Role and impact of prophages in *Paenibacillus larvae*

1 CEB - Centre of Biological Engineering, LIBRO – Laboratory of Research in Biofilms Rosário Oliveira, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.
2 Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Henrique G. Ribeiro[1], Anna Nilsson[2], Joana Azeredo[1], Luís D. R. Melo[1] & Ana Oliveira[1]

Introduction

Paenibacillus larvae is a spore-forming Gram-positive bacterium that causes American Foulbrood disease (AFB), the most destructive bacterial infectious diseases of honeybee brood[1]. Bacteriophages (phages) are bacterial viruses that parasitize bacteria and play a key role in the evolution of most bacterial communities in all ecosystems[2]. Temperate phages – prophages – follow a lysogenic lifecycle and are able to integrate into the host genome, making rearrangements, disrupting gene function or adding new features to the bacteria[3]. Other studies describe prophage-host relationships as advantageous to improve the host toxicity, as described for *E. coli* with the Shiga toxin[3]. In *B. subtilis* the presence of prophages made host unable to sporulate[4], and in *A. baumannii*, prophage converted strains susceptible to antibiotics into resistant[5]. So far, no study has evaluated the impact of prophages in *P. larvae* ecology.

Goal of the study

The main goal was to **understand the impact of prophages on *P. larvae* virulence and fitness.**

Methods

Data collection

GenBank (Last accessed April 2020)
- *P. larvae* genomes (n=14)
  - n=11 unique sequence
  - n=4 several contigs

Detection and curing of prophages in *P. larvae* strains

Accession no. for bacterial genomes
PHASTER output: Prophages grouped as intact or defective (incomplete and questionable)

Manual curing:
- attachment sites (*attR/attL*)
- Presence of lysin (endolysin or other)
- Structural genes enough
- Assembly genes (small and large terminase)

BLASTp default and tailed phages

CD-Search Tool with E-value $1E^{-5}$

tRNAscan-SE

Prophages characterization and analysis

Taxonomic classification (according to strutural proteins)

BLASTn homology

Prophages genome alignments
- MAUVE

Virulence / fitness features provided by prophages

Antibiotic resistance genes:
- ResFinder
- Resistance Gene Identifier (RGI) of CARD (The Comprehensive Antibiotic Resistance Database)
  - W/ perfect, strict and loose hits

BLASTp

Results

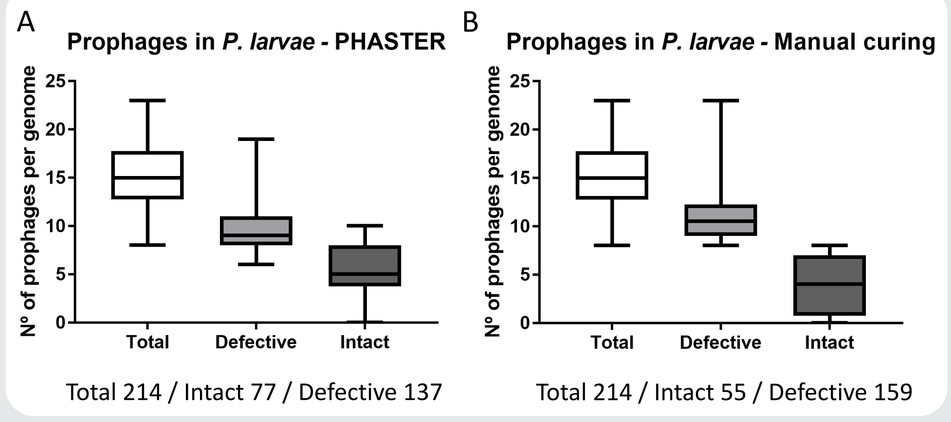

Figure 1. Prevalence of prophages in *P. larvae* genomes after PHASTER **(A)** and manual curing **(B)**. **(C)** Average of total, defective and intact prophages per host genome. **(D)** Prevalence in percentage of total, intact, defective prophages, ≥ 8 defective prophages and ≥ 5 intact prophages in hosts.

