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Completed Genome Sequence of the Anaerobic Iron-Oxidizing Bacterium *Acidovorax ebreus* Strain TPSY▽

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***Acidovorax ebreus* strain TPSY is the first anaerobic nitrate-dependent Fe(II) oxidizer for which there is a completed genome sequence. Preliminary protein annotation revealed an organism optimized for survival in a complex environmental system. Here, we briefly report the completed and annotated genome sequence of strain TPSY.**

Microorganisms from diverse anoxic environments are capable of nitrate-dependent Fe(II) oxidation at circumneutral pH (4, 11, 17, 18, 20, 21). Despite their geochemical importance (22), little is known of the underlying biochemical and genetic mechanisms. Genome sequencing of several nitrate-dependent Fe(II) oxidizers will provide insight into this process. By comparing Fe(II) oxidation mechanisms in various organisms, we hope to identify both the conserved and disparate aspects of the metabolism. The genome of *Acidovorax ebreus* strain TPSY is the first of these to be sequenced.

Strain TPSY is a motile, Gram-negative facultative anaerobe isolated from groundwater collected from the U.S. Department of Energy Integrated Field Research Challenge site at Oak Ridge, TN. Growth experiments performed as previously described (21) revealed TPSY's incapacity for lithoautotrophic growth, which was supported by a lack of genes in the genome encoding any known CO_2 fixation pathways. TPSY did grow mixotrophically with Fe(II) as the electron donor and a 0.1 mM acetate carbon source. 16S rRNA gene sequence analysis placed TPSY in the class *Betaproteobacteria* with 99.8% similarity to *Acidovorax* sp. strain JS42 in the family *Comamonadaceae*.

The completed genome consisted of a single circular chromosome of 3,796,573 bp with an average 66.8% G+C content. A total of 3,479 protein-encoding genes were predicted, and 34 (0.98%) had no similarity to public database sequences. Sequencing performed at the Department of Energy Joint Genome Institute (JGI) used Sanger sequencing and 454 pyrosequencing to a depth of 20× coverage. All JGI library construction and sequencing techniques can be found at http://www.jgi.doe.gov/. Sequence assembly, quality assessment, and annotation were performed using the software Phred/Phrap/Consed (www.phrap.com) (6–8), Dupfinisher (10), CRITICA (2), GLIMMER, and GENERATION (5) and the JGI Integrated Microbial Genomes site (12). The completed genome sequence contained 33,341 reads and had an average of ninefold coverage per base and an error rate of <1 in 100,000.

TPSY was named in part for its meandering motility, and its genome confirmed the twitching phenotype with the presence of *pilT*, *pilU*, and a complete set of flagellar and chemotaxis genes. The ability of TPSY to oxidize simple alcohols and acids with oxygen or nitrate respiration was confirmed by the genome. In addition, biosynthetic pathways for all amino acids except tyrosine and phenylalanine were present. No homologues of chorismate mutase (EC 5.4.99.5), an enzyme required for tyrosine and phenylalanine anabolism, were identified. The genome contained both intact Embden-Meyerhof-Parnas and Entner-Doudoroff pathways, in addition to a pentose phosphate pathway and a trichloroacetic acid cycle.

In support of its facultative anaerobicity, a complete set of genes for denitrification and three different terminal oxidases (cytochrome aa_3, cbb_3, and cytochrome *d* quinol oxidase) were present. The cbb_3 and cytochrome *d* oxidases, with their high oxygen affinity, putatively enable survival in microaerobic environments (14).

TPSY had sequences encoding 30 transposases, 11 integrases, and 11 phage/prophage-related genes. A region of particular interest putatively conferred resistance to lead, arsenate, and mercury: *pbrRATARTBC*, *arsRDAB*, and *merRPCADE*. Evidence suggests horizontal transfer and insertion of this region, as it was flanked on the 5′ end by λ prophage-related genes and the 3′ end encoded a putative Tn*21* transposase. Phenotypic studies by the method of Wang et al. (19) revealed MICs of 16 μM phenylmercuric acetate and 250 μM $MgCl_2$. TPSY was also capable of growth in the presence of arsenate (10 mM) but did not use it as an electron acceptor.

Related to phage infection, one CRISPR (clustered, regularly interspaced, short palindromic repeats) region (3, 16) was predicted. The core proteins, the *cas1* and *cas2* genes, and a *csn1* gene formed the CRISPR subtype Nmeni, which is associated with vertebrate pathogens and commensals (9). However, the lack of typical pathogenic type I or III secretion systems such as the *hec* cluster of *Dickeya chrysanthemi* (15) or the *inv*/*spa* system of *Salmonella enterica* serovar Typhimurium

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(13) indicated that TPSY would probably not exhibit a pathogenic lifestyle.

Nucleotide sequence accession number. The genome sequence of *Acidovorax ebreus* strain TPSY (formerly *Diaphorobacter* sp. strain TPSY) reported in this paper has been deposited in the GenBank database under accession number NC_011992.

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