Complete Genome Sequence of the Universal Killer Salmonella enterica Serovar Typhimurium UK-1 (ATCC 68169)[∇]

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The Salmonella enterica serovar Typhimurium strain UK-1 exhibits the highest invasion and virulence attributes among the most frequently studied strains. S. Typhimurium UK-1 has been used as the foundation for developing recombinant vaccines and has been used extensively on virulence and colonization studies in chickens and mice. We describe here the complete genome sequence of S. Typhimurium UK-1. Comparative genomics of Salmonella Typhimurium will provide insight into factors that determine virulence and invasion.

Salmonella enterica serovar Typhimurium is the principal cause of food-related illness (3). More than 200 different *S*. Typhimurium strains have been identified, which are principally adapted to niches in the environment and the intestines of different animal species (12).

S. Typhimurium strain UK-1 (UK stands for universal killer) was first isolated from the spleen of a chick orally inoculated 3 days earlier with a highly virulent *S.* Typhimurium strain retrieved from an infected horse in 1991 (4). UK-1 is not only highly invasive and virulent for chickens and mice, but it is also capable of lethal infections in calves, pigs, and horses. Because of the high virulence of UK-1, attenuated derivatives of the UK-1 strain would probably induce a higher level of protective immunity after oral administration than the attenuated derivatives of less-virulent *S.* Typhimurium strains with the same route of infection as UK-1 (13). This strain has been used as the foundation for developing recombinant vaccines (2, 6, 11).

Whole-genome sequencing of UK-1 was performed on 454 GS-FLX titanium by SeqWright, Inc. A 3-kb Mate-Pair library was constructed for the sequencing. Three separate one-quarter runs were performed to minimize sequencing errors. In total, 189,174,669 bases of 592,216 random reads with an average read length of 311 nucleotides were obtained. SeqWright performed the assembly using Newbler Assembler software resulting in 11 scaffolds with 37 gaps. The approximate coverage of UK-1 genome is 38-fold. An optical map of the UK-1 strain was generated to help with genome completion (14). All gaps were closed using Sanger DNA sequencing.

UK-1 genome annotation depended mainly on that of *S*. Typhimurium LT2 (9). The UK-1-specific genes were determined by three methods: GeneMark.hmm (8), Glimmer v3.02 (5), and Prodigal (7). The genes in repetitive and prophage regions and genes shorter than 100 bp were manually examined to ensure a complete annotation with a high degree of

* Corresponding author. Mailing address: The Biodesign Institute, Center for Infectious Diseases and Vaccinology, Arizona State University, P.O. Box 875401 Tempe, AZ 85287-5401. Phone: (480) 727-0445. Fax: (480) 727-0466. E-mail: rcurtiss@asu.edu. confidence. The description for each gene was investigated by comparing it to the annotation of the LT2 gene (9). For the genes that were absent in LT2, we performed BLASTP searches using these genes against the nr database and annotated each with the function of the best homologs (1). rRNA and tRNA genes were predicted using BLASTN against rRNA and tRNA genes from LT2, respectively.

The genome of *S*. Typhimurium UK-1 is composed of a 4,817,868-bp chromosome and a 93,277-bp large virulence plasmid, designated pSTUK-100. The chromosome contains 4,478 open reading frames (ORFs) including 22 pseudogenes, and pSTUK-100 contains 101 ORFs with one pseudogene. Comparison of UK-1, LT2, and three other *Salmonella* Typhimurium strains (14028s, D23580, and SL1344) revealed high colinearity. However, UK-1 carries the smallest number of prophage elements, but it exhibits the highest virulence among the frequently studied strains. Two unique genes were predicted in UK-1, which are highly homologous to the genes involved in the type III secretion system (10).

Nucleotide sequence accession numbers. The complete *S*. Typhimurium UK-1 genome has been deposited in GenBank under accession no. CP002614 for the chromosome and CP002615 for the plasmid.

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REFERENCES

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
- Bollen, W. S., B. M. Gunn, H. Mo, M. K. Lay, and R. Curtiss III. 2008. Presence of wild-type and attenuated Salmonella enterica strains in brain tissues following inoculation of mice by different routes. Infect. Immun. 76:3268–3272.
- Centers for Disease Control and Prevention. 2008. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food–10 states, 2007. MMWR Morb. Mortal. Wkly. Rep. 57:366–370.
- Curtiss, R. I., et al. 1991. Colonization control of human bacterial enteropathogens in poultry, p. 169–198. *In* L. C. Blankenship, J. S. Bailey, N. A. Cox, S. E. Craven, R. J. Meinersmann, and N. S. Stern (ed.), Nonrecombinant and recombinant avirulent Salmonella live vaccines for poultry. Academic Press, Inc., San Diego, CA.

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^{5.} Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg. 1999.

Improved microbial gene identification with GLIMMER. Nucleic Acids Res. 27:4636–4641.

- Gunn, B. M., S. Y. Wanda, D. Burshell, C. Wang, and R. Curtiss III. 2010. Construction of recombinant attenuated Salmonella enterica serovar Typhimurium vaccine vector strains for safety in newborn and infant mice. Clin. Vaccine Immunol. 17:354–362.
- 7. Hyatt, D., et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119.
- Lukashin, A. V., and M. Borodovsky. 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. 26:1107–1115.
- McClelland, M., et al. 2001. Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature 413:852–856.
- Miao, E. A., and S. I. Miller. 2000. A conserved amino acid sequence directing intracellular type III secretion by Salmonella typhimurium. Proc. Natl. Acad. Sci. U. S. A. 97:7539–7544.
- Shi, H., S. Wang, K. L. Roland, B. M. Gunn, and R. Curtiss III. 2010. Immunogenicity of a live recombinant Salmonella enterica serovar Typhimurium vaccine expressing pspA in neonates and infant mice born from naive and immunized mothers. Clin. Vaccine Immunol. 17:363–371.
- Winfield, M. D., and E. A. Groisman. 2003. Role of nonhost environments in the lifestyles of Salmonella and Escherichia coli. Appl. Environ. Microbiol. 69:3687–3694.
- Zhang, X., S. M. Kelly, W. Bollen, and R. Curtiss III. 1999. Protection and immune responses induced by attenuated Salmonella typhimurium UK-1 strains. Microb. Pathog. 26:121–130.
- Zhou, S. J. H., and D. C. Schwartz. 2007. A single molecule system for whole genome analysis, p. 265–300. *In* K. R. Mitchelson (ed.), New high throughput technologies for DNA sequencing and genomics. Elsevier Scientific Publishers, Amsterdam, the Netherlands.