# *Bacillus thuringiensis* receptors recognized by Vp4 tail proteins upon adsorption

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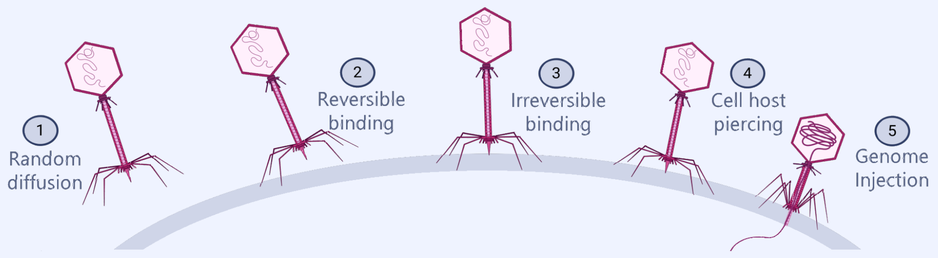
## Adsorption: getting inside the host




The adsorption allows the phage recognition of sensitive host cell through specific interactions between **Receptor Binding Proteins** (RBPs) located in the baseplate and **receptors** on the surface of their host. It is generally described as a three-step process:

- Initial contact by random collisions between the 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 initial receptors or through interactions with secondary receptors

## The Vp4 myophage

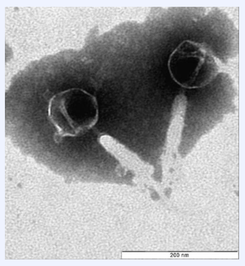


**Fig. 1.** Vp4 TEM microscopy.

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, with 269 CDS displaying almost the same gene synteny as Deep-Blue myophage [1].

## The RBP - Gp112 and BW - Gp119 are involved in Vp4 adsorption

Homologues of proteins implicated in the baseplate architecture of most contractile devices could be identified in Vp4 and Vp4 tail proteins (TP) are mostly encoded in the same order as those of *Listeria* phage A511 [2] (Fig. 2).

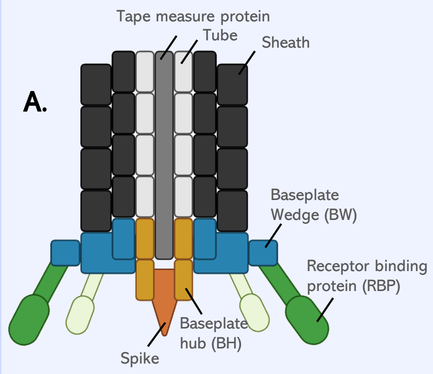
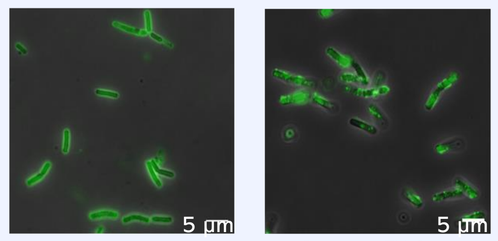



**Fig. 2.** Tail proteins encoded by Vp4. **A.** Schematic representation of Vp4 tail. **B.** Tail protein-encoding region of Vp4. The colour coding corresponds to the one of part A.

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).

- As they recognize variable components at the host surface, RBP-encoding genes are often the least conserved in the tail region. Gp112 (895 aa) was the best RBP candidate. 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. Gp119 (831 aa) displays carbohydrate-binding module (CBM) folds. It showed a decoration of some *B. cereus* strains, suggesting a binding of sugar moieties upon adsorption.



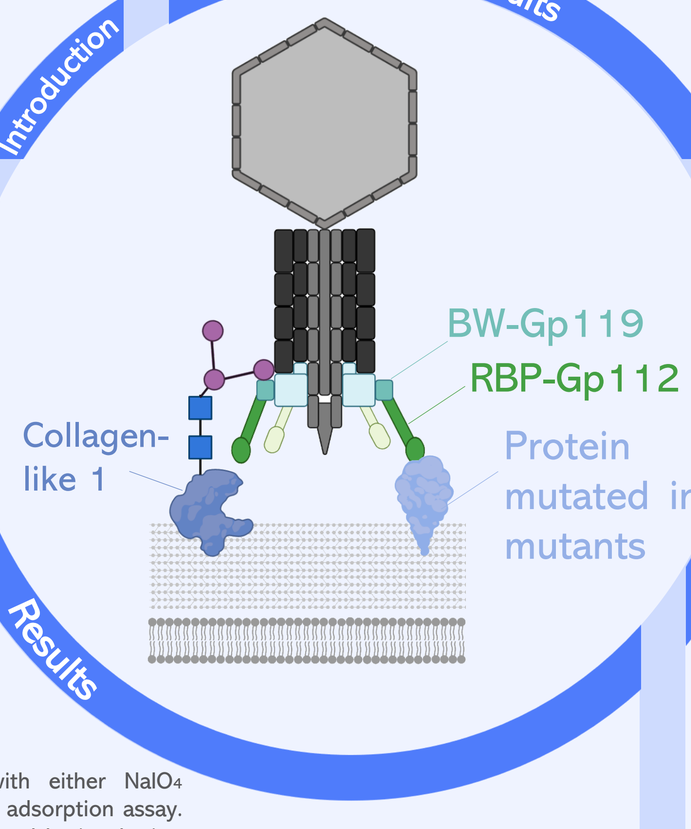

*B. cytotoxicus* SM2.8
RBP - Gp112

*B. cereus* TIAC139
BW - Gp119

**Fig. 3.** CWDA of Gp112 (left, 60 µg proteins) and Gp119 (right, 90 µg proteins) to bacteria of the *B. cereus* group.

Introduction

Results




Results

Conclusion

## Vp4 recognizes saccharidic structures

Alteration of surface carbohydrate structures of bacterial hosts strongly impaired Vp4 adsorption (Fig. 4A). To further document the sugars potentially involved, a competition assay was performed with various sugars found in secondary cell wall polysaccharides which suggested the implication of mannose and N-acetylglucosamine structures (Fig. 4B).

