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GENOME SEQUENCES

Complete Genome Sequence of *Myxococcus* Phage Mx4

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ABSTRACT *Myxococcus xanthus* is a bacterial model in microbial developmental biology and social evolution. Here, I present the 57.0-kb circular genomic sequence of the wild-type *Myxococcus* phage Mx4, with a GC content of 70.1%. Annotation predicted 97 protein-coding genes. Head-neck-tail protein classification assigns Mx4 to the tailed, Mu-like members of the family *Myoviridae* of group type 1 (cluster 8).

Myxococcus xanthus is a predatory *deltaproteobacterium* in soil (1). It is a well-studied model organism in developmental biology and social evolution (2), and various aspects of its multicellular life cycle have been studied in molecular detail (3–14). Bacteriophages of *Myxococcus* have been isolated and used experimentally (15); however, a complete genome sequence is available for only one, Mx8 (unpublished; GenBank accession number AF396866). Here, I present the whole-genome sequence of the lytic wild-type *Myxococcus* phage Mx4. The sample traces back to the wild-type isolate from the 1970s, originally derived by infecting the susceptible laboratory strain *Myxococcus xanthus* DZ1 with resuspended and filtered soil and manure of mixed origins (16). Mx4 was kept in MOPS (3-morpholinopropanesulfonic acid)-based phage buffer at 4°C and was frequently passaged in the aforementioned host (17). A fresh high-titer stock was prepared from one clear plaque at 32°C in the *Myxococcus xanthus* host strain DZ1, following standard methods for lytic myxophage propagation (17, 18). To extract genomic DNA, I infected strain DZ1 (5×10^8 CFU/ml) with Mx4 at a multiplicity of infection (MOI) of ~2 in modified CTT broth (8 mM $MgSO_4$, 10 mM Tris [pH 8.0], 20 g/liter Casitone, 1 mM KPO_4, and 1 mM $CaCl_2$) for 60 min without agitation at room temperature prior to setting up a double agar overlay (18). After 2 days of incubation (32°C, 90%rH), the confluently lysed DZ1 lawn was covered with 2 ml genome buffer B1 (Qiagen) and kept at 4°C for 24 h to dissolve free phage particles. Genomic DNA from this suspension, as well as a pellet of the uninfected DZ1 control, were purified using Qiagen's blood and tissue kit following the manufacturer's recommendations. DNA was sheared to ~350-bp lengths using a multifunctional bioprocessor (EpiSonic) and prepared for Illumina short-read sequencing in 150-bp paired-end mode on the same flow cell lane on a HiSeq 4000 machine using the NEBNext Ultra DNA library prep kit. The samples were demultiplexed using Illumina's bcl2fastq v2.20.0. Reads from both treatments were quality checked using fastqc v0.11.8 (19) and trimmed to lengths of >80 bp using Trimmomatic v0.32 (20) with default parameters, leaving ~70% of the sequence information (Table 1). To remove any host-derived reads from the Mx4 infection treatment, I devised the following approach. The trimmed DZ1 control reads were *de novo* assembled using SPAdes v3.11.1 (21) with "-k 21,33,55,77,99,127 --careful" and the resulting contigs served as a reference to which trimmed reads of the Mx4 infection treatment were mapped using breseq v0.28.1 (22). The 175,376 reads (average length, ~145.1 bp) of the Mx4 infection treatment (~29%) (Table 1) that did not match the DZ1 control contigs, i.e., the enriched Mx4 reads, were assembled using SPAdes v3.11.1 (21), with the same parameters as above. This procedure resulted in a total of 22 contigs, and the removal of low coverage contigs (k-mer coverage, <10) and manual curation led to a single contig with overlapping ends, which indicates a circular genome. The completed

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TABLE 1 Illumina sequencing read statistics

Treatment	SRA library name	SRA accession no.	No. of raw reads	No. of trimmed reads	No. of unmatched reads[a]	Size of raw sequences (Mbp)[b]	Size of trimmed sequences (Mbp)[b]	Size of unmatched sequences[a] (Mbp)[b]
DZ1	CONTROL	SRR15834097	8,772,050	6,038,535		1,315.0	870.4	
Mx4 infection	MIX	SRR15834096	838,202	604,983		125.7	87.7	
Enriched Mx4[a]	UNMATCHED	SRR15834095			175,376			25.4

[a]Unmatched reads after mapping the MIX reads against the assembled CONTROL contigs.
[b]Mbp, million base pairs.

