microPublication BIOLOGY

2/3/2026 - *Open Access*

Genome Sequence of *Corynebacterium glutamicum* Phage MicyPS

Ombeline Rossier[1§], Florence Constantinesco-Becker[1], Anne Lopes[1], Daniel Delaruelle[2], Ana A Arteni[1], Lydia Hassissene[1], Malika Ouldali[1], Laura Pieri[1], Avril Zappini[3], Katia Zaidi[3], Armand Tomasella[3], Perla Tannous[3], Michel Nouhra[3], Mickael Marques[3], Cindy Goodur[3], Louis Gachot[3], Erine Dumond[3], Alizée Dias[3], Heather Desolle[3], Meissan Chikhi[3], Chahine Belhachem[3], Adel Amriche[3], Christophe Regeard[1§]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

[2]Faculté des Sciences d'Orsay, Université Paris-Saclay, Orsay, France

[3]Ecole Universitaire de Premier Cycle, Faculté des Sciences d'Orsay, Université Paris-Saclay, Orsay, France

[§]To whom correspondence should be addressed: ombeline.rossier@universite-paris-saclay.fr; christophe.regeard@universite-paris-saclay.fr

Abstract

MicyPS is a bacteriophage with siphovirus morphology infecting *Corynebacterium glutamicum* strain MB001. It was isolated from soil near a henhouse in Villiers-sur-Marne, France. Its 78,208-bp genome encodes 115 predicted protein-encoding genes and 5 tRNAs. Based on gene-content similarity with actinobacteriophage PSonyx, MicyPS was assigned to the new cluster EQ.

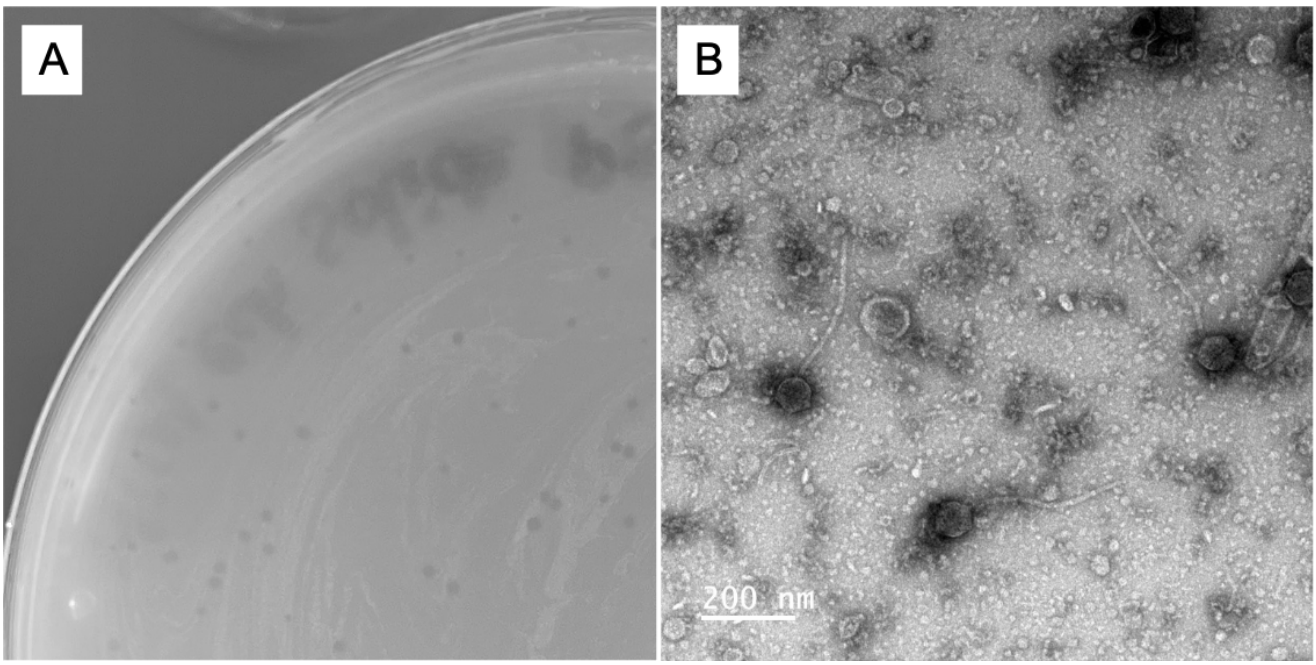

Figure 1. Plaque and virion morphology of phage MicyPS:

