*viruses*

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*Supplementary Information*

# Genome sequence of the bacteriophage CL31 and analysis of its characteristics and host interactions

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**Table S1.** Oligonucleotides used in this study.

| Number | Sequence (5′-3′) |
|---|---|
| 1 | CCTGCAGGTCGACTCTAGAGAGCATGGAATCGCCACCGAT |
| 2 | CATTCCACTATAGAAACTGTTTTTTAAAGGAGAACCATGTCTGA |
| 3 | TAAAAAACAGTTTCTATAGTGGAATGTTACCGTGCTTTTTAGC |
| 4 | CCCAGTTCGCTGACTGCTTGGATAT |
| 5 | CAAGCAGTCAGCGAACTGGGTTGGC |
| 6 | CTAAGAAACCATGACTGCAGCACAGACCA |
| 7 | CTGCAGTCATGGTTTCTTAGTCTAAATCCTGAAGG |
| 8 | TTGTAAAACGACGGCCAGTGAGACCACATGCTCAACCCGT |
| 9 | CCTGCAGGTCGACTCTAGAGGAGGTCTCGCACAGCCTTGGT |
| 10 | GCCGACAAGGACGCCCGCTTCATCC |
| 11 | AAGCGGGCGTCCTTGTCGGCGGCGT |
| 12 | TTGTAAAACGACGGCCAGTGACCTGTGACCACTGACTTTGTTATCGGCGT |
| 13 | CCTGCAGGTCGACTCTAGAGAATCTGAGGGCTGCGGCC |
| 14 | GGAAGAATTAGTAACGGAGAGCTGACGGAAG |
| 15 | CTCTCCGTTACTAATTCTTCCGAGAAATCTCATCAATGC |
| 16 | AGTGGTCCACGACGCCGATGACATG |
| 17 | CATCGGCGTCGTGGACCACTGCTCC |
| 18 | AGGCCTCAATATGGAACAGAGCCAAT |
| 19 | TCTGTTCCATATTGAGGCCTCAGCCT |
| 20 | TTGTAAAACGACGGCCAGTGCGCTCCGCCGTTGGC |
| 21 | GTGTCCAATTCCAGATCCACATCGT |
| 22 | ACGATGTGGATCTGGAATTGGACAC |
| 23 | CTGGTTGGTGCCAGGCAGGAAG |
| 24 | GGTGGTCACGTGTACTCCCCTG |
| 25 | CTGCAGCCTTGGTGTCCTGAGT |
| 26 | CACACAAGAACCCTGCACAACGC |
| 27 | GGTCATGCATGTCGTAGCCCTGGTTA |
| 28 | TAACCAGGGCTACGACATGCATGACC |
| 29 | CCACGACGAAGATGATCGGGAT |
| 30 | ATGTATGTCACCGGCCCAGCC |
| 31 | CCCGACAGAACTGCGAAGATGG |
| 32 | GGAGCTCATTTGAGTCGCCGC |
| 33 | GCATGTTCTTCTTCTCTGGTAGCGG |
| 34 | CGCGATACCATGCTCATTGCCAG |
| 35 | GTCGCTGACGTGGTAATCATGGTGG |
| 36 | CGGATCCTTTGTGGAGATCGGCG |
| 37 | CCGCTACCAGAGAAGAAGAACATGC |
| 38 | CTGGCAATGAGCATGGTATCGCG |
| 39 | CCACCATGATTACCACGTCAGCGAC |
| 40 | CGCCGATCTCCACAAAGGATCCG |
| 41 | GATTTCAATTCCGGAACGCTCGGTGAG |
| 42 | GGCTACGACGGATTCAACACCGAT |
| 43 | ATGCCAGCGATTTCGCCAGC |
| 44 | CGAGGAAGCCGTAAAGCGCC |
| 45 | GCGTCAACATATGCACGCTGCA |
| 46 | GCCAACATGGATCCTCAGCAGC |
| 47 | GCGCAGATCCGCTAGCTTCT |
| 48 | CCTCGTTTTGGTTGAGGCGG |
| 49 | GCTTTCGTACTGAGATCCGCGATCC |
| 50 | CGCAGGTGGGGGAGGATTCTTGAA |

