



The New Zealand Institute for Plant & Food Research Limited

Plant & Food
RESEARCH

RANGAHAU AHUMĀRA KAI



Detection, Pathogenicity and Molecular typing

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Te Puke 03 March 2011

Part of a master plan

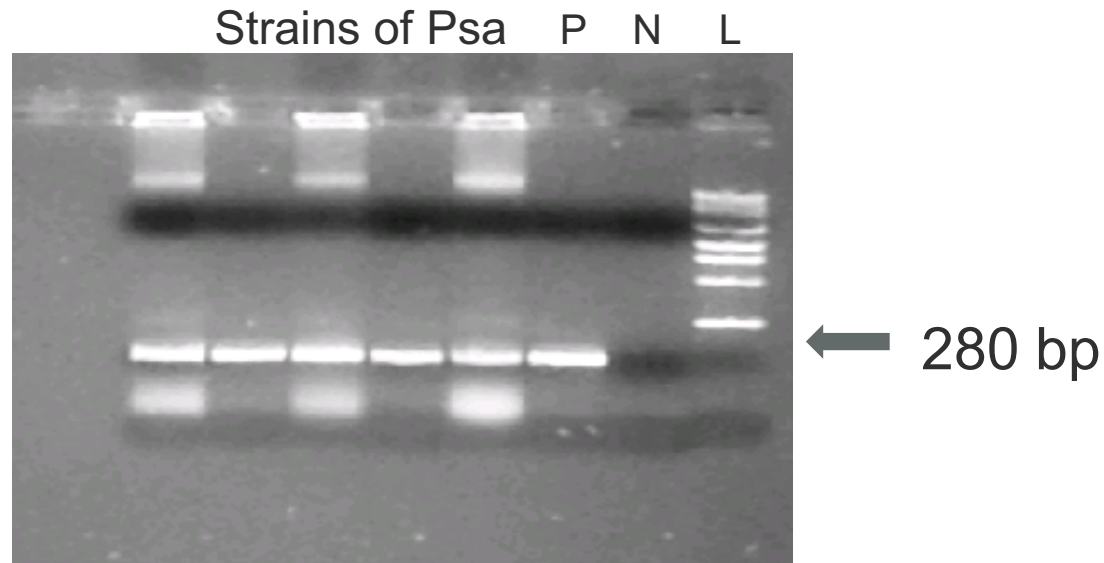
Since early 2009 when Psa detected in Italy, ZESPRI and PFR developed some research programmes to control Psa

Collaborations between Italy and New Zealand between ZESPRI, PFR and Bologna University

Most of our knowledge about Psa and the disease is coming from the work done over the last two years

Tools to study Psa had to be developed, including tools for detection identification and typing

Identification of *Pseudomonas syringae* pv *actinidiae* by PCR using the primers PsaF1/R2



All strains of Psa tested gave a 280 bp band

None of non *Pseudomonas* strains gave a 280 bp band

Only very few of the 25 different pathovars tested might give a 280bp, usually not thought of being associated with kiwifruit

