API480: features towards therapy in honeybee hives

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Introduction

American foulbrood disease (AFB) is a devastating bacterial disease affecting honeybees. It is caused by *Paenibacillus larvae*, a worldwide-distributed spore forming Gram-positive bacterium which spread easily across apiaries producing highly resistant spores. When AFB symptoms are found the burning of contaminated hives is mandatory causing serious economic losses [1]. Bacteriophages (phages) are being considered valuable solutions to the control of this infection [2-5]. So far, 48 *Siphoviridae P. larvae* phages sequences are known and most encode integration genes suggesting a temperate lifestyle. All of these 48 phages seem to have a common evolutionary ancestor showing an overall common structure. Their genomes were grouped into four clusters (with Fern, Harrison, Vegas and Halcyone as representative phages) and one singleton (phage Lily) [6].

Goal of the study

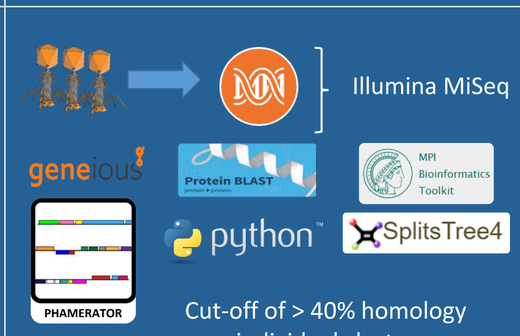
The main goal of this study was the characterization of the first podovirus infecting *P. larvae* (API480), envisaging AFB control.

Methods

Phage isolation

Culture condictions:
- MYPGP medium
- 37ºC, 5% CO_2, O/N

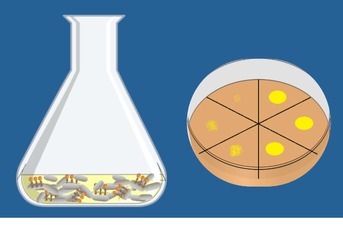
Genome sequencing, annotation and compative analysis



Cut-off of > 40% homology individual cluster

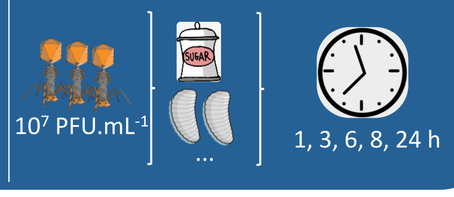
Phase characterization

- TEM
- Lytic spetra, EOP
- Adsorption, OSGC (MOI=0.1)
- Specificity test

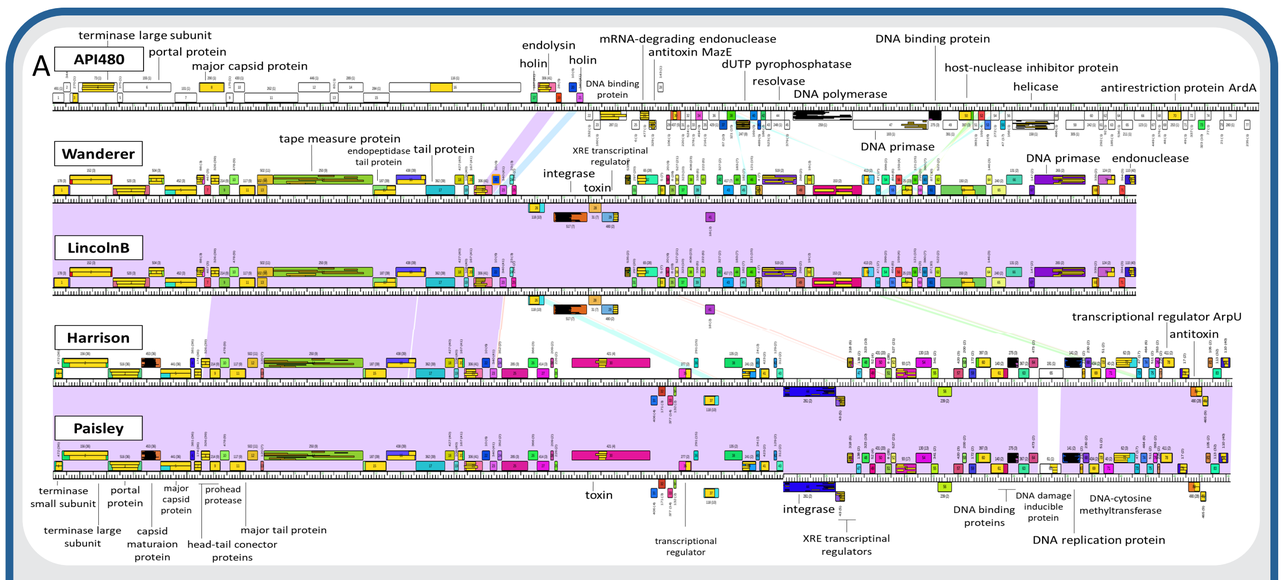


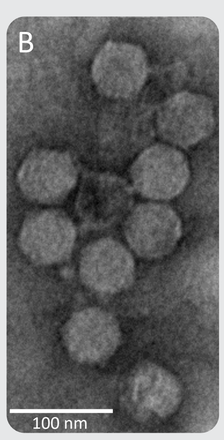
Phage viability in simulated field conditions

