

Complete Genome Sequence of the B₁₂-Producing *Shimwellia blattae* Strain DSM 4481, Isolated from a Cockroach

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Here we announce the complete genome sequence of the coenzyme B₁₂-producing enteric bacterium *Shimwellia blattae* (formerly *Escherichia blattae*). The genome consists of a single chromosome (4,158,636 bp). The genome size is smaller than that of most other enteric bacteria. Genome comparison revealed significant differences from the *Escherichia coli* genome.

The enteric bacterium *Shimwellia blattae* (formerly *Escherichia blattae*) has been isolated from the hindgut of a cockroach (2). In contrast to *Escherichia coli* and other enteric bacteria, *S. blattae* is nonpathogenic for humans. In addition, it is able to synthesize coenzyme B₁₂ *de novo*, and molecular tools developed for *E. coli* such as cloning and expression systems are functional in *S. blattae* (3). Thus, this organism is an interesting alternative to *E. coli* with respect to biotechnological applications. The complete genome sequence of *S. blattae* DSM 4481 was determined by whole-genome shotgun Sanger sequencing. To establish a shotgun library, genomic DNA was sheared and the resulting DNA fragments (1.5 to 4 kb) were cloned into the vector pBluescript SKII (Stratagene, San Diego, CA). Subsequently, plasmid inserts (approximately 57,000) were sequenced from both ends. PCR-based techniques and primer walking on recombinant plasmids were applied to close remaining gaps.

The manually curated and annotated sequence of *S. blattae* DSM 4481 comprises a single chromosome of 4,158,636 bp and no plasmids. The chromosome encodes 3,904 predicted protein-encoding genes and 88 tRNA genes. The genome size of *S. blattae* is smaller than those of other enteric bacteria such as *Klebsiella pneumoniae* 342 (CP000964), *Citrobacter koseri* ATCC BAA-895 (CP000822), and *E. coli* strain K-12 substrain MG1655 (U00096), which range from 4.6 to 5.6 Mbp. *S. blattae* harbors 8 rRNA operons, whereas most other enteric bacteria, such as members of the genera *Escherichia*, *Enterobacter*, *Salmonella*, *Shigella*, *Yersinia*, and *Erwinia*, contain 7. The average GC content of 56.5% is significantly higher than that of *E. coli* (approximately 51%). This indicated that *S. blattae* and *E. coli* are not very close relatives. This was confirmed by phylogenetic analysis of 16S rRNA gene sequences (data not shown).

To identify orthologous genes and the strain-specific gene content, a combination of bidirectional BLAST and a global sequence alignment was employed (4). Comparison of *S. blattae* strain DSM 4481 with genome sequences of *K. pneumoniae* 342 (CP000964), *Enterobacter* sp. 638 (CP000653), *C. koseri* ATCC BAA-895 (CP000822), *E. coli* strain K-12 substrain MG1655 (U00096), *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain LT2 (AE006468), and *Shigella flexneri* 2a strain 301 (AE005674) revealed in all genomes a set of 1,183 orthologous genes, which show more than 85% similarity. *S. blattae* shares the most orthologous

genes (1,742) with *K. pneumoniae* and the fewest (1,441) with *Sh. flexneri*. A total of 666 putative genes were specific for *S. blattae*. Most of these genes encode proteins of unknown function or hypothetical proteins. A significant number of mobile elements, such as transposases (37), putative recombinases (11), and phage-related genes (34), were identified within the strain-specific genes. These results and further analysis revealed a mosaic structure of the *S. blattae* genome and the occurrence of horizontal gene transfer. One example of genes acquired by horizontal gene transfer is the *cob* operon, which encodes the major reactions of B₁₂ synthesis (1).

Nucleotide sequence accession number. The genome sequence of *S. blattae* DSM 4481 has been deposited in GenBank under accession number CP001560.

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