Bacillus thuringiensis receptors recognized by Vp4 tail proteins upon adsorption

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Getting inside the host: the adsorption process

Adsorption is a key stage in phage recognition of sensitive host cell implying the specific interaction between **Receptor Binding Proteins (RBPs)** often located at the distal part of the phage tail (*i.e.* tail fibers, spikes or baseplate proteins) and **receptors** on the surface of their bacterial host. Adsorption is generally described as a three-step process:

- Initial contact by **random collisions** between phage and its host,
- **Reversible binding** which maintains the phage close to the cell surface,
- **Irreversible binding** through tighter binding of the phage RBP to the initial receptors or through interactions with secondary receptors.

The Vp4 myophage

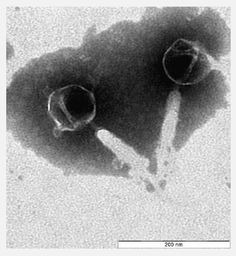


Fig. 1. Vp4 TEM microscopy.

Vp4 (Fig. 1) is a virulent myophage belonging to the *Herelleviridae* family infecting members of the *Bacillus cereus* group. It features a dsDNA genome of *ca.* 163 kb (Fig. 2) , with 269 putative Coding DNA Sequences (CDS) displaying almost the same gene synteny as Deep-Blue myophage [1].

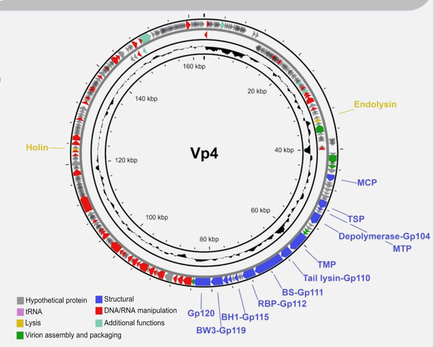


Fig. 2. Vp4 genomic map

Phage tail proteins involved in the adsorption process

Homologues of proteins implicated in the baseplate architecture of most contractile devices could be identified in Vp4 and Vp4 tail proteins (TP) and are mostly encoded in the same order as those of *Listeria* phage A511 [2] (Fig. 3). To evaluate the binding capacity of TP potentially involved in the adsorption process, GFP-fused proteins were tested in Cell Wall Decoration Assay (CWDA).

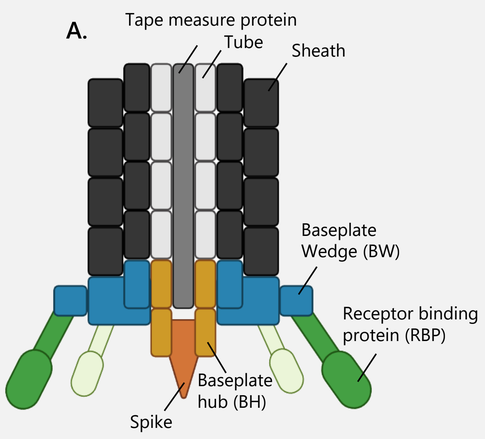


Fig. 3. Tail proteins encoded by Vp4. **A.** Schematic representation of Vp4 tail. **B.** Tail protein-encoding region of Vp4. The colour coding corresponds to the one of part A.

- As they recognize variable components at the host surface, RBP encoding genes are often the least conserved in the tail region. Gp112 was the best RBP candidate as no structural homologue could be identified except for the intramolecular chaperone (IMC) at the very C-terminus. The C-terminal variable part of the protein was fused to a GFP and tested in CWDA (Fig. 4). Strains insensitive to Vp4 were not bound by Gp112 while Gp112 was able to bind to sensitive and lysed from without strains.
- Three putative baseplate wedge proteins (BW) are present in Vp4, presumably acting as components of the peripheric wedges surrounding the central hub and anchoring tail fibers. Interestingly, Gp119 displays carbohydrate-binding module (CBM) folds. Its binding capacity was assessed through CWDA using GFP-fused proteins. It showed a decoration of some *B. cereus* strains, suggesting an additional binding of sugar moieties upon adsorption even if the exact mechanism remains unclear.
- Gp120 is homologous to phage A511 Gp106 that forms pyramidal structures potentially interacting with host surface upon A511 adsorption. Its exact role in Vp4 adsorption remains to be investigated.

