

API480: features towards therapy in honeybee hives

1 Centre of Biological Engineering, LIBRO – Laboratory of Rosário Oliveira
University of Minho, Campus de Gualtar, 4700-057 Braga, Portugal

Henrique G. Ribeiro¹, Luís D. R. Melo¹, Hugo Oliveira¹, Maarten Boon²,
Rob Lavigne², Joana Azeredo¹ & Ana Oliveira¹

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.

Results

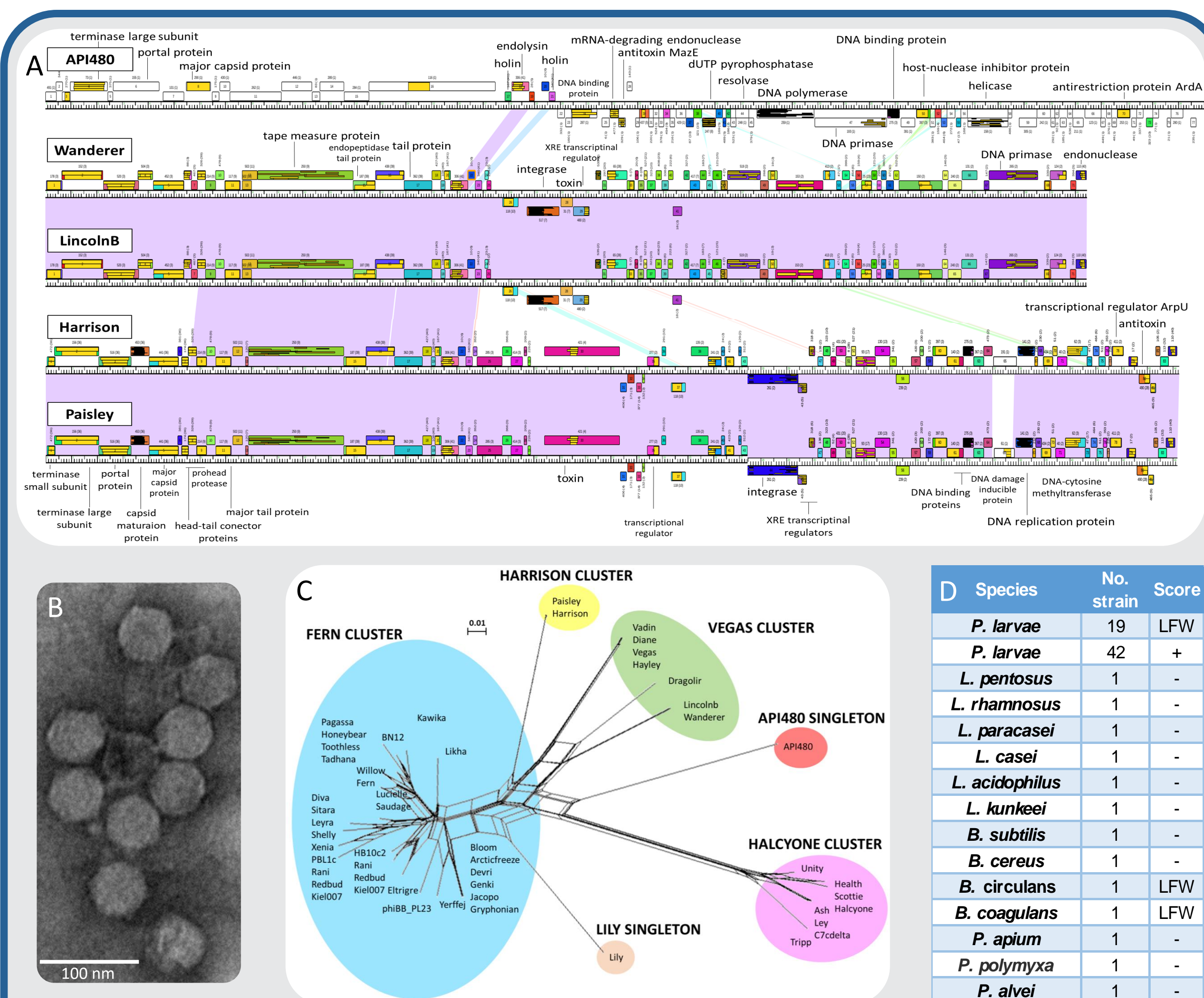
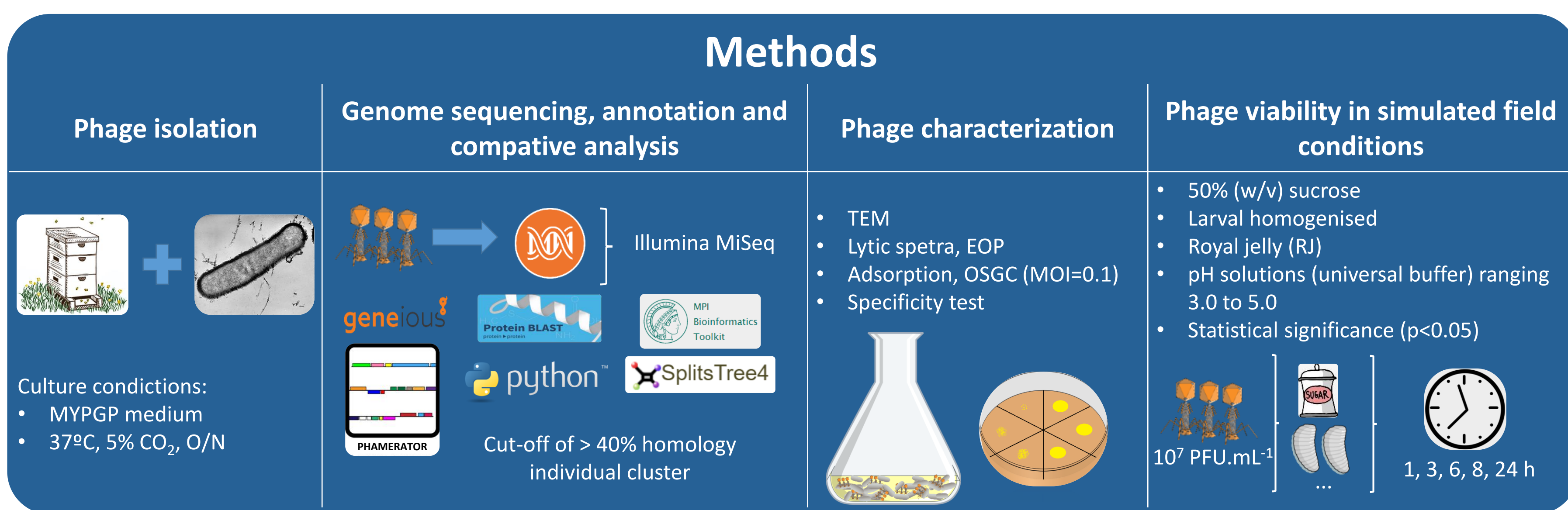
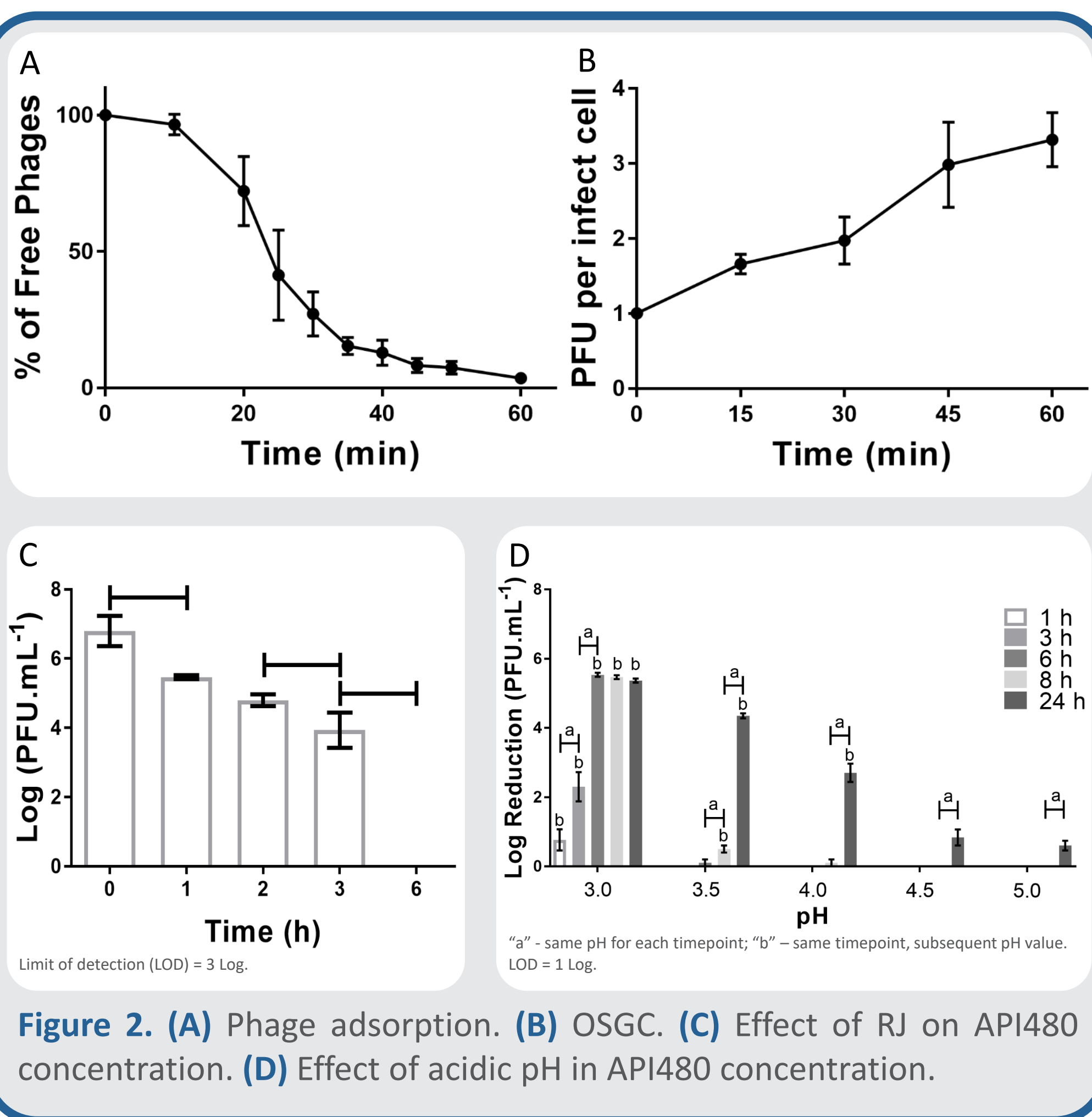