**Table S2:** Construction of plasmids used in this study. Numbers represent oligonucleotide pairs used for PCR (see Table A1). The restriction enzymes were used for linearization of the vectors and plasmids were assembled using Gibson assembly (multiple primer pairs indicate that constructs were assembled using multiple fragments). To verify the plasmids and the integrations using sequencing, the following primers were used: *accD2:* 21 – 26; *accD3*: 27 -32; *pks*: 33 – 48.

| Plasmid | Template | Primers | Vector | Restriction Enzymes |
|---|---|---|---|---|
| pK19*mobsacB-accD2*_G1049A | *C. glutamicum* chromosome | 1 + 2, 3 + 4, 5 + 6, 7 + 8 | pK19*mobsacB* | *BamHI *EcoRI |
| pK19*mobsacB-accD3*_G1022A | *C. glutamicum* chromosome | 9 + 10, 11 + 12 | pK19*mobsacB* | *BamHI *EcoRI |
| pK19*mobsacB-pks*_T2656G | *C. glutamicum* chromosome | 13 + 14, 15 + 16, 17 + 18, 19 + 20 | pK19*mobsacB* | *BamHI *EcoRI |

**Table S6**. Prediction and (if available) experimental proof of the life style of different corynephages. Please note, we did not identify an integrase gene in the genome of CL31. Therefore, the life style predictions have to be treated with care and further experiments are required.

| Phage | Domains (temperate) | Domains (virulent) | Predicted Life Style | Experimental | Reference |
|---|---|---|---|---|---|
| CL31 | 5 | 1 | Temperate | - | This work |
| Phi16 | 7 | 3 | Temperate | Temperate | [1,2] |
| Phi673 | 0 | 3 | Virulent | Virulent | [3] |
| Phi674 | 0 | 6 | Virulent | Virulent | [3] |

