A rapid shotgun metagenome protocol based on Oxford Nanopore Technology applied to soil biodiversity analysis

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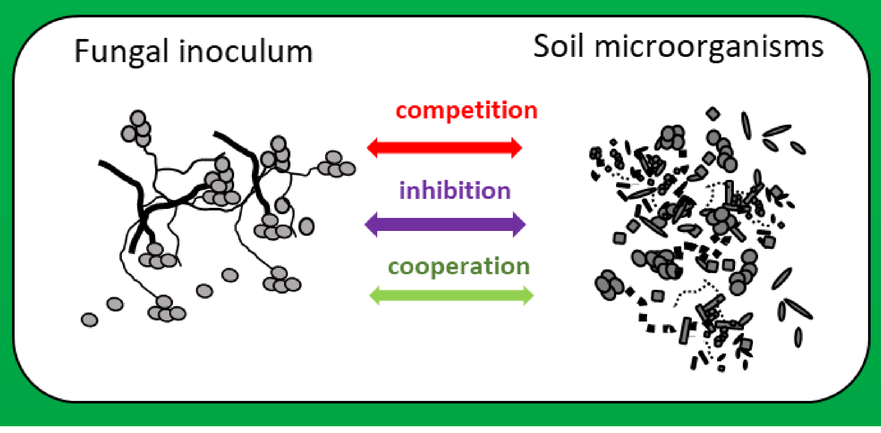
Introduction

Beneficial microorganisms are increasingly used in modern agriculture with significant benefits on yields and sustainability of the agricultural system. However, the massive introduction of non-indigenous bacterial and fungal species in the form of biofertilizers and bioinoculants can result in their uncontrolled spread with undesirable effects on the ecology of agricultural soils and nearby environments.



Aims

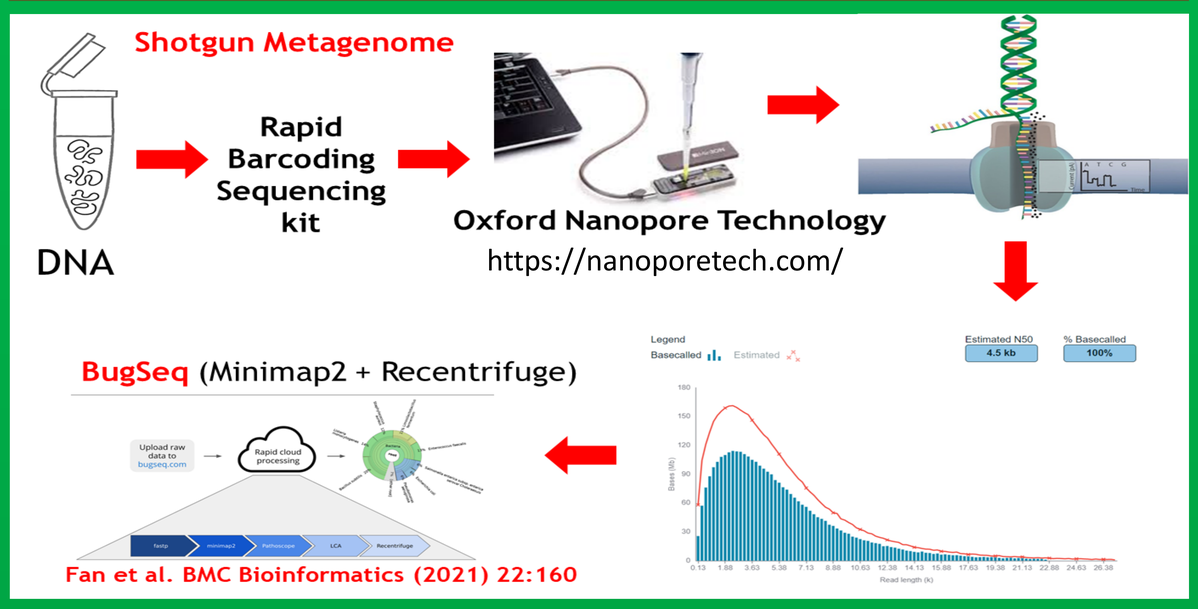
This study aims to develop a protocol for monitoring soil biodiversity after applying bioinoculants with beneficial action. The basis of the experimentation is a third-generation DNA sequencer (Oxford Nanopore Technology, ONT) and a shotgun metagenome sequencing of the microbial community (technique that relies on massive sequencing of total DNA extractable from the soil).

Methodology

Soils cultivated with industrial tomatoes and treated with a commercial *Trichoderma*-based bioinoculum were compared to the untreated control system. A platform developed by Oxford Nanopore was used for sequencing. Total soil DNA was extracted by using a protocol based on Qiagen Powerlyzer® soil extraction kit, followed by solid-phase reversible immobilisation (SPRI) beads size selection with a 0.70X ratio to select long DNA fragments (Cummings et al., 2017).

