Examining the risk of disease outbreaks in a diseased population using ash as a model

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INTRODUCTION

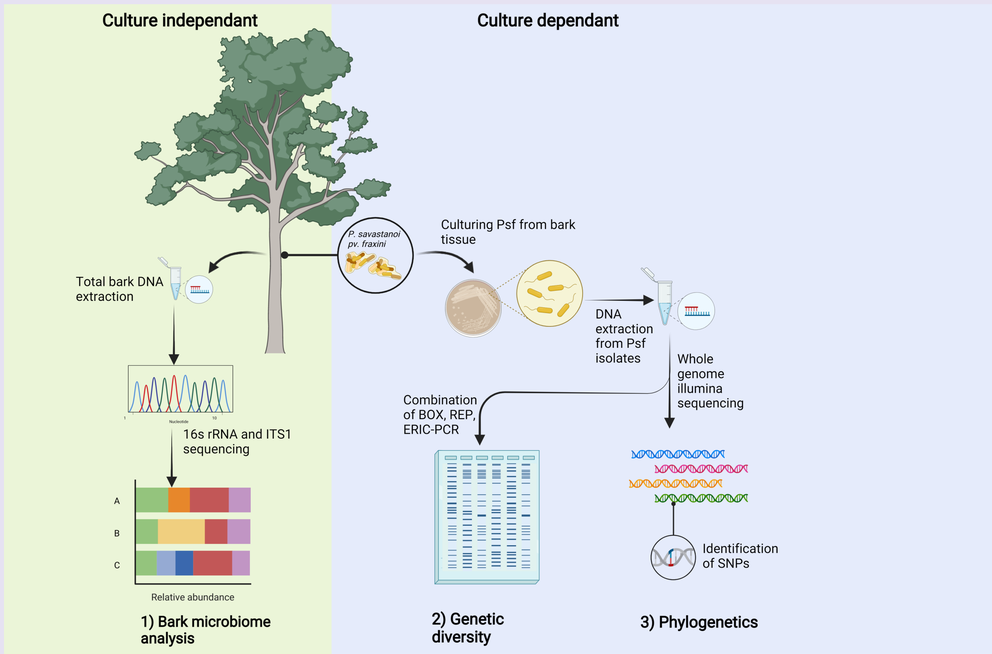
The bacterium *Pseudomonas savastanoi pv. fraxini* causes cankers on the trunk and stems of ash trees (Fraxinus exclesior). The disease has been present in the UK for decades and is widespread[2]. Little is known about the bacterium's disease cycle, its virulence factors, or epidemiology, so this project aims to develop high-throughput tools for the isolation and identification of this pathogen for further study. With the declining health of ash trees due to ash dieback there is the potential of an increase in virulence.

Ash trees are the second most common broadleaf species in the UK, forming a dominant part of woodlands, urban landscapes and hedgerows. They also provide a habitat for over 953 species, 44 of which are obligate species[1]. Ash are resilient to drought and are predicted to survive well under future conditions caused by climatic change[2]. However, populations globally are facing high rates of mortality due to invasive species, namely the ash dieback fungus, Hymenoscyphus fraxineus, and the emerald ash borer beetle. The future of ash populations relies on breeding programmes and the planting of genetically diverse woodlands.

AIMS

1) Understanding the genetic diversity of *Pseudomonas savastanoi pv. fraxini* in the UK
2) Identify key virulence genes in the Psf genome to understand its pathogenicity
3) Assess whether bacterial infection affects the bark microbiome

METHODOLOGY



1) The impact of bacterial disease on the bark microbiome will be assessed by sequencing the 16s rRNA and ITS1 regions from DNA extracted from bark samples. ASVs will be calculated and taxonomic alignments will be performed using SILVA and UNITE. Alpha and beta diversity will be calculated and compared between healthy and diseased trees.

2) The genetic diversity of Pseudomonas savastanoi pv fraxini will be investigated using REP-PCR. REP-PCR amplifies repetitive regions in the bacterial genome, resulting in a fingerprinting profile. Genetic diversity will be evaluated by the quantification of different fingerprints.

3) A phylogenetic analysis of Psf isolates from the UK will be perfomed by conducting core genome alignments. SNPs will be identifed to produce a phylogenetic tree.

STUDY DESIGN

1) Six sites across a broad geographical range have been chosen. Two are planted provenance trials, owned by Forest Research, four are semi-natural ancient woodlands.

2) Six diseased and six healthy trees will be sampled. From diseased trees, 3 bark samples will be taken directly from the lesion and 3 will be taken from healthy tissue. From healthy trees, 3 bark samples will be taken from healthy tissue.

3) Outer bark tissue sampled. Epiphytic populations will be isolated using a PBS wash. Endophytic populations will be isolated by plating sterile tissue onto agar plates

4) Psf-like colonies will be isolated and identified using species specific PCR primers.

FUTURE WORK

1) Ash sapling glasshouse experiment: ash saplings will be inoculated with Psf isolates from sampling to confirm Koch's postulate and identify variations in virulence.

2) Transposon mutagenesis of Psf will be conducted to identify key virulence genes associated with pathogenicity.

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