(A) Clear plaques observed in the LB agar overlay supplemented with 1 mM $CaCl_2$ and *C. glutamicum* strain MB001. A portion of a standard 100-mm Petri dish is shown. **(B)** Negative staining of MicyPS phages with 2% w/v uranyl acetate observed with a JEOL1400 microscope operated at 80 kV and equipped with RIO9 direct electron detection camera (Ametek/Gatan). The magnification used was 12,000x with a pixel size of 0.56 nm at the level of the specimen. Bar, 200 nm.

Description

Despite the industrial significance of *Corynebacterium glutamicum* in the production of glutamate and lysine, only seven bacteriophages infecting this species have been fully characterized to date with respect to both genome sequence and virion morphology (Moreau et al. 1995; Bukovska et al. 2006; Chen et al. 2008; Lobanova et al. 2017; Yomantas et al. 2018; Hünnefeld et al. 2021; Rossier et al. 2024). This limited number underlines an important gap in our understanding of phage diversity, biology, and their potential impact on fermentation processes involving *C. glutamicum*.

Phage MicyPS was isolated from soil near a henhouse in Villiers-sur-Marne, France (GPS 48.829132 N, 2.537859 E). Soil was incubated for 1 hour with 25 mL LB broth (5 g/L NaCl; 5 g/L yeast extract; 10 g/L tryptone), whilst shaking at 30° C. Following centrifugation at 4000 xg for 15 minutes, the supernatant was passed through a 0.45-µm filter and

supplemented with 1 mM $CaCl_2$ and inoculated with *C. glutamicum* strain MB001, a derivative of the reference strain ATCC 13032 in which the prophages CGP1, CGP2, and CGP3 were deleted (Hünnefeld et al. 2021). After shaking for 24 hours at 30° C, enrichment cultures were centrifuged and the supernatant was filtered through a 0.22-µm filter. Five mL of the supernatant were then spotted on double agar plate supplemented with 1 mM $CaCl_2$ and strain MB001. After 24 hours of incubation at 30° C, individual plaques were visible and further purified through three rounds of single plaque picking and plating. Plaques were clear and between 0.5 and 3-mm in diameter (Figure 1A). Negative-staining electron microscopy revealed a siphovirus morphology with capsids 75 (±4) nm in diameter and tails 320 (±26) nm long (n=20).

Viral DNA was extracted using the PCI/SDS protocol (see Methods). NEB Ultra II FS Kit was used to prepare the library that was sequenced using Illumina NextSeq 1000, yielding 1,078,713 single-end 100-base reads. Raw reads were trimmed with cutadapt 4.7 (using the option: –nextseq-trim 30) and filtered with Skewer 0.2.2 (using the options: -q 20 -Q 30 -n -l 50) prior to assembly (Martin 2011; Jiang et al. 2014). De novo assembly was performed with Newbler v2.9 as previously described (Russell 2018) and further checked with Consed v.29 and Unicycler (Gordon et al. 1998; Wick et al. 2017). The resulting genome was 78,208-bp in length, with a G+C content of 53.2% and circularly permutated ends. Based on gene content similarity of 76.2% with the previously singleton phage PSonyx (Rossier et al. 2024), phage MicyPS was assigned to the new cluster EQ in the Actinophage database PhagesDB (Russell and Hatfull 2017).

