

Genome Sequence of *Salmonella enterica* Serovar Typhi Oral Vaccine Strain Ty21a

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Attenuated *Salmonella enterica* serovar Typhi strain Ty21a is an important vaccine for controlling typhoid fever and serves as an oral vector for delivering heterologous antigens. The key attenuating features of this randomly mutated strain remain in question. Genome sequencing has revealed 679 single nucleotide polymorphisms (SNPs), and will help define alterations contributing to Ty21a safety and immunogenicity.

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Prior genomic sequencing of *Salmonella enterica* serovar Typhi (1–3) has both stimulated research and increased our understanding of typhoid pathogenesis. Nevertheless, typhoid fever remains a major cause of worldwide morbidity and mortality, with an estimated global annual incidence of 22 million cases and >600,000 deaths (4, 5). The observed increasing antibiotic resistance of typhoid isolates in nature leaves typhoid vaccination as a critically important control measure.

Development of the live, attenuated *Salmonella enterica* serovar Typhi strain Ty21a (6) validated the concept that orally delivered, attenuated enteric bacteria can mimic natural infection in stimulating both mucosal and systemic immune responses without causing disease symptoms (7). Ty21a has been shown to be highly safe (i.e., no reversion to virulence) following administration to >200 million recipients over >25 years. Ty21a, tested in several large clinical trials totaling >500,000 adults and children, has demonstrated overall typhoid protective efficacies of 67 to 96%, which were maintained at high levels through at least 7 full years (8). Importantly, Ty21a attenuation resulted in an enviable balance of reduced virulence and enhanced immunogenicity relative to immunity derived following natural typhoid infections (i.e., only 30 to 35% protection following acute typhoid disease). Ty21a has also been employed as an oral delivery system to protect against other disease agents, such as shigellosis (9–11) and the biodefense threat anthrax (12).

Derived from parent strain Ty2, Ty21a was primarily mutated to *galE* negativity and Vi-capsule nonexpression by random mutagenic methods (6) but was also selected for downregulation of other genes in galactose metabolism (13, 14). Additionally, other mutations (e.g., in *rpoS* and *ilvD*) are also known to exist in Ty21a (14). A full accounting of Ty21a genomic mutations has, to date, been lacking. Thus, the intent of these studies has been to enumerate, locate, and analyze all mutations in Ty21a relative to the Ty2 parent. The resulting data are being used to gain a better under-

standing of Ty21a attenuation (i.e., safety) and enhanced immunogenicity over wild-type strains, information that might be helpful in the development of new attenuated bacterial vaccines.

DNA was prepared with a genomic isolation kit (Promega) from L-broth-grown Ty21a obtained from commercially available Vivotif capsules (Crucell/Berna Biotech, Ltd., Switzerland). Initially, a 98% complete Ty21a genomic sequence was derived using a NimbleGen chip-based (high-density microarray) resequencing analysis compared to the published Ty2 genome (NCBI accession number AE014613); this approach resulted in numerous uncalled nucleotides. Separately, a Ty21a genomic sequence obtained from 4× coverage by random 454-based sequencing was determined. These two sequences were used integratively to verify identical genomic regions and to detect areas of disparity. All incongruous regions were resequenced using double-strand sequence analysis for clarification. The Ty21a genome comprises a single circular contig consisting of 4,791,958 bp, with an average G+C content of 52.06%. A total of 4,339 coding regions (open reading frames [ORFs]) were defined in Ty21a using GeneMark (<http://opal.biology.gatech.edu/GeneMark>) and compared with known Ty2 ORFs by BLAST analysis. A total of 679 SNPs was identified in Ty21a, relative to the published Ty2 genome (2). A detailed analysis of these mutations will be included in future publications.

Nucleotide sequence accession number. The *Salmonella enterica* serovar Typhi strain Ty21a genome sequence has been deposited in GenBank under the accession number CP002099.

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