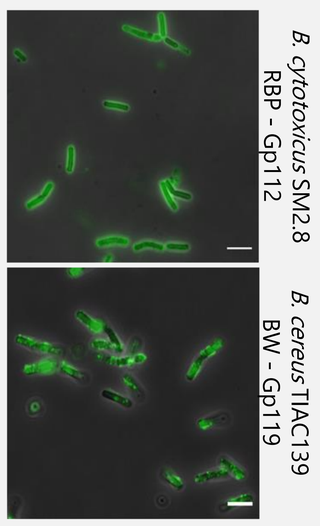
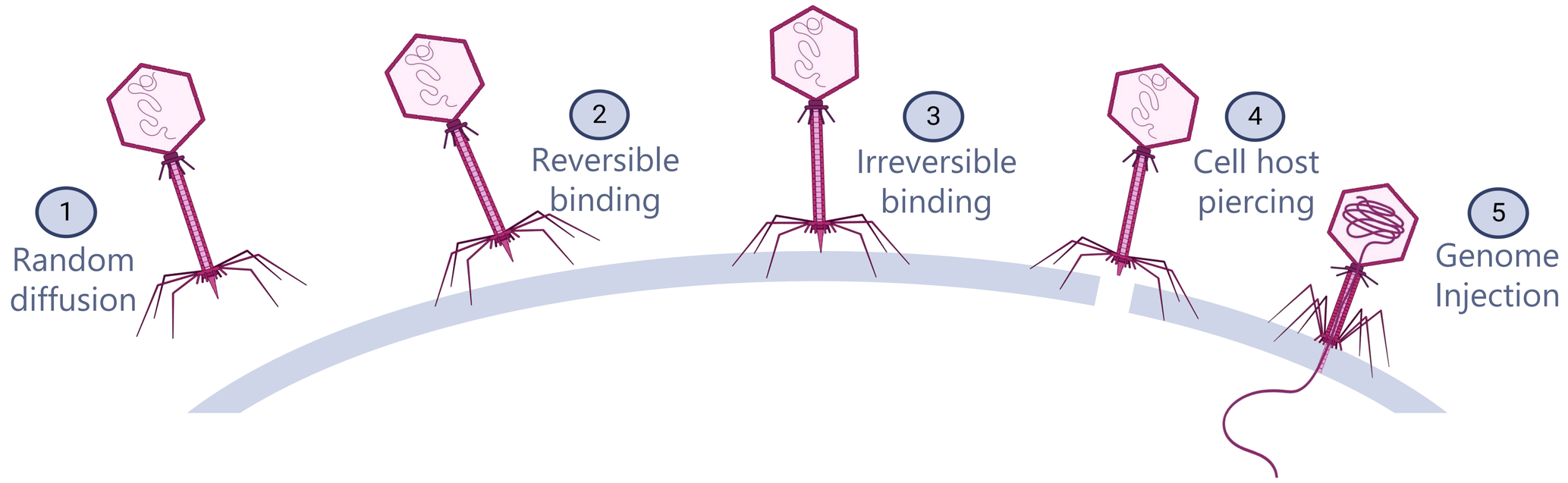


Fig. 4. CWDA of Gp112 and Gp119 to bacteria of the *B. cereus* group.



Bacterial surface structures involved in the adsorption process

As key determinant of the adsorption process, the identification and characterization of the cognate receptors recognized by Vp4 upon adsorption have also been addressed.