Rees-George J, Vanneste JL *et al.* 2010 Plant Pathology 59:453-464

Vanneste et al. 2010 New Zealand Plant Protection 63:7-14

Limitations of detection by PCR

Specificity

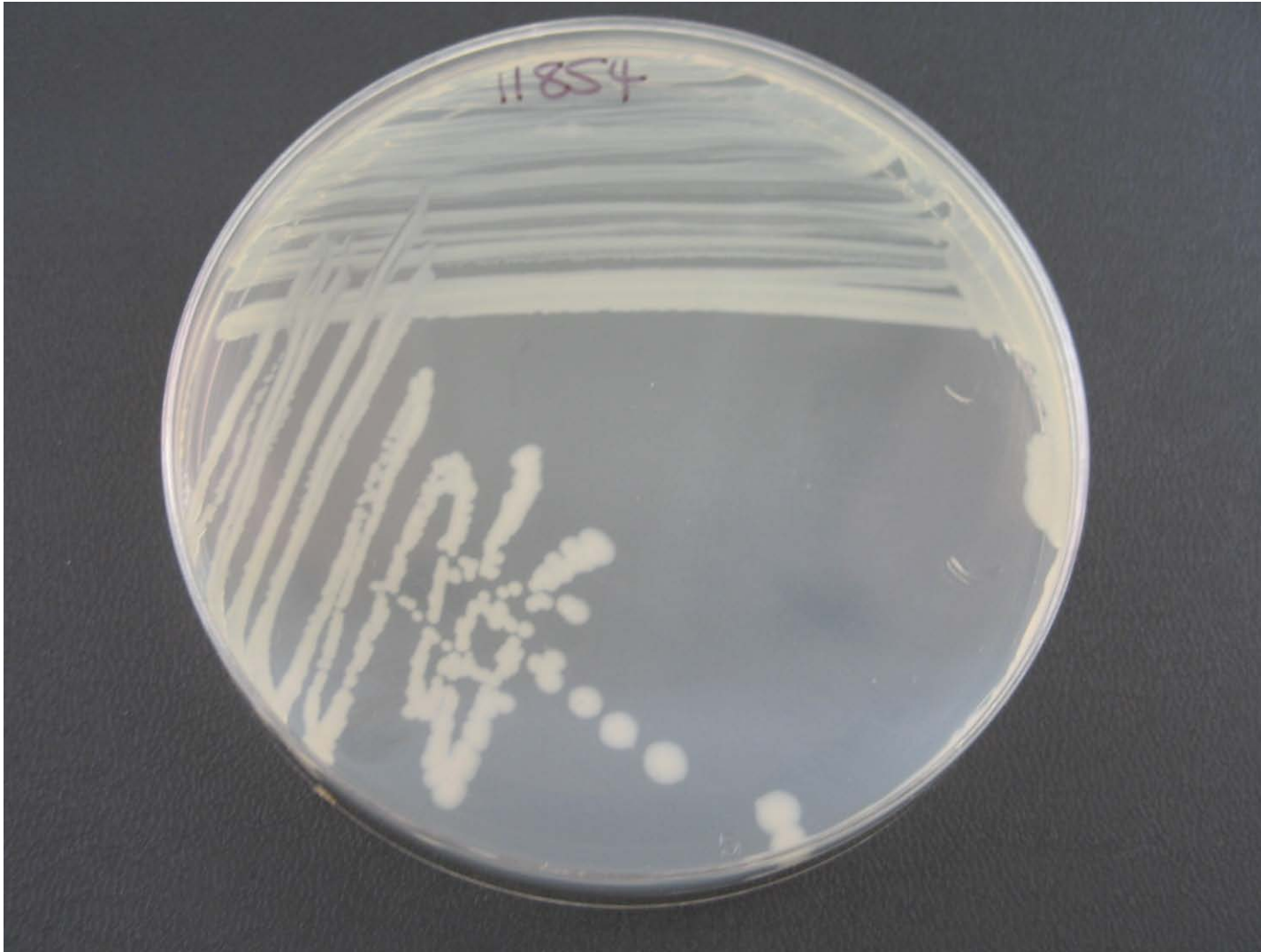
Sensitivity

Not quantitative

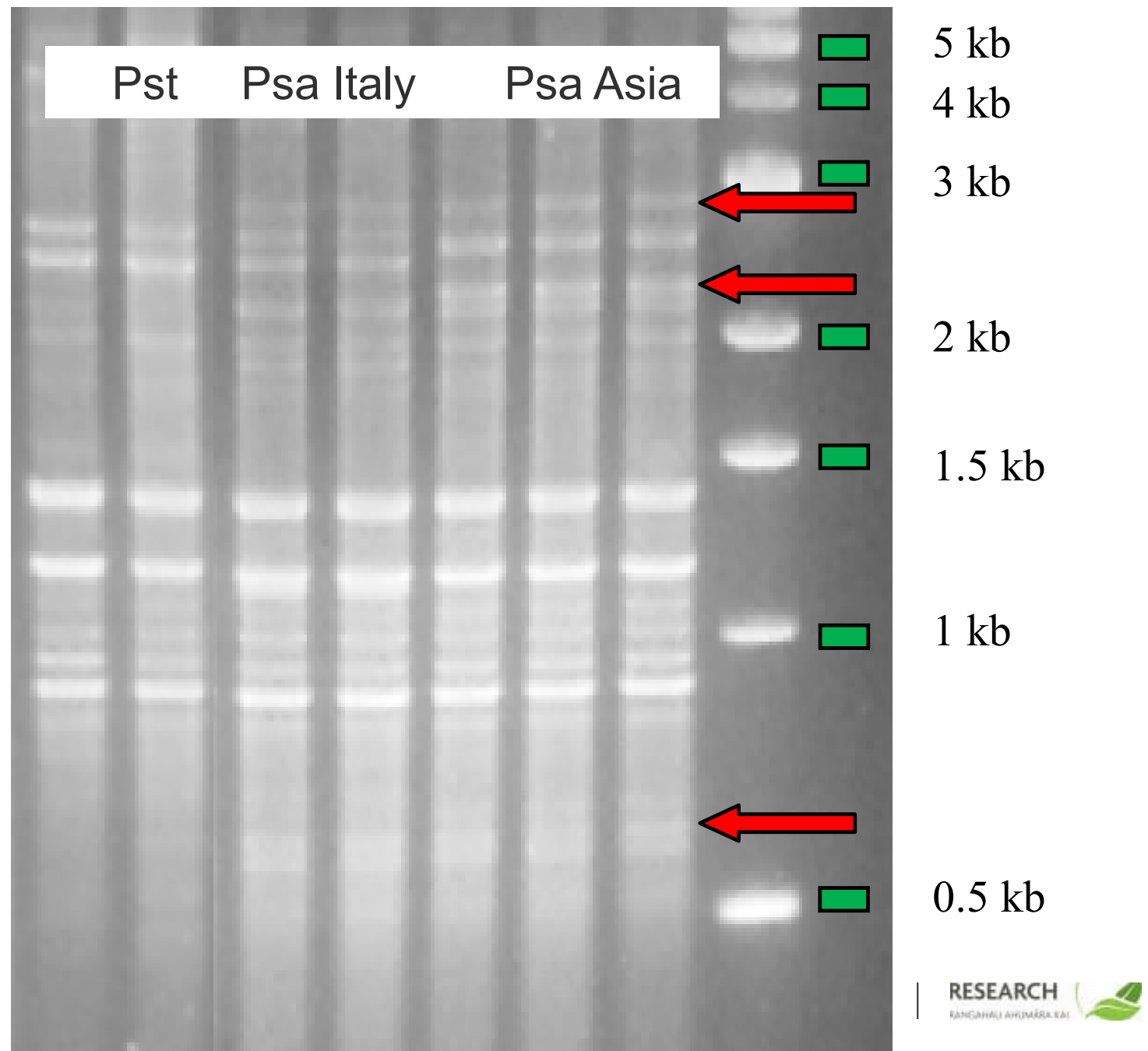
No difference between dead and live cells

