

Genome Sequence of *Listeria monocytogenes* Scott A, a Clinical Isolate from a Food-Borne Listeriosis Outbreak[∇]

Yves Briers, Jochen Klumpp, Markus Schuppler, and Martin J. Loessner*

Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

Received 17 May 2011/Accepted 6 June 2011

***Listeria monocytogenes* is an opportunistic food-borne pathogen and the causative agent of listeriosis in animals and humans. We present the genome sequence of *Listeria monocytogenes* Scott A, a widely distributed and frequently used serovar 4b clinical isolate from the 1983 listeriosis outbreak in Massachusetts.**

Listeria monocytogenes is an infectious organism and member of the *Firmicutes*. Listeriosis manifests itself as septicemia or meningoenzephalitis in elderly and immunocompromised patients, while neonatal listeriosis can lead to abortion and stillbirth. *L. monocytogenes* is transmitted through raw and ready-to-eat foods. Due to changes in food processing and distribution over the last few decades, the rate of listeriosis in Europe is increasing (1, 11).

Since its isolation during the Massachusetts listeriosis outbreak in 1983 (3), the serovar 4b strain Scott A has been widely used as a reference strain for efficacy testing of food processing and preservation techniques (4, 6, 7), establishment of detection methods in foods (12), growth and heat resistance studies (2), and virulence studies (8). Scott A also harbors the temperate phage PSA, which employs unusual decoding mechanisms (14), and its integration into the 3' end of single-copy tRNA^{Arg} was used for design of a now widely used integration vector (9). However, its genome sequence has not been determined.

A paired-end library (200-bp fragments) was sequenced on an Illumina genome analyzer II, yielding 6,152,268 reads of 31 bp (62-fold total coverage). A high-quality draft genome was assembled with CLC Genomics Workbench 4.6.1 (CLC Bio, Aarhus, Denmark) using a combinatorial approach of reference assembly against *L. monocytogenes* F2365 (GenBank accession number AE017262.2), *de novo* assembly of nonassembled reads, and manual editing. The length of this draft genome is 3,021,822 bp, distributed in 5 contigs (from which the last two form a scaffold), with an average GC content of 37.9%. The final pseudogenome corresponds unequivocally with the physical macrorestriction map of Scott A assembled previously, based on restriction analysis and contour-clamped homogeneous electric field electrophoresis (5). Gene prediction and functional annotation were performed using the DIYA pipeline (13) with manual editing, taking into account the presence of ribosome binding sites, BLASTx results, and the open reading frame (ORF) prediction tool of CLC Genomics Workbench. The chromosome features 2,953 pre-

dicted ORFs, at least 65 tRNA genes, two pseudo-tRNAs, six copies of 16S-23S-5S operons, and two prophage sequences.

Scott A possesses a conserved *prfA* virulence gene cluster with high sequence identity (99%) to *Listeria* isolates of lineage I. Comparative analysis of the multilocus sequence typing (MLST) determinants (10) showed that this strain is closely related to lineage I clonal complex 2 (CC2), sequence type 290 (ST290). The only previous isolate of type ST290 is another human clinical serovar 4b strain from Canada (10). Scott A carries *inlA*, *inlB*, *inlD* (2×), *inlE*, *inlI*, *inlJ*, and 18 other leucine-rich internalin-like coding sequences. InlA is produced from a full-length allele type 22-23-24, which has been linked to human illness (10). A gene cluster unique to Scott A (CDS 2150-2470, a putative mobile genetic element from *Enterococcus faecalis*), comprises 32 genes, including a complete arsenic resistance operon and a cadmium-translocating ATPase.

Nucleotide sequence accession number. The sequencing project has been deposited at the Genomes OnLine Database (GOLD) under Gi09588 and at DDBJ/EMBL/GenBank under the accession number AFGI00000000. The version described in this paper is the first version, AFGI01000000. The complete annotation has been assigned GenBank accession number CM001159.

This work was funded by the Competence Center Environment and Sustainability (CCES) of the ETH Domain (ERU FEH, BactFlow). Yves Briers holds an EMBO long-term fellowship of the European Molecular Biology Organization, Heidelberg, Germany.

We are grateful to Shanmuga Sozhamannan and Mohit Patel, Naval Medical Research Center, Rockville, MD, for running the DIYA analysis pipeline.

REFERENCES

1. Allerberger, F., and M. Wagner. 2010. Listeriosis: a resurgent foodborne infection. *Clin. Microbiol. Infect.* **16**:16–23.
2. Bereksi, N., F. Gavini, T. Benezech, and C. Faille. 2002. Growth, morphology and surface properties of *Listeria monocytogenes* Scott A and LO28 under saline and acid environments. *J. Appl. Microbiol.* **92**:556–565.
3. Fleming, D. W., et al. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* **312**:404–407.
4. Guenther, S., D. Huwyler, S. Richard, and M. J. Loessner. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl. Environ. Microbiol.* **75**:93–100.
5. He, W., and J. B. Luchansky. 1997. Construction of the temperature-sensitive vectors pLUCH80 and pLUCH88 for delivery of Tn917::NotI/SmaI and use of these vectors to derive a circular map of *Listeria monocytogenes* Scott A, a serotype 4b isolate. *Appl. Environ. Microbiol.* **63**:3480–3487.
6. Ita, P. S., and R. W. Hutkins. 1991. Intracellular pH and survival of *Listeria monocytogenes* Scott A in tryptic soy broth containing acetic, lactic, citric, and hydrochloric acids. *J. Food Prot.* **54**:15–19.
7. Jin, T. 2010. Inactivation of *Listeria monocytogenes* in skim milk and liquid

* Corresponding author. Mailing address: Institute of Food, Nutrition and Health, ETH Zurich, Schmelzbergstrasse 7, CH 8092 Zurich, Switzerland. Phone: (41) 44 632 33 35. Fax: (41) 44 632 12 66. E-mail: martin.loessner@ethz.ch.

[∇] Published ahead of print on 17 June 2011.

- egg white by antimicrobial bottle coating with polylactic acid and nisin. *J. Food Sci.* **75**:M83–M88.
8. **Lammerding, A. M., K. A. Glass, A. Gendron-Fitzpatrick, and M. P. Doyle.** 1992. Determination of virulence of different strains of *Listeria monocytogenes* and *Listeria innocua* by oral inoculation of pregnant mice. *Appl. Environ. Microbiol.* **58**:3991–4000.
 9. **Lauer, P., M. Y. N. Chow, M. J. Loessner, D. A. Portnoy, and R. Calendar.** 2002. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J. Bacteriol.* **184**:4177–4186.
 10. **Ragon, M., et al.** 2008. A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog.* **4**:e1000146. doi:10.1371/journal.ppat.1000146.
 11. **Schlech, W. F.** 2000. Foodborne listeriosis. *Clin. Infect. Dis.* **31**:770–775.
 12. **Schmelcher, M., et al.** 2010. Rapid multiplex detection and differentiation of *Listeria* cells by use of fluorescent phage endolysin cell wall binding domains. *Appl. Environ. Microbiol.* **76**:5745–5756.
 13. **Stewart, A. C., B. Osborne, and T. D. Read.** 2009. DIYA: a bacterial annotation pipeline for any genomics lab. *Bioinformatics* **25**:962–963.
 14. **Zimmer, M., E. Sattlberger, R. B. Inman, R. Calendar, and M. J. Loessner.** 2003. Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed +1 translational frameshifting in structural protein synthesis. *Mol. Microbiol.* **50**:303–317.