The *Polymyxa* genome, a new tool for understanding of their role as virus vector

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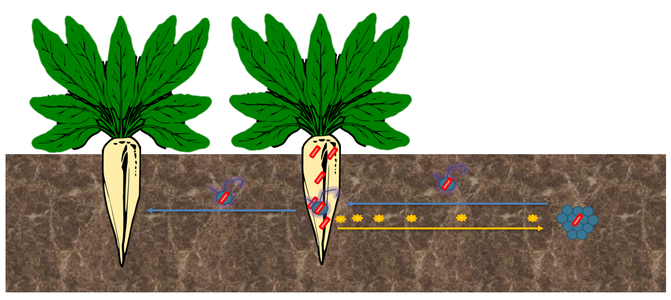


Figure 1. Virus transmission to sugar beet by *Polymyxa betae.*

Introduction

The *Beet necrotic yellow vein virus (*BNYVV) is the causal agent of the rhizomania disease (figure 1). This virus is considered a major concern for sugar beet breeding and production. It is often found along with two pomoviruses, *Beet soil-borne virus* (BSBV) and *Beet virus Q* (BVQ), in the same diseased roots. Those viruses share the same vector, *Polymyxa betae,* a soil-borne protist belonging to the *Plasmodiophorida* (figure 2). Unlike some insect-transmitted phytoviruses, very few control methods based on interference with *P. betae* transmission are available (Bragard et al., 2013). Molecular determinants of the *Plasmodiophorid* mediated virus transmission have been studied from the virus side (BNYVV and BSBMV on sugar beet and PMTV on potato) through mutant and reverse genetic approaches (D'Alonzo et al., 2012; Reavy et al., 1998; Rahim et al., 2007; Tamada et al., 1996). However, the transmission mechanism remains a complete mystery from the vector side. One reason could be found in their soil-borne, intracellular, obligate biotrophic nature, which makes in depth molecular studies particularly challenging.

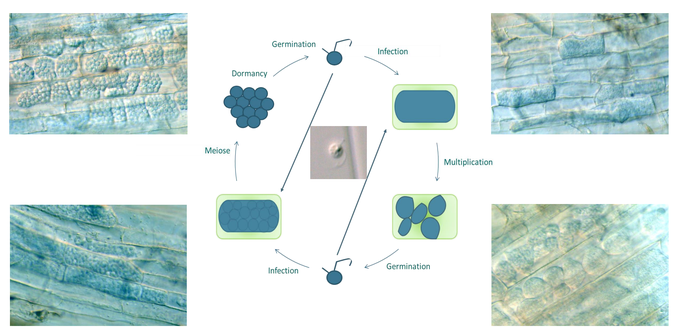
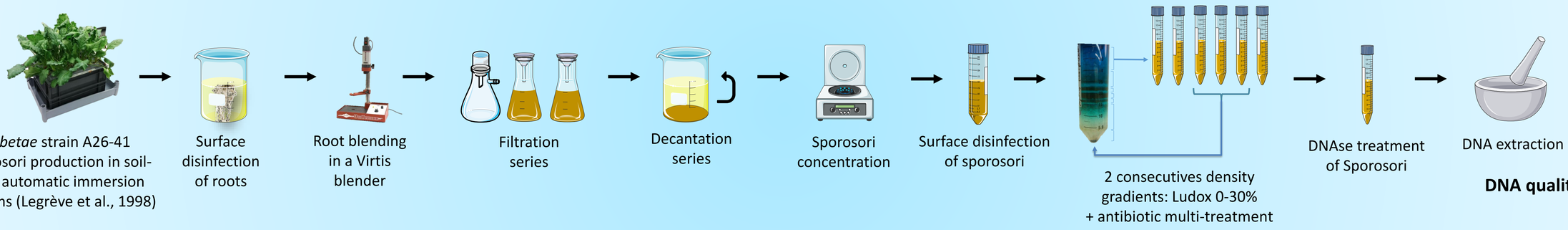


Figure 2. The life cycle of *Polymyxa betae.*

Objectives

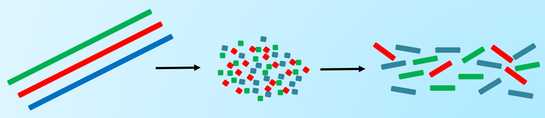
The first goal of this project was to release the first full genome sequence of *Polymyxa betae,* in order to provide new insights in plamodiophorid related research. This new tool will then be used to study *Polymyxa*-virus interactions from the vector side through comparative genomic and transcriptomic approaches.

Sporosori production and purification



Whole genome sequencing

Shotgun sequencing and assembly



A TruSeq Nano DNA library was prepared from a sporosori DNA sample and paired-end sequenced (2x250 bp) using an Illumina MiSeq instrument. Adapter trimming was performed with the Trimmomatic package. Quality trimming (figure 3) and sequences assembly were performed using the CLC Genomics Workbench. The high number of resulting contigs can be explained by a significant sequence contamination.

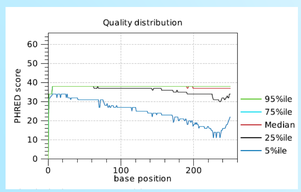


Figure 3. Mean sequencing quality score distribution relative to base position.