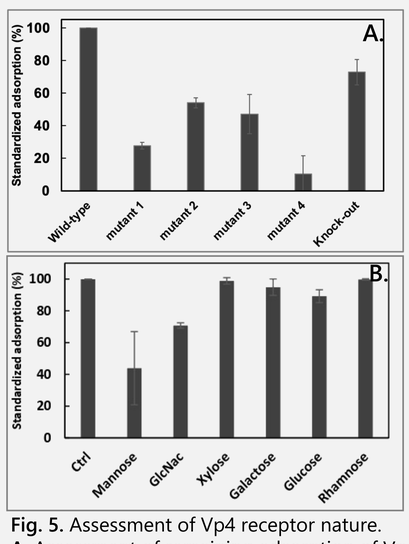
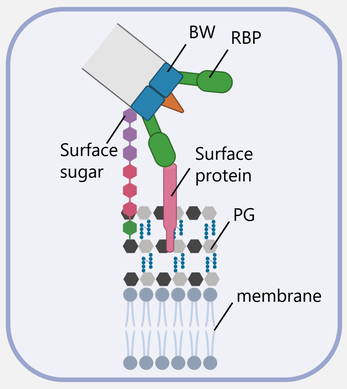


Fig. 5. Assessment of Vp4 receptor nature. **A**. Assessment of remaining adsorption of Vp4 to spontaneous BGSC4BA1 and KO mutants. **B**. Assessment of sugar structure involved in Vp4 adsorption. Competition assays were performed by incubating Vp4 with different sugars followed by an adsorption assay with GSX002 strain. GlcNAc: N-acetyl glucosamine.

- Four independent spontaneous mutants of the *Bacillus thuringiensis* BGSC 4BA1 strain were isolated and characterized genetically (whole genome sequencing, WGS) and phenotypically. All mutants showed impaired adsorption of Vp4 (Fig. 5A) and Gp112 was not able to decorate the mutants. However, no significative difference could be seen in cell morphology, growth kinetics and swimming capacity. WGS pinpointed an alanine to glutamic acid mutation in a CDS containing a collagen triple helix repeat domain. To assess the potential implication of this protein in the adsorption process, the gene was knocked out (KO). The KO mutant showed a decrease in Vp4 adsorption, to a lesser extent than for the spontaneous mutants (Fig. 5A). Complementation experiments are in progress.
- Interestingly, when bacterial hosts were treated with periodate (alteration of carbohydrate structures), Vp4 adsorption was previously shown to be seriously impaired, suggesting that carbohydrate moieties play a crucial role. To further document the sugars potentially involved in Vp4 adsorption, a competition assay was performed using various sugars found in secondary cell wall polysaccharides of *B. cereus*. As shown in Fig. 5B, mannose and N-acetyl glucosamine interfered with adsorption, which was reduced by ca. 60 and 30%, respectively. This suggests the implication of an additional receptor of saccharidic nature containing mannose and/or N-acetylglucosamine units or glycosylated proteinaceous receptor.

Take-home message



Taken together, all these experiments suggest that:

- Gp112 acts as a *bona fide* RBP, potentially interacting with a bacterial surface protein putatively containing collagen triple helix repeat domains.
- Gp119 is a BW binding to saccharidic structures upon adsorption, likely of mannose and/or N-acetylglucosamine nature.

References
[1] Hock, L., Leprince, A., Tournay, M., Gillis, A., & Mahillon, J. (2019). Biocontrol potential of phage Deep-Blue against psychrotolerant *Bacillus weihenstephanensis*. *Food Control*, *102*, 94-103.
[2] Guerrero‐Ferreira, R. C., Hupfeld, M., Nazarov, S., Taylor, N. M., Shneider, M. M., Obbineni, J. M., ... & Leiman, P. G. (2019). Structure and transformation of bacteriophage A511 baseplate and tail upon infection of *Listeria* cells. *The EMBO journal*, *38*(3), e99455.