Can integrated prophages affect virulence and fitness of the honeybee pathogen *Paenibacillus larvae?*

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INTRODUCTION

Paenibacillus larvae **is a spore-forming Gram-positive bacterium, with five distinct genotypes (ERIC I-V), that causes one of the most destructive bacterial honeybee brood diseases – American Foulbrood (AFB)[1]. Temperate phages (prophages) play an important role in the evolution of bacterial populations across all ecosystems, often providing new genes and host genome rearrangements[2,3,4]. They are, thus, able to alter bacterial phenotypic traits, at fitness and virulence level, or give host protection through superinfection[3]. So far, the impact of prophages on *P. larvae* ecology has not been evaluated. Our main goal was to understand the potential role or impact of prophages on fitness and virulence of *P. larvae*.**

METHODS

Data collection

GenBank (accessed April 2020)
- *P. larvae* genomes (n=14)
  - n=11 unique sequences
  - n=3 sequences excluded
    (2: high no. of contigs; 1: repeated)

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

Accession no. for bacterial genomes
PHASTER output: Intact or defective (incomplete and questionable) prophages

Manual curing:
- Presence of lysin (endolysin or other)
- Structural CDSs enough
- Assembly CDSs (small and large terminase)

BLASTp default and tailed phages (Tax id: 28883)

CD-Search Tool: E-value cut-off of 1×10^{-5}

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

Adapted COG (Cluster of Orthologous Groups) analysis
- Proteins grouped according to the function

ResFinder 4.1

RGI Resistance Gene Identifier

RESULTS

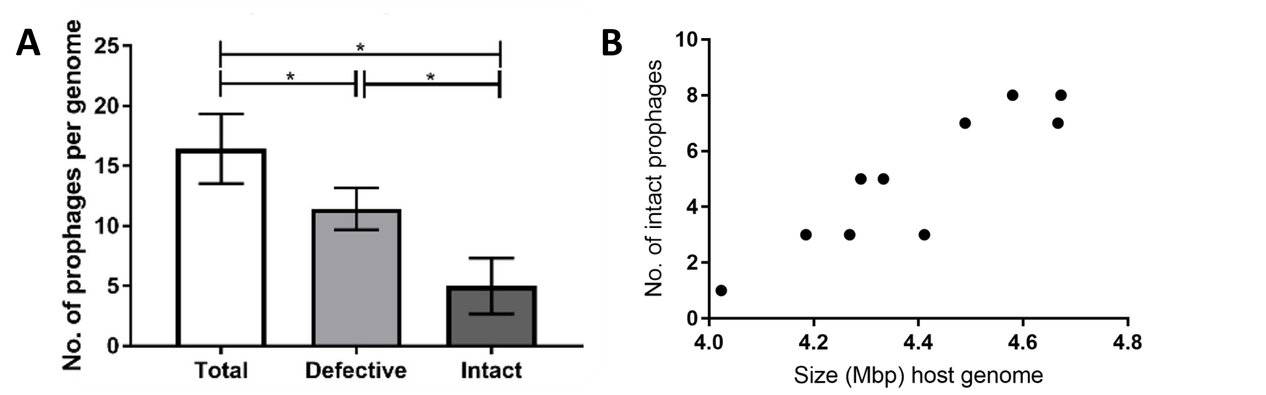
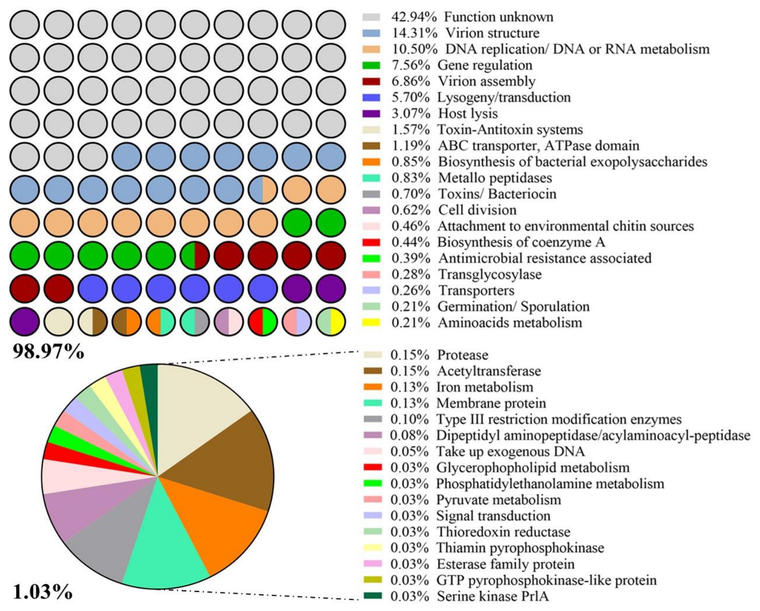


Figure 1. (A) Total, defective and intact prophages/ host genome. **(B)** No. of intact prophages/ size of host genome.

- Each *P. larvae* genome held **5.0±2.3 intact** and **11.5±1.8 defective** prophages;
- The prophage frequency of occupation was **5.8±2.5%**.
- *P. larvae* with larger genomes harboured a higher number of intact prophages.



- 3876 prophage proteins.
- **36 functional categories**. 95% of proteins ≥ 1 homologous sequence with tailed phages.
- **43%** proteins- unknown function.
- **Lysogeny/transduction** group is **5.7%** of the proteins, **transposase** (n= 112) was the most often identified CDS.
- **TA systems:** virulence and fitness-related category with **the highest % -> 1.6%.**

Figure 2. List of COG categories. % of prophage-derived CDS with a given function/ group.

- **ResFinder / RGI – no functional antimicrobial resistance genes.** Yet, TetR family transcriptional regulator, metallo-β-lactamase and β-lactamase inhibitory proteins were identified.
- **CDS provided by prophages only found in:**

Genotype	No. of unique CDS found in intact prophage - e.g. of CDS
ERIC I	21 CDS – Efflux transporter, bacteriocin-like closticin, DNA internalization protein ComEC/Rec2, enhancin-like protein, histidine kinase-like protein, pyruvate dehydrogenase E1
ERIC II	5 CDS – Antitoxin SocA, FtsX-like permease, MazG-like nucleotide pyrophosphohydrolase, outer spore coat protein (CotE) and DNA mismatch repair protein MutS
ERIC III	2 CDS – DNA binding YncE and phosphatidylglycerophosphatase
ERIC IV	2 CDS – Iron–sulfur (Fe-S) uptake (SufB) and nitrogen fixation (NifU)
ERIC V	7 CDS – Aromatic acid exporter family, leukocidin subunit LukF-PV precursor, toxins (2), membrane protein, chitin-binding protein GbpA and thioredoxin reductase

CONCLUSIONS

- 30.4% of detected prophages were intact.
- The high number of proteins associated with transport and exchange of genomic DNA fragments **can be responsible** for prophage and host **genomes rearrangements**.
- Some CDS were widely distributed across **all genotypes**, e.g.: HicB and MazE antitoxins, YopX protein.
- For each function associated with virulence and fitness, the percentage of trait was **less than 2%** in the COG analysis.
- Only **ERIC V** strains appear to have a competitive advantage since prophages contained **multiple CDS** that could contribute to **a more aggressive infection** (LukF-PV and GbpA).

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