➔ **Results**:
Sequencing yield = 12292 Mb
12450 contigs assembled (≥ 1kbp)
Total length = 66.7 Mbp
N50 = 28,325 bp

Polymyxa betae contigs extraction

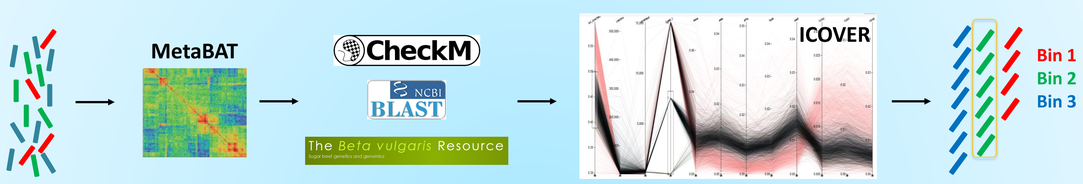


Figure 4. Metagnomic binning workflow used to isolate *P. betae* contigs from DNA contamiations.

A metagenomic approach was performed to isolate *P. betae* contigs from unavoidable contaminations (figure 4). Assembled contigs were binned using MetaBAT and resulting bins were assessed for genome completeness, contamination and assigned bin higher-level taxonomy with CheckM. This strategy, along with further BLAST annotation, allowed us to remove bacterial and viral sequences. In addition sugar beet leaf DNA reads were then mapped to the remaining contigs in order to detect and remove plant contaminations. Putative *P. betae* contigs were eventually selected based on coverage, tetranucleotide composition, GC content and coding density, with the help of the ICoVeR tool.

➔ **Results**
1001 contigs selected
Total length = 27,086 Mbp
N50 = 61 339
Min = 1513 ; Max = 531 965
Coverage = ~225X

Genome completeness evaluation

Genome completeness evaluation with CEGMA revealed that 91% of the 248 eukaryotic core genes were present in the assembly (figure 5). Similarly, a complete genome size of 29,354 Mbp was estimated using the 17-mer coverage distribution inferred with Jellyfish, and reads mapping to the final *P. betae* assembly. Genome completeness evaluation based on the ratio of the cumulated assembly length to the total estimated genome size equaled around 92%, in agreement with the CEGMA evaluation.

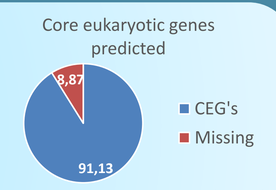


Figure 5. CEGMA genes % (CEG's) found in our draft genome.

What's next ?

Genome annotation

A first RNA-Seq assisted gene prediction was performed. To this end, total RNA was extracted from heavily infected sugar beet roots and sequenced. A TopHat read alignment was used as an input for gene prediction with the Braker1 pipeline. As shown in table 1, our *P. betae* draft genome shares main genomic features with other plasmodiophorid genomes available. The use of additional genomic and/or transcriptomic data will allow us to refine our draft genome. Functional annotation will then be performed on the new reference genome.

Table 1. Comparison of our *P. betae* draft genome with *Plasmodiophora brassicae* and *Spongospora subterranea* available genomes.

Features	*Pl. brassicae*	*P. betae*	*S. subterranea*
N° of contigs	165	**1001**	2340
N50	473 Kb	**61 Kb**	28 Kb
Assembly length	24 Mb	**27,09 Mb**	28,08 Mb
GC content	58,5 %	**45,1 %**	45,7 %
Number of genes	9 730	**~10000**	10778
Coding density	66 %	**~70%**	/
% repeats	~5 %	**~6%**	/

Polymyxa-virus interactions

An new functional soil-free virus-vector-host model developed in the lab (M. Mahillon's *et al.,* this congress) will be valued to study the molecular interactions between viruses and *P. betae* during its life cycle through a transcriptomic approach. The experimental set-up is presented below.

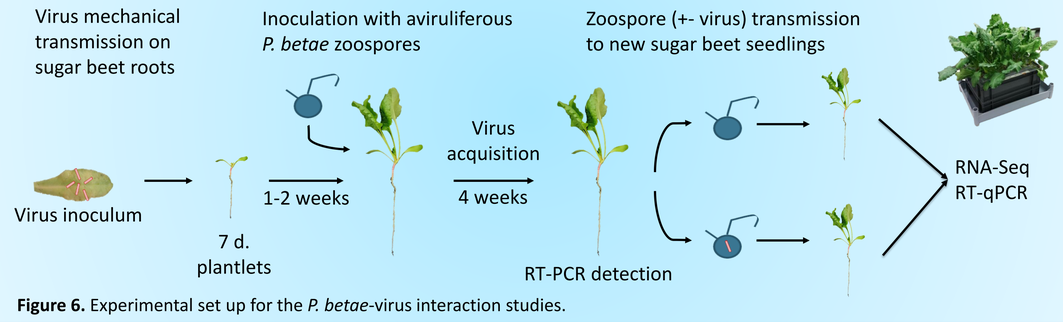


Figure 6. Experimental set up for the *P. betae*-virus interaction studies.

Conclusions

The first draft genome sequence of *Polymyxa betae* is now available (Decroës *et al.,* 2019). This significant resource provides new insights into the virus-vector interactions study. It also brings unique perspectives into *Polymyxa*-host research and opens the way for *P. graminis* sequencing.

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