Genome annotation identified 120 putative genes including 5 tRNAs with the following tools: DNA master v5.23.6 (Pope and Jacobs-Sera 2018) which incorporates Glimmer v3.02 (Delcher et al. 2007) and GeneMark v2.5p (Besemer and Borodovsky 2005), Aragorn v1.2.40 (Laslett 2004), tRNAscan-SE v2.0 (Chan et al. 2021) and Phamerator (Cresawn et al. 2011). Start sites were selected based on gene length, minimal gaps or overlaps, RBS scores, and BLASTP alignment. Functional annotation was performed with BLASTP (Altschul et al. 1990) using PhagesDB and NCBI non-redundant databases (e values < 0.001) and with HHpred (Soding et al. 2005) using databases PDB_mmCIF70_30_Mar, Pfam-A_v37, Uniprot-Swissprot-viral70_3_Nov_2021, and NCBIConservedDomain(CD)_v3.19. Using DeepTMHMM v1.0.42 (Hallgren et al. 2022), nine proteins were predicted to localize to the membrane. Only 35% of putative genes had a predicted function. One region (representing 40% of the genome) is transcribed in the rightward direction and encodes proteins involved in virion assembly and host lysis. The next 40%, transcribed leftwards, contains tRNAs and genes likely involved in DNA replication. Located in this region, nine (of a total of 15) predicted genes have no significant similarity with any other predicted genes in the Actinophage database. These genes are termed orphams. The final 20%, transcribed rightwards, end with a large putative gene which is predicted to encode a 2,873-amino-acid protein of unknown function. Both phages in cluster EQ (MicyPS and PSonyx) have no identifiable immunity repressor or integrase functions suggesting the use of the lytic cycle for replication.

Nucleotide sequence accession numbers

MicyPS is available at GenBank with Accession No. PV876931 and Sequence Read Archive (SRA) No. SRX28943171.

Methods

Viral DNA was extracted using the PCI/SDS protocol (https://phagesdb.org/media/workflow/protocols/pdfs/PCI_SDS_DNA_Extraction_2.2013.pdf).

Acknowledgements: We thank Véronique Delesalle, Becky Garlena, Deborah Jacobs-Sera, Dan Russell, Vic Sivanathan and the SEA-PHAGES program for training and support.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410. DOI: 10.1016/S0022-2836(05)80360-2

Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Research 33: W451-W454. DOI: 10.1093/nar/gki487

Bukovska G, Klucar L, Vlcek C, Adamovic J, Turna J, Timko J. 2006. Complete nucleotide sequence and genome analysis of bacteriophage BFK20 — A lytic phage of the industrial producer *Brevibacterium flavum*. Virology 348: 57-71. DOI: 10.1016/j.virol.2005.12.010

Chan PP, Lin BY, Mak AJ, Lowe TM. 2021. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Research 49: 9077-9096. DOI: 10.1093/nar/gkab688

Chen CL, Pan TY, Kan SC, Kuan YC, Hong LY, Chiu KR, et al., Hu. 2008. Genome sequence of the lytic bacteriophage P1201 from *Corynebacterium glutamicum* NCHU 87078: Evolutionary relationships to phages from *Corynebacterineae*. Virology 378: 226-232. DOI: 10.1016/j.virol.2008.05.027

Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23: 673-679. DOI: 10.1093/bioinformatics/btm009

Gordon D, Abajian C, Green P. 1998. *Consed:* A Graphical Tool for Sequence Finishing. Genome Research 8: 195-202. DOI: 10.1101/gr.8.3.195

Hallgren J, Tsirigos KD, Pedersen MD, Almagro Armenteros JJ, Marcatili P, Nielsen H, Krogh A, Winther O. 2022. DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. : 10.1101/2022.04.08.487609. DOI: 10.1101/2022.04.08.487609

Hünnefeld M, Viets U, Sharma V, Wirtz A, Hardy Al, Frunzke J. 2021. Genome Sequence of the Bacteriophage CL31 and Interaction with the Host Strain *Corynebacterium glutamicum* ATCC 13032. Viruses 13: 495. DOI: 10.3390/v13030495

Jiang H, Lei R, Ding SW, Zhu S. 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15: 10.1186/1471-2105-15-182. DOI: 10.1186/1471-2105-15-182