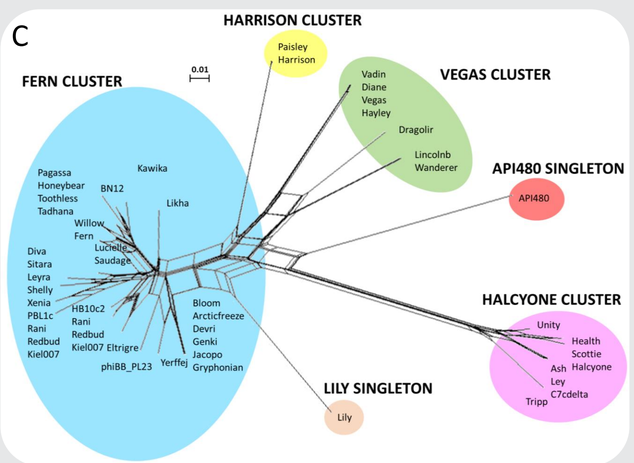
- 50% (w/v) sucrose
- Larval homogenised
- Royal jelly (RJ)
- pH solutions (universal buffer) ranging 3.0 to 5.0
- Statistical significance ($p<0.05$)



Results

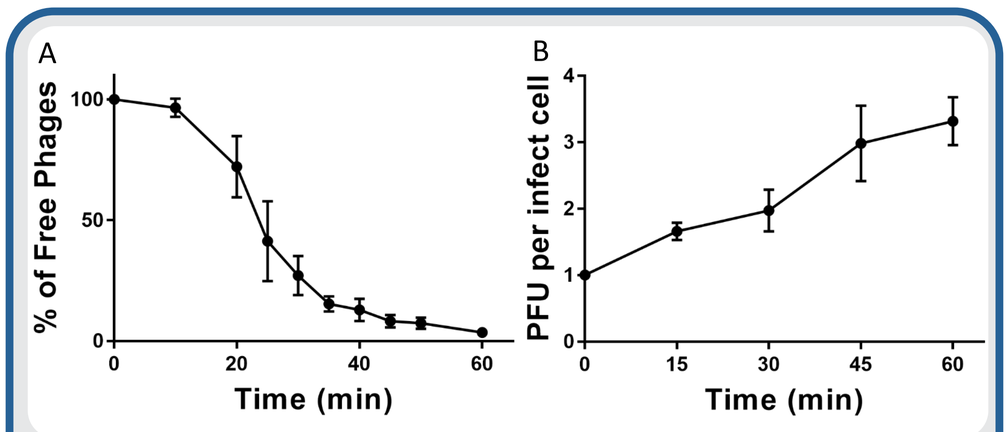


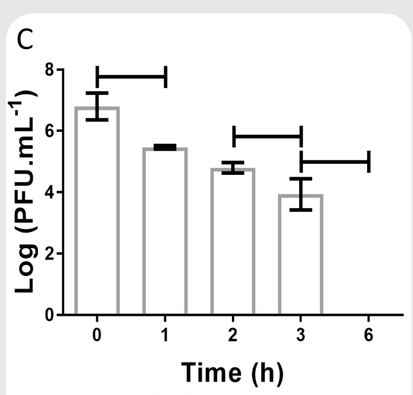




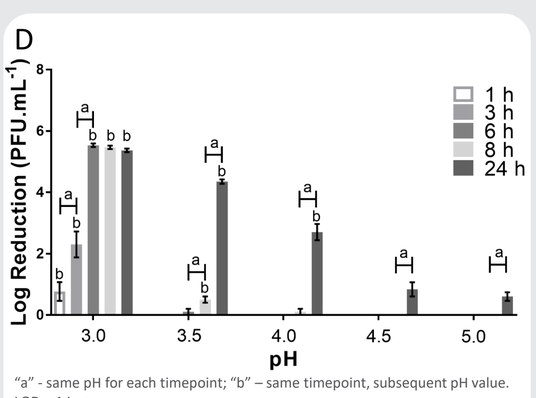
D Species	No. strain	Score
P. larvae	19	LFW
P. larvae	42	+
L. pentosus	1	-
L. rhamnosus	1	-
L. paracasei	1	-
L. casei	1	-
L. acidophilus	1	-
L. kunkeei	1	-
B. subtilis	1	-
B. cereus	1	-
B. circulans	1	LFW
B. coagulans	1	LFW
P. apium	1	-
P. polymyxa	1	-
P. alvei	1	-

Figure 1. (A) Pairwise genome maps of API480 with the closest relatives phages. **(B)** TEM micrograph showing the virion particle morphology. **(C)** Relationship of shared gene content from a total 49 *P. larvae* phages. **(D)** API480 lytic spectra and EOP (susceptible (+), non-susceptible (-) and lysis from without (LFW)).





Limit of detection (LOD) = 3 Log.



"a" - same pH for each timepoint; "b" – same timepoint, subsequent pH value.
LOD = 1 Log.

Figure 2. (A) Phage adsorption. **(B)** OSGC. **(C)** Effect of RJ on API480 concentration. **(D)** Effect of acidic pH in API480 concentration.

No negative effects caused in API480 phage viability by sucrose and larval homogenate after 24 hours.

Main conclusions

- **API480 is a distinct phage (4% genome coverage and 14% shared proteins), suggesting the creation of a new species within the *Podoviridae* family.**
- **Besides no lysogenic module being identified, API480 is a temperate phage.**
- **API480 has a broad lytic spectrum and is specific to *P. larvae*. The larval commensal (*L. kunkeei* and *P. apium*) are not affected by this phage.**
- **This phage is very stable when exposed to high sucrose concentration and to larval homogenized content.**
- **Overall, results suggest that this phage holds potential to be used in the biocontrol of American Foulbrood disease.**

References

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Acknowledgements

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