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Complete Genome Sequences of T4-Like Bacteriophages RB3, RB5, RB6, RB7, RB9, RB10, RB27, RB33, RB55, RB59, and RB68

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t4-like bacteriophages have been explored for phage therapy and are model organisms for phage genomics and evolution. Here, we describe the sequencing of 11 T4-like phages. We found a high nucleotide similarity among the T4, RB55, and RB59; RB32 and RB33; and RB3, RB5, RB6, RB7, RB9, and RB10 phages.

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T4-like bacteriophages have been explored for phage therapy and are model organisms for phage genomics and evolution. Here, we describe the sequencing of 11 T4-like phages. We found a high nucleotide similarity among the T4, RB55, and RB59; RB32 and RB33; and RB3, RB5, RB6, RB7, RB9, and RB10 phages.

We prepared phage lysates as previously described (2) from host Escherichia coli B (CGSC 3565), extracted DNA with the Phage DNA isolation kit (bioWORLD, Dublin, OH), and sequenced the samples as paired-end 250-bp reads on the MiSeq instrument (Illumina, San Diego, CA). A total of 789,300 (for RB6) to 6,985,449 (for RB7) paired reads were generated per sample. On average, 82.8% of the pairs survived quality control and trimming with Trimmomatic (3). The insert sizes were ~330 bp; the median coverage of sequenced phages was 2,966×, ranging from 259× (for RB55) to 6,985× (for RB7). We performed de novo assembly using Velvet version 1.2.08 (4), with k-mer lengths of K51, K57, and K63, and we were able to obtain a single ~168-kbp contig from at least one of the assemblies. We used Geneious version 7.1.7 for postassembly processing and filled any assembly gaps by iterative mapping of reads to the scaffold.

The circularly permuted linear double-stranded DNA genomes of the 11 RB phages have lengths of ~168 kbp. Approximately 270 open reading frames (ORFs) per phage were predicted with Glimmer 3 (5). The annotations were transferred from the published genomes of T4 and T4-like phages with at least 98% similarity. The remaining ORFs were annotated by lowering the similarity cutoff to 70% or performing BLAST searches (6). Eight to 10 tRNAs were predicted in each genome by tRNAscan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/). According to the convention in T4-like phages, we oriented completed genomes to start with rIIA.

The sequenced phages have a similar genome organization and nucleotide identity. Using progressiveMauve alignment (8), we found that RB7, RB27, RB33, and RB68 are 73 to 86% similar to one another and are ~75% identical to T4. Furthermore, RB33 shares 99.93% similarity with RB32. RB55 and RB59 are 99.8% similar to T4 and are 99.96% identical to each other. We noted a high nucleotide similarity (99.99%) among RB3, RB5, RB6, RB7, RB9, RB10, RB55, RB59, and RB68. The RB phages were sequenced the genomes of the RB3, RB5, RB6, RB7, RB9, RB10, RB27, RB33, RB55, RB59, and RB68 phages. The RB phages were originally isolated by Rosina Berry in 1964 from six sewage treatment plants in Long Island, NY, for studies on speciation among the RB phages.

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TABLE 1  Genome features of the sequenced strains

| Enterobacteria phage | Accession no. | Genome size (bp) | Coverage (×) | No. of CDSs\textsuperscript{a} | No. of tRNAs |
|----------------------|---------------|------------------|--------------|-------------------------------|--------------|
| RB3                  | KM606994      | 168,402          | 2,831        | 273                           | 10           |
| RB5                  | KM606995      | 168,394          | 3,449        | 271                           | 10           |
| RB6                  | KM606996      | 168,394          | 1,474        | 271                           | 10           |
| RB7                  | KM606997      | 168,395          | 6,985        | 272                           | 10           |
| RB9                  | KM606998      | 168,395          | 2,826        | 272                           | 10           |
| RB10                 | KM606999      | 168,401          | 2,798        | 272                           | 10           |
| RB27                 | KM607000      | 165,179          | 2,966        | 271                           | 10           |
| RB33                 | KM607001      | 166,007          | 3,355        | 274                           | 8            |
| RB55                 | KM607002      | 168,896          | 259          | 272                           | 8            |
| RB59                 | KM607003      | 168,966          | 3,158        | 276                           | 8            |
| RB68                 | KM607004      | 168,401          | 3,187        | 276                           | 9            |

\textsuperscript{a}CDSs, coding sequences.

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