*Figure S1*

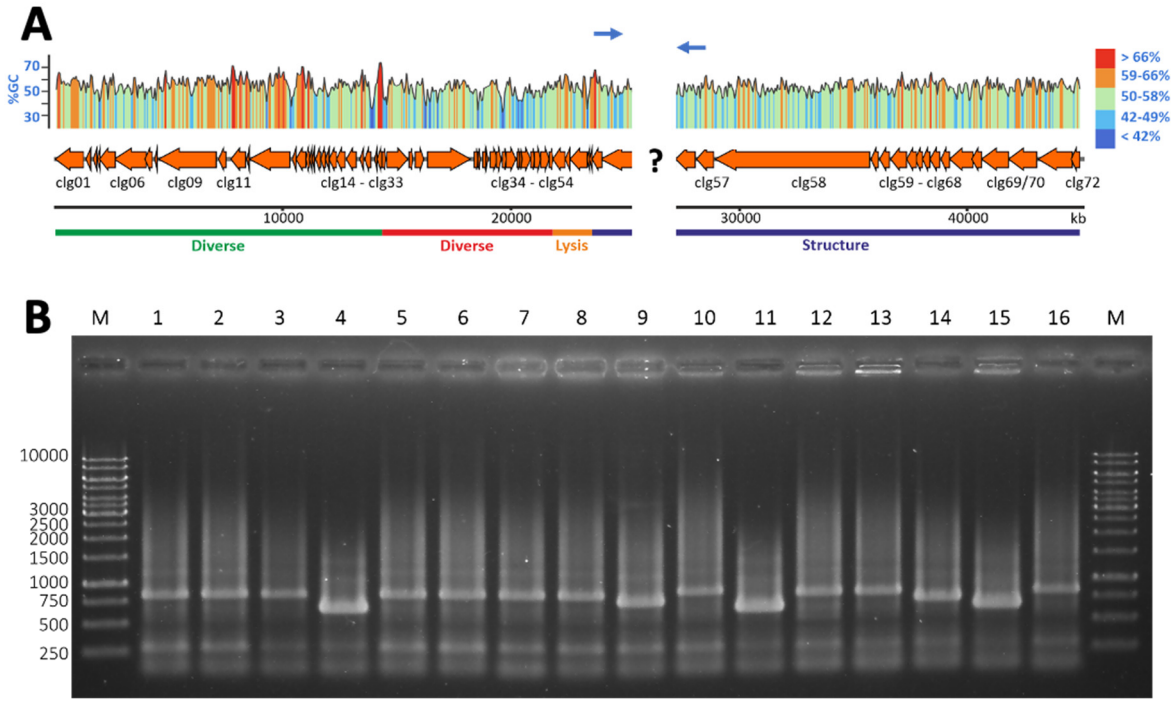


Figure S1: PCR and Sanger sequencing of singles plaques to close the CL31 genome. (A) Schematic representation of the position of a repeat region in the CL31 genome. This region could not be correctly assembled based on Illumina reads and thus was further analyzed with Sanger sequencing. The blue arrows indicate the approximate position of the primers used for PCR amplification and sequencing. (B) Primers 49 and 50 were used to amplify the area shown in A from single CL31 plaques (exemplary plate shown in Figure 2A of the main text). Three different product sizes were obtained. For sequencing three different sizes were chosen (Lane 1, 4, 14).

*Figure S2*

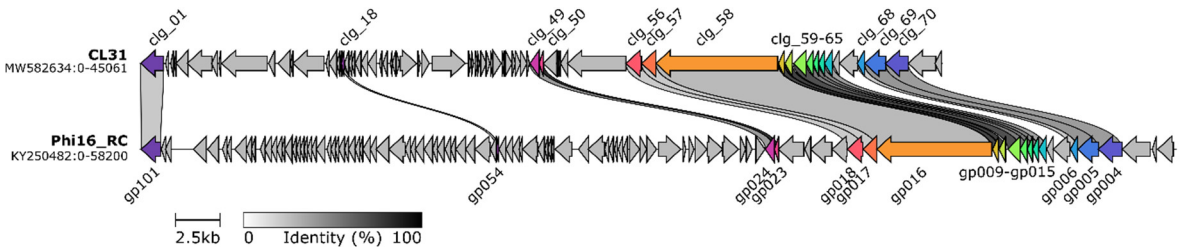


Figure S2: Synteny plot comparing the corynephages CL31 and Φ16. The genomes of the corynephages CL31 and Phi16 (as reverse complement) were compared with regard to identity of single genes. For this purpose, the tool Clinker was used with standard parameters [4].