Mx4 genome contained 56,975 bp (Fig. 1) with ~458-fold coverage, in line with the predicted size range for Mx4 (15). The genome's GC content, ~70.1%, precisely matched the early predictions based on buoyant density measurements (23). RASTtk-enabled gene annotation with the Virus option in PATRIC (24, 25) predicted 97 protein-coding genes. The coding density was ~95.5%. Head-neck-tail protein classification in Virfam (26) assigned Mx4 to the Mu-like tailed viruses of the *Myoviridae* group type 1 (cluster 8), in line with the physical characterization of Mx4 morphology (23).

Data availability. The whole-genome sequence of *Myxococcus* phage Mx4 was deposited in DDBJ/ENA/GenBank under accession number OK085710. The raw sequencing reads were deposited in the Sequence Read Archive (SRA) under accession numbers

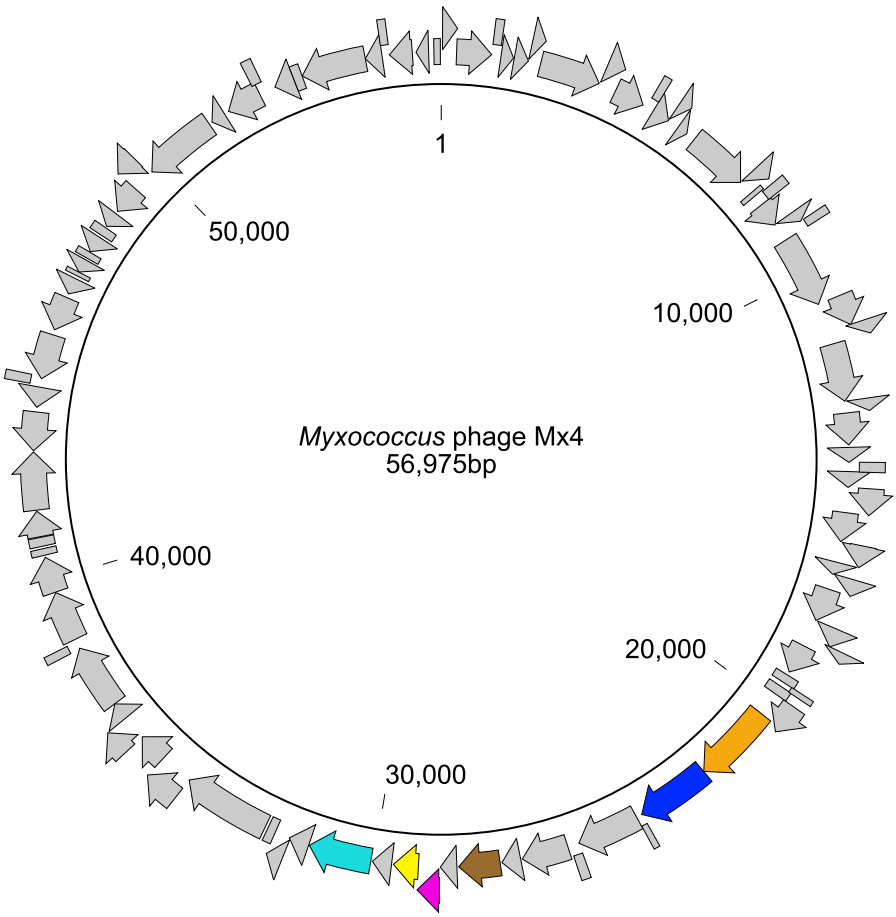

FIG 1 Circular genome of *Myxococcus* phage Mx4, containing 97 protein-coding genes (arrows). The colors are based on the protein classification in Virfam (26): orange, large terminase protein (UAW08042); blue, portal protein (UAW08043); brown, major capsid protein (UAW08049); magenta, adaptor protein (UAW08051); yellow, neck protein (UAW08052); mint, tail sheath (UAW08054). The inner ring labels depict the position in base pairs.

SRR15834095 (phage), SRR15834096 (infected host), and SRR15834097 (uninfected host).

