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Next-generation DNA sequencing shows a microbiota shift after an incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) on a single kiwifruit orchard

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A virulent strain of *Pseudomonas syringae* pv. *actinidiae* (Psa) is a major pathogen for New Zealand's \$3B kiwifruit (*Actinidia* spp.) industry, and was first identified from a Te Puke orchard on 5 November 2010. Psa was first found on the Kerikeri research orchard (KRO) of Plant & Food Research on 19 September 2014. The samples for this study were collected from the same orchard on 7 December 2012 and 25 November 2014, i.e. before and after the Psa incursion. Polymerase chain reaction (PCR) was conducted on total genomic DNA from four leaf discs of 15 individual vines sampled from two kiwifruit cv. 'Hort16A' orchard blocks at KRO, using modified PCR primers complementary to bacterial 16S ribosomal DNA and the fungal inter-transcribed spacer (ITS) region. The microbiota present before and after the Psa incursion were investigated by Illumina MiSeq™ next-generation sequencing to produce 2 × 300 bp pair end reads, followed by metabarcoding analysis using QIIME2 software. Populations of fungi from the Basidiomycete orders Filobasidiales, Sporidiobolales, Tremellales and Leucosporidiales, and genera of bacteria with known biological control activity, such as *Erwinia*, *Pantoea*, *Methylobacterium*, *Sphingomonas* and *Paenibacillus*, increased in the presence of Psa.

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Testing new PCR primers and a TaqMan™ probe for detection of *Phlyctema vagabunda* syn. *Neofabraea alba*

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Phlyctema vagabunda syn. *Neofabraea alba* is a fungal pathogen that causes bull's eye rot (BER) of apples. Polymerase chain reaction (PCR) primers complementary to the inter-transcribed spacer region of ribosomal DNA (ITS) and the β -tubulin gene region, and a TaqMan™ probe assay were developed to detect this pathogen. These assays were compared in quantitative PCR (qPCR) reactions for amplification of DNA extracted from several fungal species and from apple tissue. Although the ITS and the β -tubulin primers amplified all *N. alba* isolates, both primers also amplified a few other fungal species. The TaqMan™ probe used with published primers for *N. alba* only amplified *N. alba* isolates. The TaqMan™ assay resulted in the lowest crossing threshold (Ct) values for DNA extracted from apple fruit, leaves, and spores collected on cellophane from eight apple orchards. The TaqMan™ results were correlated with percentage BER (%BER) in a 400-apple sample harvested from the same orchards. The TaqMan™ probe assay was the most sensitive and specific qPCR protocol tested, and Ct values showed the best correlation with %BER.