*Figure S3*

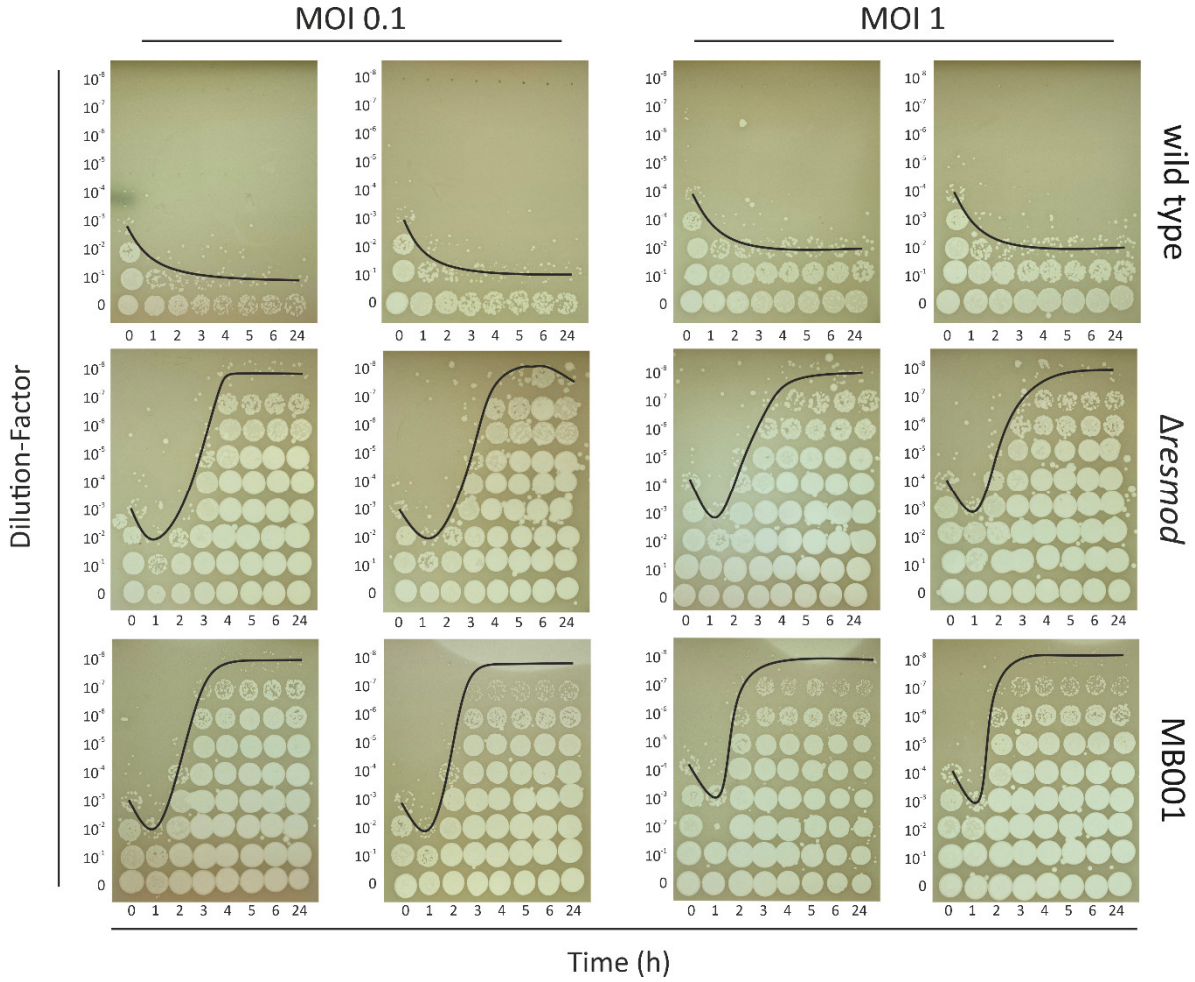


Figure S3: Plate assays as infection dynamics analysis. Infection assay of CL31 with different host strains was used to determine the phage titer at different time points of incubation. For this purpose, bacterial cells were incubated with CL31 in BHI medium with an initial MOI of 0.1 or 1. At the described time points, samples were extracted and 3 µl of the centrifuged supernatant were spotted on a MB001 lawn ($OD_{600}$ 0.5).

