Nanopore sequencing for the detection of plant pathogens: Longer reads, accurate results

Venbrux Marc[1*], Sam Crauwels[1, 2], Hans Rediers[1, 2]

(1) Laboratory for Process Microbial Ecology and Bioinspirational Management. Department of Microbial and Molecular Systems, KU Leuven, B-3001, Leuven, Belgium;
(2) KU Leuven Plant Institute, B-3001, Leuven, Belgium; * Presenting author

INTRODUCTION

Plant pathogens

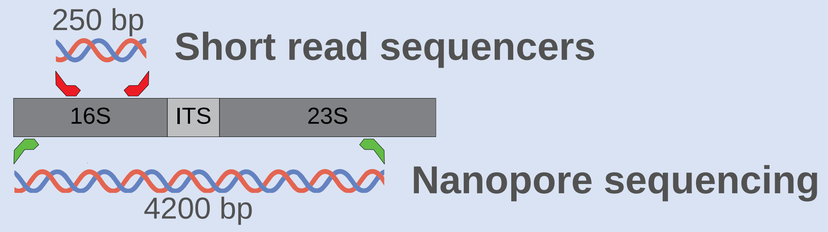
Plant pathogens can cause up to **40% loss** in crop yield, accounting for an annual economic loss of **220 billion US $**

Timely detection of plant pathogens is **crucial** in **integrated pest management**

Sensitive diagnostic tools for simultaneous detection of bacterial and fungal plant pathogens are still lacking

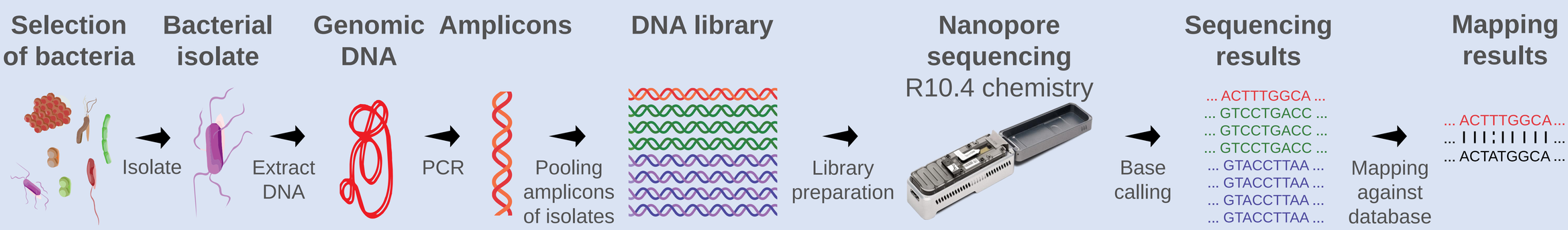
OBJECTIVE

Evaluate **Nanopore sequencing** for the **multiplex detection** of bacterial and fungal **plant pathogens** using an **amplicon sequencing approach,** capitalizing on the platform's ability to generate **ultra–long reads**



Hypothesis: Amplification of a larger part (16S-ITS-23S) of the ribosomal RNA operon provides an increased taxonomic resolution compared to conventional short read sequences

METHOD



RESULTS

The choice of reference database considerably affects taxonomic assignments of the reads.

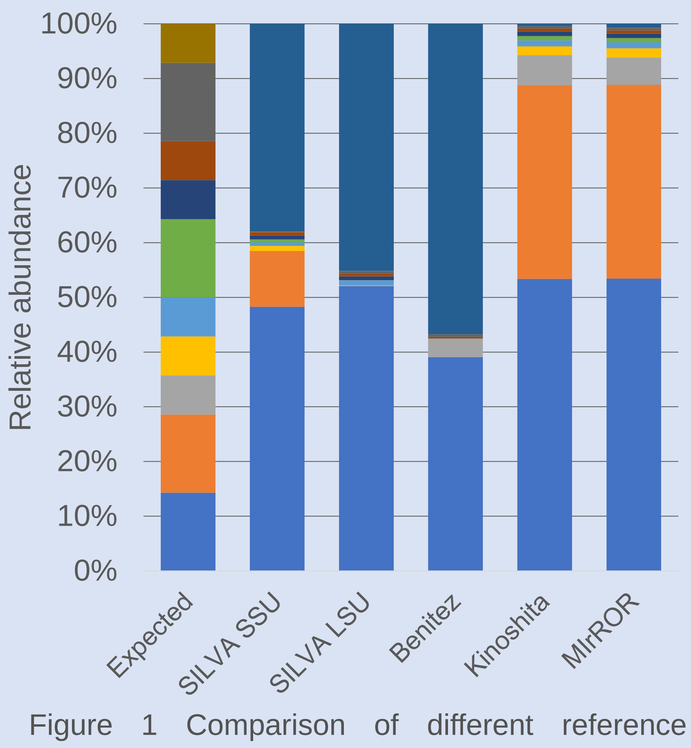


Figure 1 Comparison of different reference databases regarding taxonomic identification of reads obtained after Nanopore sequencing. The figure shows relative abundances of bacterial species assigned using 16S (SILVA SSU), 23S (SILVA LSU), and 16S-ITS-23S (Benítez, Kinoshita and MIrROR) reference databases.

