Université de Montréal

MICROBIOLOGIE, INFECTIOLOGIE ET IMMUNOLOGIE



CONFERENCE

November 19th at 11.30AM
Room N-833
Université de Montréal, Pavillon Roger-Gaudry.
2900 boul. Édouard Montpetit (Chemin de la tour), Montréal.

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The neck passage structure is involved in phage adsorption in *Lactococcus*

Members of the *Skunavirus* viral genera are the most isolated lactococcal phages in Canadian cheese factories and have been extensively studied because of their negative impact on milk fermentation. Although complete failed productions due to virulent phages are now rare, their populations are still monitored in large factories to avoid production delays and to ensure cheese uniformity. Whole genome sequencing was performed on 54 cheese-plant-isolated phages all infecting the *Lactococcus* strain SMQ-1001. Comparative genomic of their genomes revealed that the neck passage structure (NPS) has a high sequence diversity.

Two-thirds (141/213, 66%) of the virulent *Skunavirus* phages that infect dairy *Lactococcus* strains, possess a neck passage structure protein located between the capsid and the tail. The NPS, spanning from 145 to 888 amino acids, contains up to three domains: a collar, a whisker, and a globular end. Notably, the globular end at the C-terminal of the NPS exhibits greater diversity among these phages and may display an unknown enzymatic activity. While the functions of most of the structural proteins within the *Skunavirus* virions are known, the role of the NPS is still elusive, although it may participate in host recognition.

Using CRISPR-Cas9 genome editing, we engineered two *nps*-knock-out phage mutants and one *nps*-knock-in phage mutant highlighting the high modularity of lactococcal phage genomes. First, a deletion of 2,486 bp or 2,091bp, encompassing the whole NPS protein and part of the flanking regions, was generated in phage JeB4 (JeB4Δ*nps*) and RH13 (RH13Δ*nps*), respectively. Second, a knock-in of the gene coding for a NPS protein and its flanking regions (2,190 bp) was performed in the model lactococcal phage p2 (p2*nps*$^{+}$). These specific phage genome editions were confirmed by Sanger and Illumina sequencing. Electron microscopy observations revealed the absence of NPS in the phage mutants JeB4Δ*nps* and RH13Δ*nps,*and its novel presence in phage p2*nps*$^{+}$.

Removing the NPS did not alter the adsorption of JeB4Δ*nps* and RH13Δ*nps* compared to the wild-type phages showing that it is not part of the irreversible adsorption step. However, the NPS addition to phage p2 led to its adsorption to a new *Lactococcus* host, highlighting its role in the initial adsorption step. However, the genome modifications had deleterious effects as the plaque diameters and burst sizes were reduced for the three phage mutants compared with the wildtype phages.