Figure 1. (A) Pairwise genome maps of API480 with the closest relatives phages. **(B)** TEM micrograph showing the virion particle morphology. **(C)** Relationship of shared gene content from a total 49 *P. larvae* phages. **(D)** API480 lytic spectra and EOP (susceptible (+), non-susceptible (-) and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



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 overlap 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. kunkei* 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

[1] Genersch, E. (2010). American Foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. [2] Beims, H. et al. (2015). *Paenibacillus larvae*-Directed bacteriophage HB10c2 and its application in American foulbrood-affected honey bee larvae. [3] Ghorbani-Nezami, S. et al. (2015). Phage therapy is effective in protecting honeybee larvae from American foulbrood disease. [4] Yost, D. G. et al. (2016). Experimental bacteriophage treatment of honeybees (*Apis mellifera*) infected with *Paenibacillus larvae*, the causative agent of American foulbrood disease. [5] Brady, T. S. et al. (2017). Bacteriophages as an alternative to conventional antibiotic use for the prevention or treatment of *Paenibacillus larvae* in honeybee hives. [6] Stammerli, C. et al. (2018). Genomic Analysis of 48 *Paenibacillus larvae* Bacteriophages.

Acknowledgements

This work was supported by the project APILYSE, PTDC/CVT-EPI/4008/2014 - POCI-01-0145-FEDER-016598, - funded by FEDER through COMPETE 2020 - Programa Operacional Competitividade e Internacionalização (POCI) and the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2019 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. The work was also supported by CEB-UM that provided the laboratorial facilities to perform this research. HR was supported by FCT through the grant SFRH/BD/12885/2017. MB and RL were supported by KU Leuven through a GOA grant [3E140356].