*Figure S4*

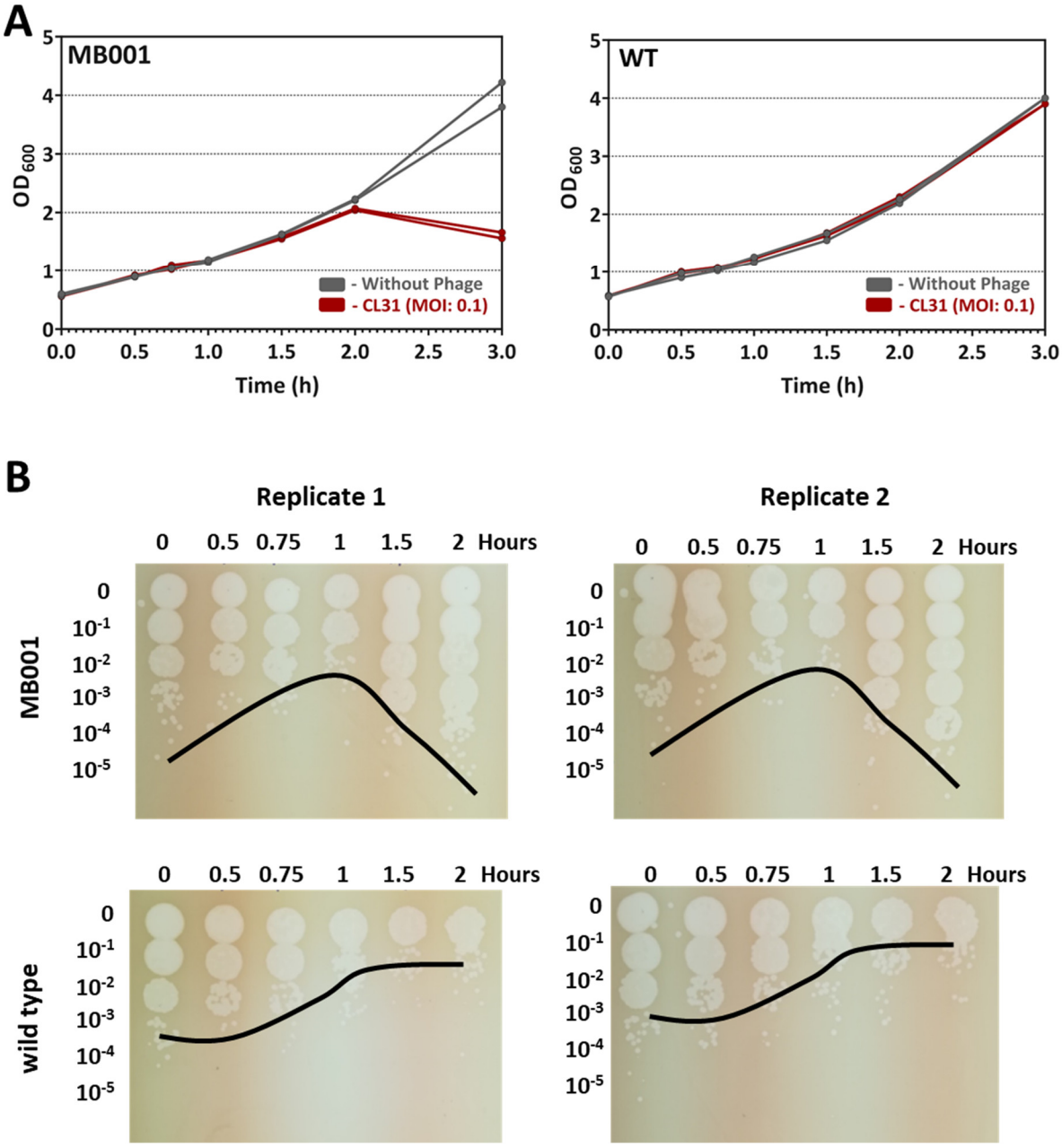


Figure S4: Infection curve for RNA-seq analysis. To determine the best point for RNA-seq analysis, an infection assay of CL31 with wild type or MB001 strains was used to determine the phage titer as well as the growth state ($OD_{600}$ measurements) during incubation. For this purpose, bacterial cells (Starting-$OD_{600}$ 0.5) were incubated with CL31 in BHI medium with an initial MOI of 0.1. At the described time points, samples were extracted, OD measurements were conducted, and 3 µl of the centrifuged supernatant were spotted on a MB001 lawn ($OD_{600}$ 0.5). The resulting plates were analysed after an overnight incubation. To cover the moment of highest intracellular phage activity, 1h after cultivation start was chosen to be used for RNA preparation. This represents the point before phage titer increase.

## References

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