
Screening for virulent phages against vancomycin-resistant *Enterococcus faecium*

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Résumé

Enterococcus faecium is a Gram-positive facultative anaerobe bacterium that commensally colonizes the human intestinal tract. It is part of the normal gut microbiota and has normally no adverse effects on healthy individuals. However, *E. faecium* can also become an opportunistic pathogen and has emerged as a leading cause of nosocomial infections, particularly in immunocompromised patients. The clinical importance of *E. faecium* is directly related to its antibiotic resistance. The bacterium has been very successful at rapidly acquiring multi resistance to antimicrobial agents commonly used in therapy. The rapid spread of vancomycin-resistant *E. faecium* (VRE) clinical isolates has been of particular concern and associated hospital acquired infections have become a growing problem. The use of bacteriophages could be a therapeutic alternative to combat VRE infections. However, the number of characterized and potential interesting virulent phages still remains scarce in literature, with five published genomes belonging to 3 different clades.

In this study, we performed a phage-screening on 14 *E. faecium* VRE clinical isolates, provided by the National Reference Center of Caen (CNR). Four raw sewage water samples from Ile de France-wastewater treatment plants (provided by the Pasteur Institute) were used as a source of phages. Following culture-enrichment and isolation steps, we were able to isolate 23 phages growing on 6 of the 14 VRE. Spot assays revealed that isolated phages have host ranges of 1-5 strains and encompass a total of 8 VRE. Interestingly, 5 of these 8 belong to the most clinical prevalent sequence types ST 17 and ST 18. Based on distinct plaque morphologies and host ranges, we selected 10 of the 23 phages for analyses on transmission electron microscopy. All belong to the *Caudovirales*-order and members all 3 families *Siphoviridae*, *Myoviridae* and *Podoviridae* were observed. A phage affiliated to each one of these families was subsequently chosen for further characterization. Primary results indicate these 3 phages, VRE-s1 (siphophage), VRE-m1 (myophage) and VRE-p1 (podophage), have distinct burst sizes (29, 122 and 58, respectively) and latent periods (15, 45 and 40 min, respectively). Genomic analyses show that while VRE-s1 and VRE-m1 are highly homologous to 2 phages already described for the closely related *Enterococcus faecalis* species, VRE-p1 could be a member of a new genus within *Picovirinae* subfamily.

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