



2018

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Recommended Citation

Greguske, E.; Nadeau, A.; Fitzmeyer, E.; and Fučíková, K. (2018). Genome Sequence of *Bacillus* Phage Saddex. *Microbiology Resource Announcement* 7: e01044-18. <https://doi.org/10.1128/MRA.01044-18>

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Genome Sequence of *Bacillus* Phage Saddex

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ABSTRACT The complete genome of *Bacillus* phage Saddex was determined and annotated in this study. Saddex has distinct sections with similarities to other *Bacillus* phages, such as Kida, even though these phages were isolated more than 800 km apart by separate laboratories.

Saddex is a novel *Bacillus* bacteriophage isolated, characterized, and annotated by students in the Howard Hughes Medical Institute (HHMI) Phage Hunters program. Saddex is able to lyse multiple *Bacillus* host species, including *Bacillus cereus*, which is of particular interest, as this bacterium lives in the gut of poultry as well as in soil and causes an estimated 2% of all cases of food poisoning (1).

Saddex was isolated from lawn soil sampled (at coordinates N42.791838, W71.069913) in Haverhill, Massachusetts. In brief, log phase *Bacillus thuringiensis* subspecies *kurstaki* cells were mixed with lawn soil and allowed to grow overnight while being shaken at 37°C. Phage were isolated via centrifugation for 10 min at 3,000 rpm, followed by filtering with a 0.22- μ m sterile syringe filter (2). DNA was isolated from purified phage with the Qiagen viral DSP spin kit version 1 and sequenced via the HiSeq 2500 Illumina platform at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH), resulting in 779,058 paired-end reads and an average 250-bp read length. Reads were trimmed with Trimmomatic (3), assembled into contigs with QUAST (4), and then refined with Geneious version 10.2 (5) reference assemblies with custom low sensitivity (allowing only 2% mismatches for precise mapping). The average depth of coverage was 539.4 \times with no areas of poor coverage noted. Saddex was autoannotated in Geneious version 10.2 with default settings, with a known *Bacillus* phage genome, Evoli (GenBank accession number [KJ489398](https://doi.org/10.1128/MRA.01044-18)), for comparison. The genome was then visually cross-checked against 11 other complete *Bacillus* phage genomes available in GenBank.

The complete genome of Saddex is 142,353 bp of linear, double-stranded DNA with a G+C content of 39.0%. All genes in Saddex were found to have at least one homolog in other published *Bacillus* phages (2, 6–10) with a BLAST nucleotide (BLASTn) analysis (11), indicating that no novel genes were identified. However, the nucleotide sequence identity similarity of these genes ranged from as low as 63% to as high as 99%, with an average 81.9% similarity; this shows that Saddex could have unique polymorphisms. Of the 208 predicted proteins, 54 were assigned a function, typically for tail and capsid structure, nuclease activity, and lytic activity. Three tRNAs were identified, all three of which were found to have 100% similarity to at least one other *Bacillus* phage with a BLASTn analysis (11).

The proposed *Bacillus* phage cluster guidelines (12), in which pairwise average nucleotide identity across the genome is used to group similar phage, place Saddex in the C1 cluster of *Bacillus* phages.

Data availability. The complete genome sequence of the *Bacillus* phage Saddex is available in GenBank under accession number [MH538193](https://doi.org/10.1128/MRA.01044-18). Raw reads are available in the SRA under accession number [SRP158918](https://doi.org/10.1128/MRA.01044-18).

Received 30 July 2018 Accepted 28 August 2018 Published 20 September 2018

Citation Greguske E, Nadeau A, Fitzmeyer E, Fucikova K. 2018. Genome sequence of *Bacillus* phage Saddex. *Microbiol Resour Announc* 7:e01044-18. <https://doi.org/10.1128/MRA.01044-18>.

Editor Frank J. Stewart, Georgia Institute of Technology

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ACKNOWLEDGMENT

This research was supported by New Hampshire-INBRE through an Institutional Development Award (IDeA), number P20GM103506, from the National Institute of General Medical Sciences of the National Institutes of Health (NIH).

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