Whole-Genome Shotgun Sequence of the Sulfur-Oxidizing Chemoautotroph *Pseudaminobacter salicylatoxidans* KCT001

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The facultatively sulfur-oxidizing chemolithoautotrophic alphaproteobacterium *Pseudaminobacter salicylatoxidans* KCT001 (MTCC 7265) belongs to the family *Phyllobacteriaceae* of the order *Rhizobiales*. Analysis of its genome offers valuable insight into the adaptive specializations and evolution of free-living soil bacteria that are phylogenetically closely related to symbiotic and invasive rhizobacteria.

Taxonomically diverse bacteria can use sulfur as an inorganic substrate (lithotrophy) by apparently distinct biochemical pathways (5). This mechanistic multiplicity notwithstanding, *sox* (sulfur oxidation) genes are omnipresent in members of the domain *Bacteria* (4, 6). However, functional essentiality of the Sox complex is proven only in facultatively lithotrophic alphaproteobacteria (5). The soil-dwelling alphaproteobacterium *Pseudaminobacter salicylatoxidans* KCT001 (family *Phyllobacteriaceae*, order *Rhizobiales* [2]) is a well-studied Sox model (9, 10), but unlike the prototypical *Paracoccus pantotrophus* (4), it can utilize tetrathionate as a substrate (10) and produces distinct ³⁴S fractionation during thiosulfate oxidation (M. Alam et al., unpublished data). Evidently, there is more to sulfur lithotrophy even within members of the class *Alphaproteobacteria* than what is explained by the Sox system alone. As such, whole-genome information on strain KCT001 would not only help us understand sulfur lithotrophy better but would also help in elucidating adaptations and evolution of free-living soil bacteria vis-à-vis their symbiotic and invasive relatives.

Genome sequencing was performed on the Ion PGM sequencer (12) using a 316 chip. MIRA 3.4.0 was used to assemble 1,640,570 reads (mean length of 226 nucleotides) into 53 contigs at 66× overall coverage. The genome was annotated by compiling results obtained from the RAST server (1) with those derived from manual analyses. In the estimated 4,612,407-bp genome of *P. salicylatoxidans* KCT001, 4,553 potential coding sequences (CDSs) could be identified, out of which ~800 were hypothetical genes. The G+C content of the genome was 62.8%. One copy each of the 16S, 23S, and 5S rRNA genes were identified together with 48 tRNA genes. Though the rRNA operon was distributed over five contigs, careful scrutiny revealed 200- to 250-nucleotide overlaps among them (*viz.*, PROY_52, PROY_44, reverse complementary [RC] of PROY_45, RC of PROY_50 and PROY_46, in that order), thereby suggesting their merger into one. Again, the 125,589-bp contig PROY_16 could be part of a large plasmid, since it harbored a *repABC* operon alongside a *parB* gene, four integrases/recombinases, and 11 transposon/IS-related genes. Notably, the putative chromosome replicating and partitioning genes were found located elsewhere.

Corroborating the phylogenetic proximity of the KCT001 strain with the nodule-forming and nitrogen-fixing mesorhizobia, its genome revealed at least one homolog each of *nodB* (involved in nodulation signal synthesis) (7) and of *nodF* and *nodE* (which define the host range of rhizobia) (3). Additionally, several *exo* genes, attributed to succinoglycan biosynthesis for infection

thread formation (8, 11), plus one *ntrYX-ntrBC* global nitrogen regulatory system were also identified.

Neither a pathogenicity island nor a gene coding for a toxin or superantigen or for a protein involved in quorum sensing, biofilm formation, or adhesion was detected. The genome, however, encompassed 14 constituents of the mycobacterial virulence operon, six genes coding for colicin V and bacteriocin, 53 flagellar motility and chemotaxis genes, 56 genes for resistance to antibiotics and toxic compounds, and 12 genes encoding components of a type II protein secretion system.

Salient genomic features reflecting KCT001's adaptation to tropical soil include the following: tetrathionate respiration and use of anaerobic respiratory reductases; oxidation of carbon monoxide and sulfur, dehydrogenation of succinate, formate, etc.; nitrate and nitrite ammonification and ammonia assimilation; methanogenesis; CO₂ fixation and photorespiration; di- and oligosaccharide biosynthesis; glycerate metabolism; lactate utilization; butanol biosynthesis; glycerol and glycerol-3-phosphate utilization; use of acetone as a carbon and energy source; and monosaccharide metabolism.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at EMBL/DDBJ/GenBank under the accession number [CAIU00000000](#). The version described in this paper is the first version, CAIU01000000.

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