

First Insights into the Completely Annotated Genome Sequence of *Bacillus licheniformis* Strain 9945A

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Strains of the species *Bacillus licheniformis* are widely used in biotechnology for the production of enzymes and antibiotics (M. Schallmeyer, A. Singh, and O. P. Ward, *Can. J. Microbiol.* 50:1–17, 2004). However, research and application of *B. licheniformis* strains are adversely affected by poor genetic accessibility. Thus, for a closer inspection of natural competence in *B. licheniformis*, the genome of strain 9945A, of which derivatives are known to be naturally competent (C. B. Thorne and H. B. Stull, *J. Bacteriol.* 91:1012–1020, 1966), was completely sequenced and manually annotated.

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Bacillus licheniformis strain 9945A, a spore-forming soil bacterium with a saprophytic lifestyle, is of biotechnological interest due to its capability to produce polyglutamic acid (1, 2) and the known ability of auxotrophic mutants to exhibit natural competence (3, 4), particularly because the *B. licheniformis* type strain DSM13 displays only faint natural competence (5). For whole-genome sequencing, the wild-type strain was grown in mineral medium (6), and chromosomal DNA was isolated employing the Masterpure DNA purification kit (Epicenter, Madison, WI). The 454 shotgun library was constructed using the GS DNA library preparation kit (Roche 454, Branford, CT) and sequenced with a genome sequencer FLX system (Roche 454). In total, 313,600 reads with 74,174,233 bases were generated and assembled using the Newbler assembly software v1.1 (Roche 454), resulting in 70 contigs >0.5 kb, with 17-fold coverage. Orientation and contig order were determined by using an end-sequenced fosmid library (EpiFos CopyControl fosmid library production kit; Epicenter) and by comparing with the genome of the type strain *B. licheniformis* DSM13 (6, 7). Gap closure was done by PCR-based techniques using the PCR extender kit (5 Prime, Hamburg, Germany), subcloning, and transposon bombing (EZ-Tn5 <TET-1> insertion kit; Epicenter). The sequence was polished to a minimal quality score of Phred 45 (Gap4 software, Staden package [8]). Gene prediction was performed with YACOP (9) and manually curated using the Artemis software (10), and tRNAs and rRNAs were identified by tRNAscan SE (11) and RNAmmer (12), respectively. The functional annotation was performed using the Ergo software (13), including comparison to related organisms, and the Swiss-Prot, NCBI (nr), and InterPro databases (InterPro Scan v4.7, release 24.0/25.0). The final annotation was checked by comparison to the *B. licheniformis* DSM13 reannotation (AE017333) and, when necessary, by additional BLAST searches (Swiss-Prot and InterPro [InterPro Scan v4.8, release 42.0]).

The complete genome sequence of *B. licheniformis* 9945A consists of 4,376,305 bp, organized in one circular chromosome with

an average GC content of 45.9%. A total of 4,359 genes have been identified, including 72 tRNAs, 7 rRNA clusters (21 genes), and 40 pseudogenes. For 80% of the 4,226 protein-coding genes (3,378), a function or putative function could be assigned. The *B. licheniformis* 9945A genome is similar to the type strain DSM13 genome with respect to GC content and the number of rRNA and tRNA genes but contains an additional 153 kb. A striking metabolic difference of 9945A and DSM13 concerns the apparent ability of the former to use urea as a nitrogen source (BaLi_c20330 to BaLi_c20410) (14).

Comparative genome analysis revealed that for all genes known to be instrumental in developing natural competence in the closely related Gram-positive model organism *Bacillus subtilis*, orthologs exist in *B. licheniformis* 9945A. Despite such findings, the 9945A wild-type strain displays only poor natural competence compared to the auxotrophic mutants generated and investigated by Thorne and Stull in 1966 (3). Thus, further functional investigations of competence system compounds are necessary and in progress (M. Jakobs, K. Hoffmann, A. Grabke, S. Neuber, M. Liesegang, S. Volland, and F. Meinhardt, submitted for publication).

Nucleotide sequence accession number. The whole-genome sequence of *Bacillus licheniformis* 9945A was deposited in GenBank under accession number [CP005965](https://www.ncbi.nlm.nih.gov/nuccore/CP005965).

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