A

Total	55	%	Tail sheath protein	Major tail protein / tail tube protein	Tail assembly A	Tail assembly B	Tail tape measure protein
Siphoviridae	4	7		MTP			
Siphoviridae	8	15		MTP			
Siphoviridae	5	9					
Myoviridae	4	7		MTP			
Myoviridae	17	31		TTP			
Inconclusive	17	31					

Siphoviridae = 17 (31%).

Myoviridae = 21 (38%).

Inconclusive = 17 (31%), (*Siphoviridae* according to BLASTn)

B

	Function (BLASTp)					
	5'			3'		
***attL/R* sequence**	**Gene before *AttL***	**Cutted genes**		**Gene after *AttR***	**No. Prophages**	**No. Cutted prophages**
GCTATGACATTC	dCMP deaminase protein	ComEC/Rec2 protein	FtsX-like permease	ABC transporter	2	2
AAAAAAGGAACT	pepF protein	O-methyl-transferase	Lantibiotic dehydratase protein	Nitroreductase protein / ABC transporter	2	2
TTGCATGGGAAAACGGATTG	MBL fold metallo-hydrolase			TetR family regulator	3	0
TGTGGGCAAAATGTGGG	Ribonuclease H			O-methyl-transferase	4	0
AACTATTAAATA	HP (DUF4064)			RNA pseudouridine synthase YhcT	2	0
TCCCACAGGATG	Hemolysin III protein			Putative permease	2	0
TTATTAAATTCATAA	CRISPR- Cas3	CRISPR- Cas5		CRISPR Cas8c/Csd1	3	3
TGCCCACATTTTGCCCAC	HP (DUF3055)			HRDC domain protein	2	0
CATCATCTACAGTATGATG	Factor Spo0A / HP (DUF4297)			HP (DUF871 family protein)	2	0
5 different att sites	5 different genes	3 distintc genes	**ImmA/IrrE family metallo-endopeptidase**	**site-specific recombinase XerD / integrase**	5	5
2 different att sites	2 different genes	2 distintc genes	LexA family regulator	**site-specific integrase**	2	2

At least 16 *att* sites to integrate 29 prophages.

ImmA/IrrE: more often (5x) interrupted gene. (highlighted in **bold**)

Integrase/recombinase often found (7x) after the prophage in the host genome. (highlighted in **bold**)

Table 1. (A) Taxonomic classification of prophages based on structural proteins present (highlighted in **gray**). **(B)** *att* sites nucleotide sequences and representation of prophages integration zones (genes before and after the *att* sites and interrupted genes).

- BLASTp: 40% proteins are hypothetical. Transposase is the gene most often identified.
- PHASTER did not identify attachment sites in 10 of the intact prophages.
- 60% of *att* sites are in intergenic regions and 40% are interrupting genes.

ResFinder – no antimicrobial resistance genes. RGI – some loose hits (30) in 15 prophages.

Host genes provided by prophages, according to BLASTp:
- TetR family transcriptional regulator, metallo-β-lactamase (MBL) and β-Lactamase inhibitory proteins (BLIP).
- Few transporters like ABC transporter, MFS transporter, SMR transporter and aromatic acid exporter.
- Two enzymes related to iron uptake, the Fe-S cluster assembly proteins SufB and NifU.
- Several toxin-antitoxin fragments as HicAB toxin-antitoxin system, mazE and SocA antitoxins.
- Some virulence factors like enhancin protein, leukotoxin LukF-PV precursor, and bacteriocin closticin.
- DNA internalization ComEC/Rec2 protein to uptake exogeneous DNA.

Main conclusions

- **25.7% of detected prophages were intact.**
- On average, each *P. larvae* genome holds **3.9 intact prophages** and **11.4 defective prophages** (15.3 prophages in total).
- Intact prophages have **several *att* sites to integrate** host genome and **some are repeated** (in 9 of these *att* sites (Table 1. B) we found 22 prophages).
- The **disrupted genes may interfere with host function.**
- The high number of **transposases can be responsible** for prophage and host **genomes rearrangements**.
- Genes involved in **host virulence and fitness were found in the prophages**.

References

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