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REFERENCES

1. Dawid W. 2000. Biology and global distribution of myxobacteria in soils. FEMS Microbiol Rev 24:403–427. https://doi.org/10.1111/j.1574-6976.2000.tb00548.x.
2. Vos M, Velicer GJ. 2009. Social conflict in centimeter- and global-scale populations of the bacterium *Myxococcus xanthus*. Curr Biol 19:1763–1767. https://doi.org/10.1016/j.cub.2009.08.061.
3. Cossey SM, Yu Y-TN, Cossu L, Velicer GJ. 2019. Kin discrimination and outer membrane exchange in *Myxococcus xanthus*: experimental analysis of a natural population. PLoS One 14:e0224817. https://doi.org/10.1371/journal.pone.0224817.
4. Dinet C, Michelot A, Herrou J, Mignot T. 2021. Linking single-cell decisions to collective behaviours in social bacteria. Philos Trans R Soc Lond B Biol Sci 376:20190755. https://doi.org/10.1098/rstb.2019.0755.
5. Kroos L. 2017. Highly signal-responsive gene regulatory network governing *Myxococcus* development. Trends Genet 33:3–15. https://doi.org/10.1016/j.tig.2016.10.006.
6. Treuner-Lange A, Chang Y-W, Glatter T, Herfurth M, Lindow S, Chreifi G, Jensen GJ, Sogaard-Andersen L. 2020. PilY1 and minor pilins form a complex priming the type IVa pilus in *Myxococcus xanthus*. Nat Commun 11:5054. https://doi.org/10.1038/s41467-020-18803-z.
7. Vassallo CN, Wall D. 2019. Self-identity barcodes encoded by six expansive polymorphic toxin families discriminate kin in myxobacteria. Proc Natl Acad Sci U S A 116:24808–24818. https://doi.org/10.1073/pnas.1912556116.
8. Bernal-Bernal D, Abellon-Ruiz J, Iniesta AA, Pajares-Martinez E, Bastida-Martinez E, Fontes M, Padmanabhan S, Elias-Arnanz M. 2018. Multifactorial control of the expression of a CRISPR-Cas system by an extracytoplasmic function sigma/anti-sigma pair and a global regulatory complex. Nucleic Acids Res 46:6726–6745. https://doi.org/10.1093/nar/gky475.
9. Wielgoss S, Wolfensberger R, Sun L, Fiegna F, Velicer GJ. 2019. Social genes are selection hotspots in kin groups of a soil microbe. Science 363:1342–1345. https://doi.org/10.1126/science.aar4416.
10. Wallace RA, Black WP, Yang X, Yang Z. 2014. A CRISPR with roles in *Myxococcus xanthus* development and exopolysaccharide production. J Bacteriol 196:4036–4043. https://doi.org/10.1128/JB.02035-14.
11. Berleman JE, Zemla M, Remis JP, Liu H, Davis AE, Worth AN, West Z, Zhang A, Park H, Bosneaga E, van Leer B, Tsai W, Zusman DR, Auer M. 2016. Exopolysaccharide microchannels direct bacterial motility and organize multicellular behavior. ISME J 10:2620–2632. https://doi.org/10.1038/ismej.2016.60.
12. Higgs PI, Jagadeesan S, Mann P, Zusman DR. 2008. EspA, an orphan hybrid histidine protein kinase, regulates the timing of expression of key developmental proteins of *Myxococcus xanthus*. J Bacteriol 190:4416–4426. https://doi.org/10.1128/JB.00265-08.
13. Mauriello EMF, Astling DP, Sliusarenko O, Zusman DR. 2009. Localization of a bacterial cytoplasmic receptor is dynamic and changes with cell-cell contacts. Proc Natl Acad Sci U S A 106:4852–4857. https://doi.org/10.1073/pnas.0810583106.
14. Nan B, Bandaria JN, Moghtaderi A, Sun I-H, Yildiz A, Zusman DR. 2013. Flagella stator homologs function as motors for myxobacterial gliding motility by moving in helical trajectories. Proc Natl Acad Sci U S A 110:E1508–E1513. https://doi.org/10.1073/pnas.1219982110.
15. Vasse M, Wielgoss S. 2018. Bacteriophages of *Myxococcus xanthus*, a social bacterium. Viruses 10:374. https://doi.org/10.3390/v10070374.
16. Campos JM, Geisselsoder J, Zusman DR. 1978. Isolation of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J Mol Biol 119:167–178. https://doi.org/10.1016/0022-2836(78)90431-x.
17. Julien B. 2003. Characterization of the integrase gene and attachment site for the *Myxococcus xanthus* bacteriophage Mx9. J Bacteriol 185:6325–6330. https://doi.org/10.1128/JB.185.21.6325-6330.2003.
18. Freund L, Vasse M, Velicer GJ. 2021. Hidden paths to endless forms most wonderful: parasite-blind diversification of host quality. Proc Biol Sci 288:20210456. https://doi.org/10.1098/rspb.2021.0456.
19. Andrews S. 2018. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
20. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
21. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
22. Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Methods Mol Biol 1151:165–188. https://doi.org/10.1007/978-1-4939-0554-6_12.
23. Geisselsoder J, Campos JM, Zusman DR. 1978. Physical characterization of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J Mol Biol 119:179–189. https://doi.org/10.1016/0022-2836(78)90432-1.
24. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
25. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. Nucleic Acids Res 48:D606–D612. https://doi.org/10.1093/nar/gkz943.
26. Lopes A, Tavares P, Petit M-A, Guerois R, Zinn-Justin S. 2014. Automated classification of tailed bacteriophages according to their neck organization. BMC Genomics 15:1027. https://doi.org/10.1186/1471-2164-15-1027.