Read length analysis illustrates the role of size selection, even though the DNA library consisted of equally sized amplicons. (A) Size selection *in vitro* before sequencing increases the yield of useful sequences for taxonomic assignment. (B) Size filtering the reads *in silico* reduces erroneous taxonomic assignments.

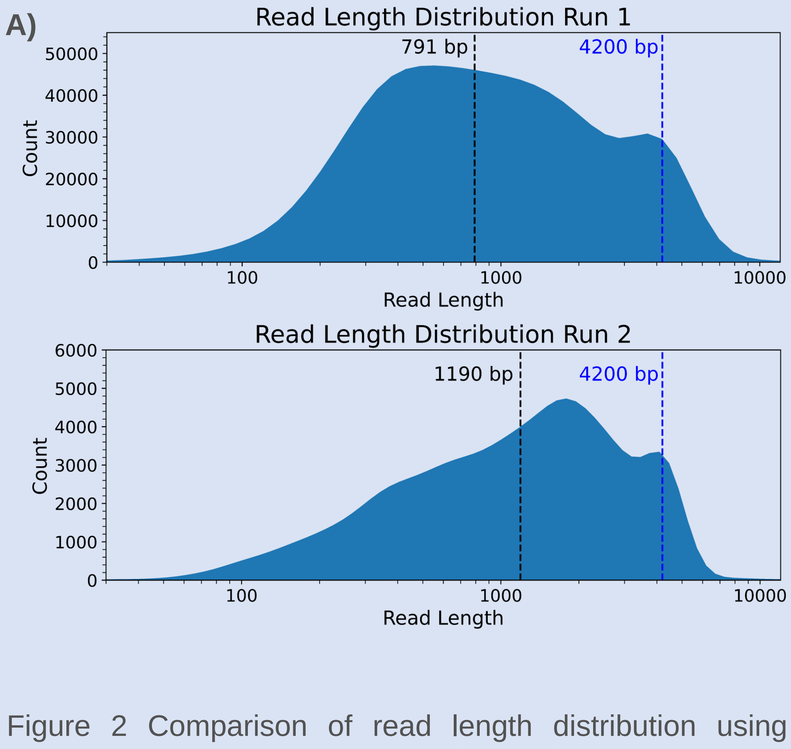


Figure 2 Comparison of read length distribution using different size selection protocols during library preparation. Run 1 used short fragment buffer, retaining DNA from all sizes, whilst run 2 used long fragment buffer, retaining large DNA fragments (>3000 bp). Black dotted line represents median read length, while blue dotted line indicates the expected amplicon size.

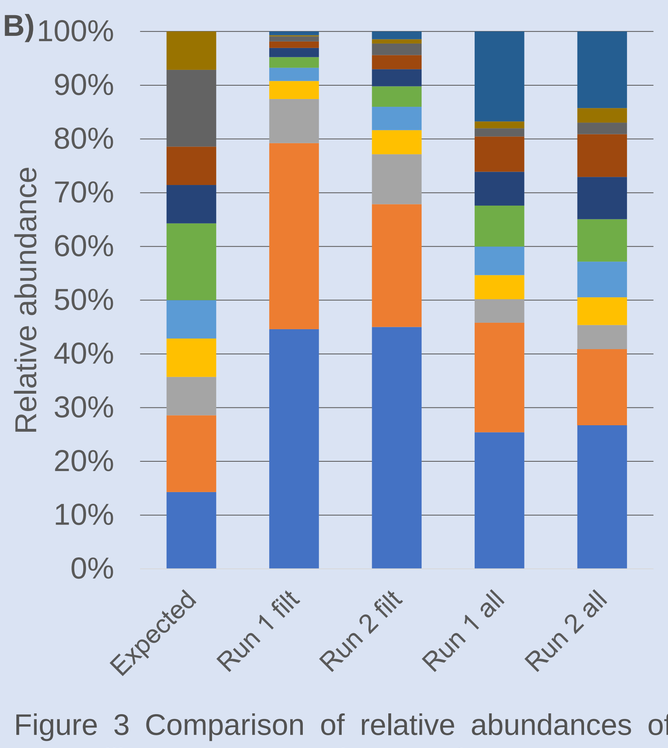


Figure 3 Comparison of relative abundances of two library preparations after Nanopore sequencing (run 1 or 2) of size filtered sequences *(in silico)* between 3500-5000 bp (filt) and unfiltered (all) sequences. The reference database used was MIrROR.

Dickeya dadantii *Acetobacter cerevisiae* *Pseudomonas synxantha* *Pseudomonas graminis* *Pseudomonas putida* *Agrobacterium biovar 1*
Erwinia billingiae *Pectobacterium atrosepticum* *Escherichia coli* *Staphylococcus aureus* Wrongly assigned

CONCLUSION

- The use of **larger than conventional genetic markers** results in an **increased taxonomic resolution**, which can accurately determine taxonomic identities up to the **species level in bacteria**.
- Similar **species level identifications** were obtained for **fungi** (data not shown).
- The **yield of full-length sequences** can be **increased** with further **optimization of library preparation** protocols.

This work was supported by KU Leuven internal funding (C1-IHO-D6451, C14/19/074 and STG/20/027), and campus GroupT funding.
Contact: marc.venbrux@kuleuven.be