D thesis. National University of Ireland, University College Cork, Cork, Ireland.
8. van Hijum SA, Zomer AL, Kuipers OP, Kok J. 2005. Projector 2: contig mapping for efficient gap-closure of prokaryotic genome sequence assemblies. *Nucleic Acids Res.* 33:W560–W566.
9. Staden R, Beal KF, Bonfield JK. 2000. The Staden package. *Methods Mol. Biol.* 1998:132–115–130.
10. Siezen RJ, Francke C, Renckens B, Boekhorst J, Wels M, Kleerebezem M, van Hijum SA. 2012. Complete resequencing and reannotation of the *Lactobacillus plantarum* WCFS1 genome. *J. Bacteriol.* 194(1):–196195–130.
11. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 8(11):119.
12. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B. 2000. Artemis: sequence visualization and annotation. *Bioinformatics* 16(10):944–945.
13. Finn RD, Mistry J, Tate J, Coggill P, Heger A, Pollington JE, Gavin OL, Gunasekaran P, Ceric G, Forslund K, Holm L, Sonnhammer EL, Eddy SR, Bateman A. 2010. The Pfam protein families database. *Nucleic Acids Res.* 38:D211–D222.
14. Kanehisa M, Goto S. 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 28(1):27–30.
15. Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN, Rao BS, Smirnov S, Sverdlov AV, Vasudevan S, Wolf YI, Yin JJ, Natale DA. 2003. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* 4:41.
16. Butler D, Fitzgerald GF. 2001. Transcriptional analysis and regulation of expression of the ScrFI restriction-modification system of *Lactococcus lactis* subsp. *cremoris* UC503. *J. Bacteriol.* 183(15):4668–4673.