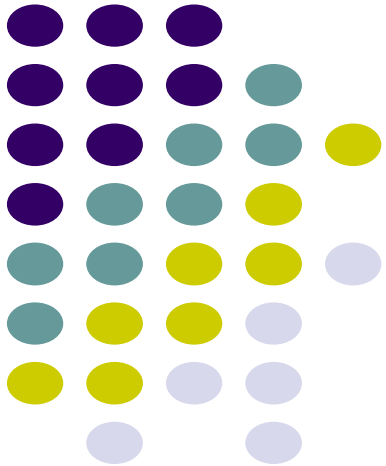
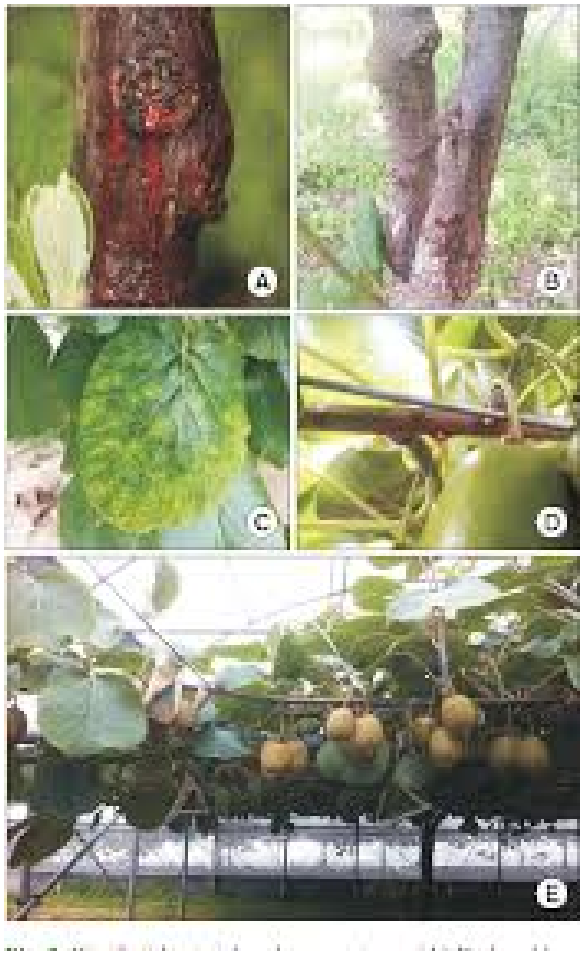
Diagnostic challenges for the detection of emerging pathogens



Case study:

- *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, is an emerging pathogen that has shown an increase in disease incidence and geographic range in New Zealand.
- Little had been published about the diagnostics, ecology and epidemiology of this pathogen prior to 2010.
- It was a challenge to diagnose the disease and stop its spreading



- The introduction of Psa into New Zealand underscored a number of challenges in the use of molecular diagnostics to detect and characterise exotic phytopathogenic bacteria.
- An ideal situation during a response to a new outbreak
  - the epidemiology of the pathogen are well understood reliable
  - specific diagnostic assays are available.
- At the time of the outbreak there were significant knowledge gaps in the biology of Psa and a lack of validated diagnostic tools for the high throughput detection of Psa in kiwifruit plant material.
- The timeframe to validate the identification of Psa was further hampered by the need to culture the causal agent and to conduct pathogenicity tests to demonstrate Koch’s postulates.

Methods for Identification of Psa

- A definitive diagnosis of Psa was obtained using a combination of biochemical, molecular and pathogenicity tests
- Colonies on nutrient agar plates as rounded, convex, glistening, translucent and creamy-white in colour but lacked a fluorescent pigment on King's medium B.
- Psa is gram-negative and exhibits characteristics of *P. syringae* LOPAT group 1a
  - i.e. levan positive, oxidase negative, potato soft rot negative, arginine dihydrolase negative and positive for tobacco hypersensitivity

- By the time of Psa outbreak in New Zealand, polymerase chain reaction (PCR) assays to identify Psa had been developed
- However, the primers used for Psa diagnosis was not Psa specific and in some situations amplified DNA from other pseudomonads found on kiwifruit as well
- Only sequencing of these amplified products could distinguish Psa from other leaf-spotting pseudomonads on kiwifruit

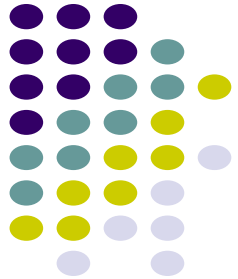
Large-Scale Testing of Psa

- DNA extraction and PCR testing protocols for the detection of Psa in large quantities of samples were not established during the New Zealand outbreak
- This required the immediate evaluation of DNA extraction methods and PCR techniques for high-throughput detection in kiwifruit plant material.
- PHEL (The public health and environmental laboratory) rapidly developed a SYBR Green qPCR assay using the primers Psa F1/R2 to detect Psa in leaf and pollen tissue. The use of the DNA-binding dye SYBR green for the detection of PCR amplicons allowed for the rapid conversion of conventional to qPCR and enabled the high-throughput detection of Psa in a response setting.

Genetic Diversity of Psa

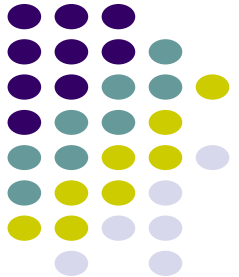
- All Psa strains isolated from Italian outbreaks shared the same rep-PCR fingerprint and MLST profile but were different to the strains previously isolated in Japan
- None of the strains from the Italian outbreaks possessed genes coding for the phytotoxins phaseolotoxin or coronatine, further differentiating these strains from those in Japan, Italy and Korea
- Analysis of the cts gene of these strains consistently detected two haplotypes that differed by two base pairs; Psa strains from Japan, Korea and the Italian 1994 outbreak belonged to one haplotype whereas the Italian strains isolated from the epidemics in 2008–2009 belonged to another haplotype
- These studies concluded that the epidemics in Italy during 2008–2009 appear to have been caused by a different Psa population than those previously recorded in Japan, South Korea and earlier Italian outbreaks.

Molecular Characterisation of Psa: Identifying the Outbreak Strain



- The identification of Psa as the causal agent of the kiwifruit disease was quickly obtained during the response investigation; however, the identification of the outbreak strain was more challenging.
- Genetic differences had been detected among overseas Psa strains and the questions was raised whether the New Zealand strain was similar to those strains causing an epidemic of bacterial canker in Italy

Role of Next-Generation Sequencing in establishing strain type



- The original outbreak strain was sequenced using Roche 454 GS Junior sequencing platform and within the first weeks of the outbreak a draft genome sequence was assembled in 3 days.
- It did allow analysis of effector and toxin genes known to be key virulence determinants in the *P. syringae* complex.
- This analysis of the draft genome sequence quickly validated the identification of the New Zealand Psa isolate and provided significant insights into possible strain type.
- The draft sequence enabled key effector and phytotoxin genes to be screened and revealed some commonalties with the New Zealand Psa strain and the more aggressive Italian strain.