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PROKARYOTES

Whole-Genome Sequences of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium Strains TT6675 and TT9097 Employed in the Isolation and Characterization of a Giant Phage Mutant Collection

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ABSTRACT We report here the genome sequences of *Salmonella enterica* subsp. *enterica* serovar Typhimurium strains TT6675 and TT9097, which we utilize for genetic analyses of giant bacterial viruses. Our analyses identified several genetic variations between the two strains, most significantly confirming strain TT6675 as a serine suppressor and TT9097 as a nonsuppressor.

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The characterization of giant bacterial viruses (phages) with genomes >200 kb and complex virions are of interest to our research group. Recent studies have indicated that giant phages are abundant in the environment, infect a broad range of bacterial hosts, and have the potential for therapeutic applications (e.g., phage therapy). However, we have a limited understanding of the biology of giant phages due to the fact that many of their genes are functionally uncharacterized. To address this problem, we utilized two strains of *Salmonella enterica* subsp. *enterica* serovar Typhimurium LT2, TT6675 and TT9097, to successfully isolate an amber mutant collection for giant phage SPN3US (1). To undertake further studies of SPN3US, particularly omics analyses of infection, we needed to define the nucleotide sequences of these two strains.

Cells were cultured in nutrient medium, and genomic DNA was isolated using the GenElute bacterial genomic kit (Sigma-Aldrich, St. Louis, MO, USA). Sequencing libraries were prepared using the Nextera XT library prep kit (Illumina, San Diego, CA, USA) and tagged with unique dual-index barcodes. Sequencing was performed on the MiSeq desktop sequencer (Illumina) located at the Monash University Malaysia Genomics Facility. Paired-end reads were error-corrected and assembled *de novo* using SPAdes version 2.7 (2). The draft genomes were subsequently annotated via the NCBI Prokaryotic Genome Annotation Pipeline (3). Transfer RNA genes were manually verified using tRNA-Scan (4) and Aragorn (5).

The genome length of each strain is 4.86 Mb, with G+C contents of 52.23% and 52.27% for strains TT6675 and TT9097, respectively. Strain TT6675 was determined to encode 77 standard tRNAs, whereas strain TT9097 was determined to encode 78 standard tRNAs. This variation was due to the identification of a single nucleotide mutation in the anticodon (CGA-CTA) of the serine tRNA in TT6675 (serU of the type strain [6]), which produces a suppressor tRNA. This resolved the genetic basis for the suppressor and

nonsuppressor phenotypes observed for TT6675 and TT9097, respectively. This is important for further work, as we can now incorporate accurate mutant peptides into mass spectral search libraries for analyses of mutant phage proteomes. Amber mutations in the histidinol-phosphate aminotransferase (*hisC*) and 2-isopropylmalate synthase (*leuA*) genes were identified in both strains, consistent with their reported phenotypes (J. Roth, personal communication). In addition, a 93.9-kb pSLT conjugative plasmid (7) was identified on a single contig in strain TT6675 (sequence no. MCGC01000009) and on five contigs in TT9097 (sequence no. MCGM0100112, MCGM01000114, MCGM01000149, and MCGM01000034). Components of the Tn*10* transposon were identified, although neither strain contains the reportedly inactive IS*10*-left transposase. TT6675 has only the IS*10*-right transposase gene, whereas TT9097 has *jemA*, *jemB*, *jemC*, *tetR*, *tetA*, *tetC*, *tetD*, and IS*10*-right transposase genes. The determination of the nucleotide sequences and variations between these two *S*. Typhimurium strains will be valuable for analyses of the molecular events during giant phage infection.

Accession number(s). Nucleotide sequences for the *S*. Typhimurium strains reported here have been deposited at DDBJ/EMBL/GenBank under the accession no. MCGL01000000 for TT6675 and MCGM01000000 for TT9097. The versions described in this paper are the first versions.

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