What do LE3 and LE4 bacteriophages tell us about Leptospira?

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Résumé

Leptospirosis is a zoonotic disease caused by infection with pathogenic species of the genus *Leptospira*-Gram-negative bacteria belonging to the *Spirochaetes* phylum. To date 22 *Leptospira* species are known in this genus; including saprophytes and pathogens which have the ability to persist in freshwater. We have isolated three bacteriophages of *Leptospira* (leptophages) called LE1, LE3 and LE4. All of them are specific to the saprophyte *Leptospira biflexa* and they belong to the *Myoviridae* family. Interestingly, prophage-like regions have been found in infectious strains but not in saprophytes. The genome of the lysogenic phage LE1 was previously sequenced (74kb) and its origin of replication was used to design a shuttle vector. The lytic phages LE3 and LE4 are characterized in this study in order to further evaluate the diversity of phages and prophages in the leptospires.
LE3 and LE4 have a similar morphology and their genomes are closely related. The 48kb genome of LE4 contains 81 open reading frames (ORFs). Putative functions could be assigned to only 12 gene products (15% of gene content) based on sequence homology and motif searches. Additionally, mass spectrometry analyses were performed on culture supernatants and we were able to consistently identify 11 gene products of phage origin. In order to identify a bacterial LE4 receptor, we selected a phage-resistant *L. biflexa* mutant strain called RLE4. Whole-genome sequencing of RLE4 allowed the identification of a single nucleotide polymorphism in a gene of the O-antigen locus that introduced a premature stop codon. This result was confirmed by targeted mutagenesis of the aforementioned gene in the WT strain, and suggests that the lipopolysaccharide is a receptor for the phage.

Finally, using comparative genomics, LE3 and LE4 exhibited homologies with other prophages in the published *Leptospira* genomes, which could, together, form a new bacteriophage/prophage group in *Leptospira*. Moreover, we identified a new LE4-like circular prophage, maintained as a plasmid, in the pathogen *L. mayottensis*.
Sequencing of these two leptophages will advance several aspects of the field including improved detection methods of prophages in *Leptospira* genomes, development of genetic tools, identification of novel genes, prediction of hypothetical proteins, and better understanding of phage contribution to bacterial evolution and virulence.

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