Laslett D. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Research 32: 11-16. DOI: 10.1093/nar/gkh152

Lobanova JS, Gak ER, Andreeva IG, Rybak KV, Krylov AA, Mashko SV. 2017. Complete nucleotide sequence and annotation of the temperate corynephage ϕ16 genome. Archives of Virology 162: 2489-2492. DOI: 10.1007/s00705-017-3383-4

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17: 10. DOI: 10.14806/ej.17.1.200

Moreau S, Leret V, Le Marrec C, Varangot H, Ayache M, Bonnassie S, Blanco C, Trautwetter A. 1995. Prophage distribution in coryneform bacteria. Research in Microbiology 146: 493-505. DOI: 10.1016/0923-2508(96)80295-6

Pope WH, Jacobs-Sera D. 2017. Annotation of Bacteriophage Genome Sequences Using DNA Master: An Overview. Methods in Molecular Biology, Bacteriophages : 217-229. DOI: 10.1007/978-1-4939-7343-9_16

Rossier O, Labarre C, Lopes A, Auberdiac M, Tambosco K, Delaruelle D, et al., Regeard. 2024. Genome sequence of PSonyx, a singleton bacteriophage infecting *Corynebacterium glutamicum*. Microbiology Resource Announcements 13: 10.1128/mra.01155-23. DOI: 10.1128/mra.01155-23

Russell DA. 2017. Sequencing, Assembling, and Finishing Complete Bacteriophage Genomes. Methods in Molecular Biology, Bacteriophages : 109-125. DOI: 10.1007/978-1-4939-7343-9_9

Russell DA, Hatfull GF. 2016. PhagesDB: the actinobacteriophage database. Bioinformatics 33: 784-786. DOI: 10.1093/bioinformatics/btw711

Soding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Research 33: W244-W248. DOI: 10.1093/nar/gki408

Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13: e1005595. DOI: 10.1371/journal.pcbi.1005595

Yomantas YAV, Abalakina EG, Lobanova JS, Mamontov VA, Stoynova NV, Mashko SV. 2018. Complete nucleotide sequences and annotations of φ673 and φ674, two newly characterised lytic phages of *Corynebacterium glutamicum* ATCC 13032. Archives of Virology 163: 2565-2568. DOI: 10.1007/s00705-018-3867-x

Funding: The present work has benefited from the platform of Cryo-EM of I2BC supported by IBiSA and by French Infrastructure for Integrated Structural Biology (FRISBI) ANR-10-INBS-05.

Conflicts of Interest: The authors declare that there are no conflicts of interest present.

Author Contributions: Ombeline Rossier: supervision, investigation, validation, writing - original draft, writing - review editing. Florence Constantinesco-Becker: supervision, investigation, validation. Anne Lopes: supervision, investigation, writing - review editing. Daniel Delaruelle: resources. Ana A Arteni: investigation, writing - review editing. Lydia Hassissene: investigation. Malika Ouldali: investigation. Laura Pieri: investigation. Avril Zappini: investigation. Katia Zaidi: investigation. Armand Tomasella: investigation. Perla Tannous: investigation. Michel Nouhra: investigation. Mickael Marques: investigation. Cindy Goodur: investigation. Louis Gachot: investigation. Erine Dumond: investigation. Alizée Dias: investigation. Heather Desolle: investigation. Meissan Chikhi: investigation. Chahine Belhachem: investigation. Adel Amriche: investigation. Christophe Regeard: supervision, investigation, validation, writing - review editing.

Reviewed By: Anonymous

History: Received November 11, 2025 **Revision Received** January 27, 2026 **Accepted** January 29, 2026 **Published Online** February 3, 2026 **Indexed** February 17, 2026

2/3/2026 - *Open Access*

Citation: Rossier O, Constantinesco-Becker F, Lopes A, Delaruelle D, Arteni AA, Hassissene L, et al., Regeard C. 2026. Genome Sequence of *Corynebacterium glutamicum* Phage MicyPS. microPublication Biology. 10.17912/micropub.biology.001936