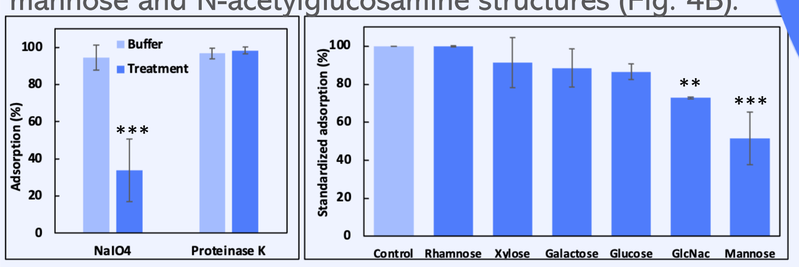



**Fig. 4.** Vp4 receptor nature. **A.** *B. thuringiensis* GSX002 was treated with either $NaIO_4$ (carbohydrates alteration) or proteinase K (proteins alteration) before performing adsorption assay. **B.** Sugar structures involved in Vp4 adsorption. Competition assays were performed by incubating Vp4 with different sugars followed by an adsorption assay with *B. thuringiensis* GSX002 strain. GlcNac = N-acetylglucosamine. ** p-val < 0,05, *** p-val < 0,001 (Tukey's law).

## A Collagen-like protein is involved in Vp4 adsorption

Four independent spontaneous mutants of *Bacillus thuringiensis* BGSC 4BA1 strain were isolated and characterized genetically (whole genome sequencing, WGS) and phenotypically. All mutants showed impaired adsorption of Vp4 (Mean remaining adsorption = 45,9 ± 9,9%, Fig. 5). Gp112 was not able to decorate the mutants (Fig. 6B) while only a faint fluorescence was observed for the mutants subjected to Gp119 (Fig. 6E). WGS pinpointed a mutation in the *collagen-like 1* gene. To assess the potential implication of Collagen-like 1 in the adsorption, the gene was knocked out (KO). The KO mutants showed a significant decrease in Vp4 adsorption (Fig. 5). Complementation experiments are in progress. However, the remaining adsorption of Vp4 to both spontaneous and KO mutants suggests the presence of another receptor. As Gp112 still binds to the KO mutant (Fig. 6C), Collagen-like 1 protein is not likely the receptor recognized by Vp4-RBP. However, the weak or no binding of Gp119 (Fig. 6F) suggests the potential recognition of saccharidic decoration on Collagen-like 1 glycoprotein (possibly also present on Collagen-like 2 and 3 proteins). KO are in progress.

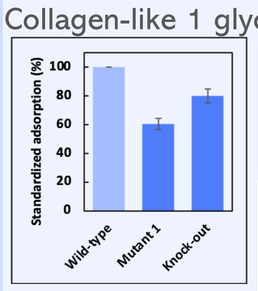



**Fig. 5.** Adsorption of Vp4 to spontaneous *B. thuringiensis* resistant and KO mutant.

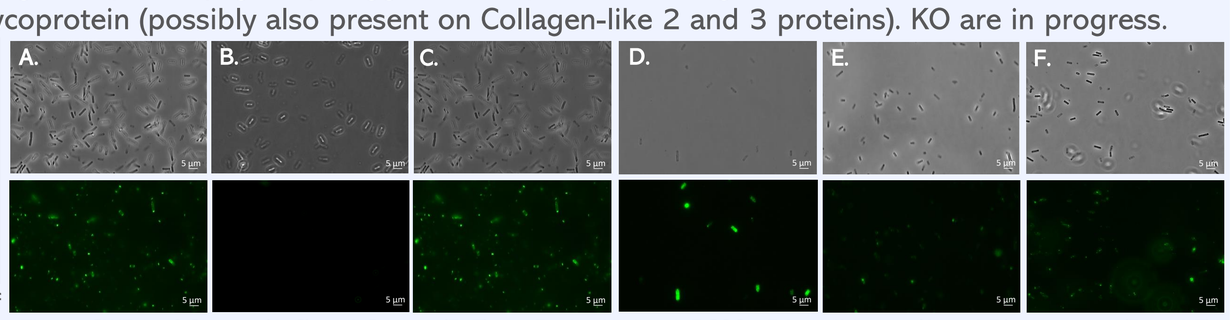



**Fig. 6.** CWDA of Gp112 (A-C) and Gp119 (D-F) on *B. thuringiensis* BGSC 4BA1 wild-type (A and D), mutant 1 (B and E) and knock-out (C and F). Top: bright field microscopy. Bottom: fluorescence micrograph. Cells were treated with *ca.* 60 µg of recombinant Gp112 and 90 µg of recombinant Gp119.

This work provided insights into phage-host interactions between Vp4 and its *B. cereus* hosts:

- Gp112 decorates both sensitive and lysis from without strains and acts as putative Receptor Binding Protein.
- Gp112 likely recognizes a receptor absent from the surface of spontaneous mutants
- Gp119 is a Baseplate Wedge protein displaying Carbohydrate Binding Modules with binding capacity.
- Vp4 adsorption relies on saccharidic structures of mannose and N-acetylglucosamine type.
- Collagen-like 1 protein is involved in Vp4 adsorption, its saccharidic decoration being putatively recognized by Gp119.

**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., ... & Leiman, P. G. (2019). Structure and transformation of bacteriophage A511 baseplate and tail upon infection of *Listeria* cells. *The EMBO J*, *38*, e99455.
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