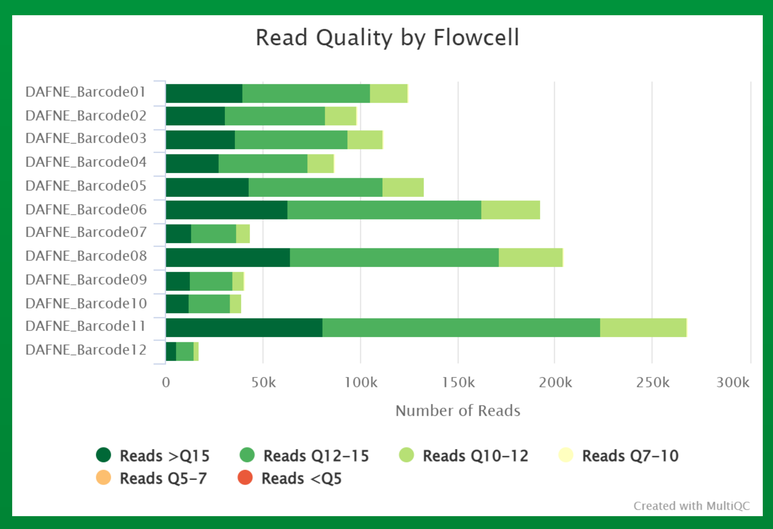
The sequencer used was an Oxford Nanopore GridION with R9.4.1 type flow cells and a rapid library preparation method based on the chemistry of the ONT SQK-RBK004 kit. The MinKNOW™ software was used to check the number of active pores available in the R9 flow cells.

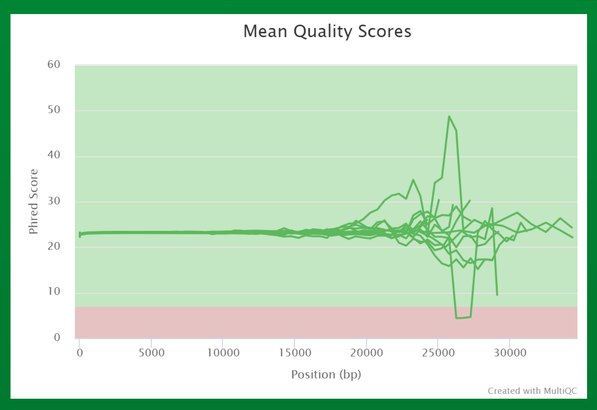
The Fast5.tmp files, obtained after sequencing, were converted to Fast5 files via the command prompt, base called using Albacore (Oxford Nanopore Technologies) and demultiplexed with EPI2ME Desktop Agent (Oxford Nanopore Technologies Metrichor).

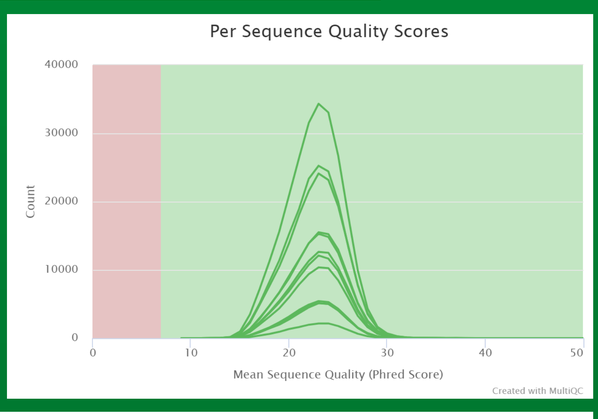


Results

With ONT sequencing, a single molecule of DNA can be "read" without the need for PCR amplification or chemical labelling of the sample. The sequence of nucleic acids is inferred from changes in the ionic current across a membrane as a single DNA molecule passes through a protein nanopore. Such sequencing technology is of interest for studying soil communities because the it allows sequencing of longer DNA fragments compared to Illumina platforms. The ONT sequencing is particularly suitable for tracing and quantifying single species of fungi or other organisms in complex samples as it allows matching with more comprehensive diagnostic markers, either found in public databases or appropriately selected for diagnostic purposes.







References

- Cummings, P. J., Olszewicz, J. & Obom, K. M. Nanopore DNA Sequencing for Metagenomic Soil Analysis. *J. Vis. Exp.* 8 (2017).
- Fan, J., Huang, S. & Chorlton, S. D. BugSeq: a highly accurate cloud platform for long-read metagenomic analyses. *BMC Bioinformatics* **22**, 160 (2021).

Acknowledgements

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