

Draft Genome Sequence and Annotation of Phyllosphere-Persisting Salmonella enterica subsp. enterica Serovar Livingstone Strain CKY-S4, Isolated from an Urban Lake in Regina, Canada

Dinah D. Tambalo, Benjamin J. Perry, Stephen F. Fitzgerald, Andrew D. S. Cameron, Christopher K. Yost University of Regina, Regina, Saskatchewan, Canada

Here, we report the first draft genome sequence of *Salmonella enterica* subsp. *enterica* serovar Livingstone. This S. Livingstone strain CKY-S4 displayed biofilm formation and cellulose production and could persist on lettuce. This genome may help the study of mechanisms by which enteric pathogens colonize food crops.

Received 29 June 2015 Accepted 30 June 2015 Published 13 August 2015

Citation Tambalo DD, Perry BJ, Fitzgerald SF, Cameron ADS, Yost CK. 2015. Draft genome sequence and annotation of phyllosphere-persisting Salmonella enterica subsp. enterica serovar Livingstone strain CKY-S4, isolated from an urban lake in Regina, Canada. Genome Announc 3(4):e00884-15. doi:10.1128/genomeA.00884-15.

Copyright @ 2015 Tambalo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Christopher K. Yost, chris.yost@uregina.ca.

Salmonella is a major cause of foodborne illness with a global burden estimated at 93.8 million cases annually (1). Fresh fruits and vegetables are recognized as important routes for transmission of foodborne pathogens (2–4). These pathogens can contaminate produce during production and handling. Following contamination, *Salmonella* has been shown to colonize and survive on vegetable crops (5–7).

Salmonella enterica subsp. enterica serovar Livingstone CKY-S4 was isolated from Wascana Lake, located in Regina, Saskatchewan, Canada. The strain was serotyped as serovar Livingstone at the Saskatchewan Disease Control Laboratory. Strains classified as Livingstone serotype have been isolated from water, poultry, and animal products (8), although human infections involving *Salmonella* Livingstone are sporadic (9). S. Livingstone CKY-S4 exhibited biofilm formation and cellulose production and was resistant to streptomycin, erythromycin, and spectinomycin. A plant colonization experiment measured the ability of CKY-S4 to persist on Romaine lettuce (cv. Paris Island), with a reduction rate of $-0.31 \log_{10}$ CFU per day. CKY-S4 persisted for at least 9 days with a major reduction of 4-log CFU/leaf, in the first 5 days.

Genomic DNA was isolated using a Bio Basic Canada DNA isolation kit, and prepared for Illumina sequencing using a NEB-Next Ultra DNA library prep kit, with size selection for approximately 700-bp fragments of genomic DNA. DNA sequencing was performed using an Illumina MiSeq, with 300-bp paired-end version 3 chemistry. Raw fastq sequence data were filtered for PhiX sequence missed by the MiSeq filtering pipeline using Bowtie2 (10), and the remaining 5,076,916 paired-end reads were used for assembly.

Genome assembly was performed using the A5-MiSeq Assembly pipeline (11) and resulted in a consensus length of 4,803,211 bp composed of 31 contigs. The assembly had an N_{50} value of 445,170 bp, an average coverage of ×260, and a GC content of 52.1% and could have 75% of the genome represented by 7 large contigs. The annotation revealed 4,473 coding sequences within the genome. In addition, 48 pseudogenes, 15 noncoding

RNAs (ncRNAs), and 2 clustered regularly interspaced short palindromic repeat (CRISPR) arrays were found.

Using the phage search tool (PHAST), three prophage-like regions were identified within the genome that shared homologous regions to phage BcepMu, SE1, and *Cronobacter* phage vB_CsaM_GAP32, with similarity scores of 20, 60, and 50, respectively (12). Antibiotic resistance genes were searched for using the antibiotic resistance genes database (ARDB). Multidrug efflux pump encoding genes *acrB*, *mdtK*, and *mdtL* were identified, as well as the macrolide-specific efflux pump *macB* and the penicillin binding protein *pbp2*. Further analysis of the annotation also confirmed the presence of the *Salmonella* pathogenicity islands required for invasion (SPI-1) and intracellular survival (SPI-2) within a mammalian host.

This is the first draft genome of a *Salmonella* strain in the serotype Livingstone. Its ability to colonize the phyllosphere of lettuce has been measured and its genome contains multiple antibiotic resistance genes, and three prophage-like regions. Availability of the draft genome increases the diversity of *Salmonella* genomes currently available for comparative analysis.

Nucleotide sequence accession numbers. The draft genome sequence of *Salmonella* Livingstone CKY-S4 was deposited in the GenBank/EMBL database with the accession number JZWK00000000. The version described in this paper is the first version, JZWK00000000.1.

ACKNOWLEDGMENTS

The serotyping of the *Salmonella* Livingstone strain CKY-S4 was conducted by Christine Turenne of the Saskatchewan Disease Control Laboratory.

Funding for the isolation and characterization of the *Salmonella* Livingstone isolate was provided by the Saskatchewan Ministry of Agriculture administered through an Agriculture Development Fund grant. Funding for the sequencing of the isolate was provided by Natural Sciences and Engineering Research Council of Canada Discovery grant 435784-2013 and Saskatchewan Health Research Foundation Establishment grant 2867.

REFERENCES

- Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882–889. http://dx.doi.org/ 10.1086/650733.
- Olaimat AN, Holley RA. 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol 32:1–19. http://dx.doi.org/ 10.1016/j.fm.2012.04.016.
- 3. Warriner K, Huber A, Namvar A, Fan W, Dunfield K, Steve LT. 2009. Recent advances in the microbial safety of fresh fruits and vegetables, p 155–208. *In* Advances in Food and Nutrition Research, vol 57. Academic Press, New York, NY.
- Heaton JC, Jones K. 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J Appl Microbiol 104:613–626. http://dx.doi.org/10.1111/j.1365 -2672.2007.03587.x.
- Kisluk G, Yaron S. 2012. Presence and persistence of Salmonella enterica serotype Typhimurium in the phyllosphere and rhizosphere of sprayirrigated parsley. Appl Environ Microbiol 78:4030–4036. http:// dx.doi.org/10.1128/AEM.00087-12.
- 6. Islam M, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X. 2004. Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with con-

taminated manure composts or irrigation water. Foodborne Pathog Dis 1:27–35. http://dx.doi.org/10.1089/153531404772914437.

- 7. Gandhi M, Golding S, Yaron S, Matthews KR. 2001. Use of green fluorescent protein expressing *Salmonella* Stanley to investigate survival, spatial location, and control on alfalfa sprouts. J Food Protect **64**: 1891–1898.
- Crichton PB, Old DC, Taylor A, Rankin SC. 1996. Characterisation of strains of *Salmonella* serotype Livingstone by multiple typing. J Med Microbiol 44:325–331. http://dx.doi.org/10.1099/00222615-44-5-325.
- Guerin PJ, De Jong B, Heir E, Hasseltvedt V, Kapperud G, Styrmo K, Gondrosen B, Lassen J, Andersson Y, Aavitsland P. 2004. Outbreak of *Salmonella* Livingstone infection in Norway and Sweden due to contaminated processed fish products. Epidemiol Infect 132:889–895. http:// dx.doi.org/10.1017/S0950268804002523.
- Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10:R25. http://dx.doi.org/10.1186/gb-2009-10-3-r25.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. http://dx.doi.org/10.1093/bioinformatics/btu661.
- Zhou Y, Liang Y, Lynch K, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. http://dx.doi.org/ 10.1093/nar/gkr485.