No possibility to check or confirm the results if
done directly from tissues

Pseudomonas syringae pv *actinidiae*



Gel electrophoresis of BOX PCR



Typing of Psa

Strains of different geographic origin show different BOX PCR patterns

Correlation between BOX PCR patterns and DNA sequence of the *cts* gene

Different BOX PCR patterns are called haplotype of the *cts* gene

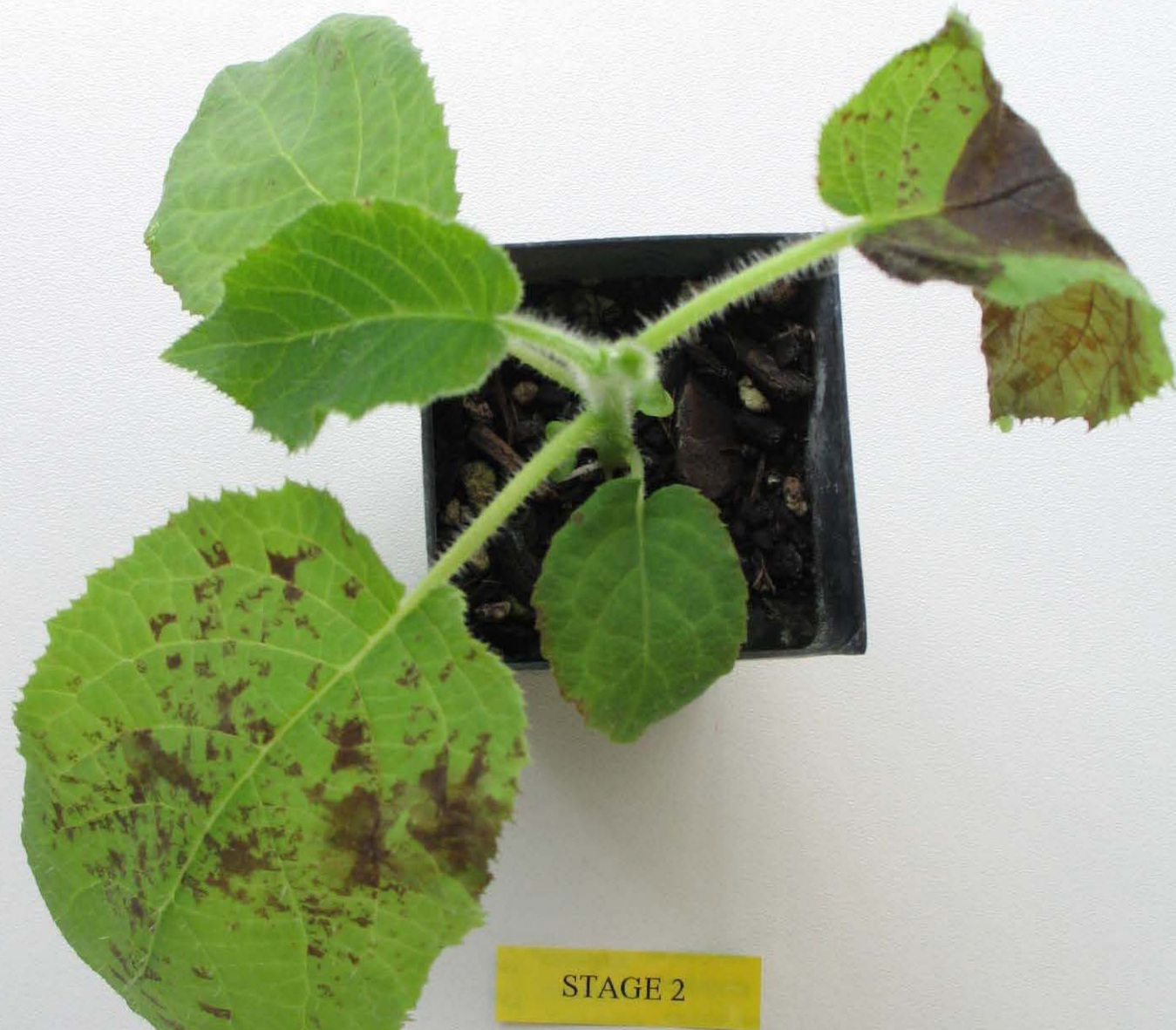
One haplotype was initially found in Italy

Different haplotypes seem to have different level of virulence

First symptoms on seedlings



Second stage of Psa symptoms on seedlings



Third stage of Psa symptoms on seedlings



STAGE 3



Fourth stage of Psa symptoms on seedlings



STAGE 4



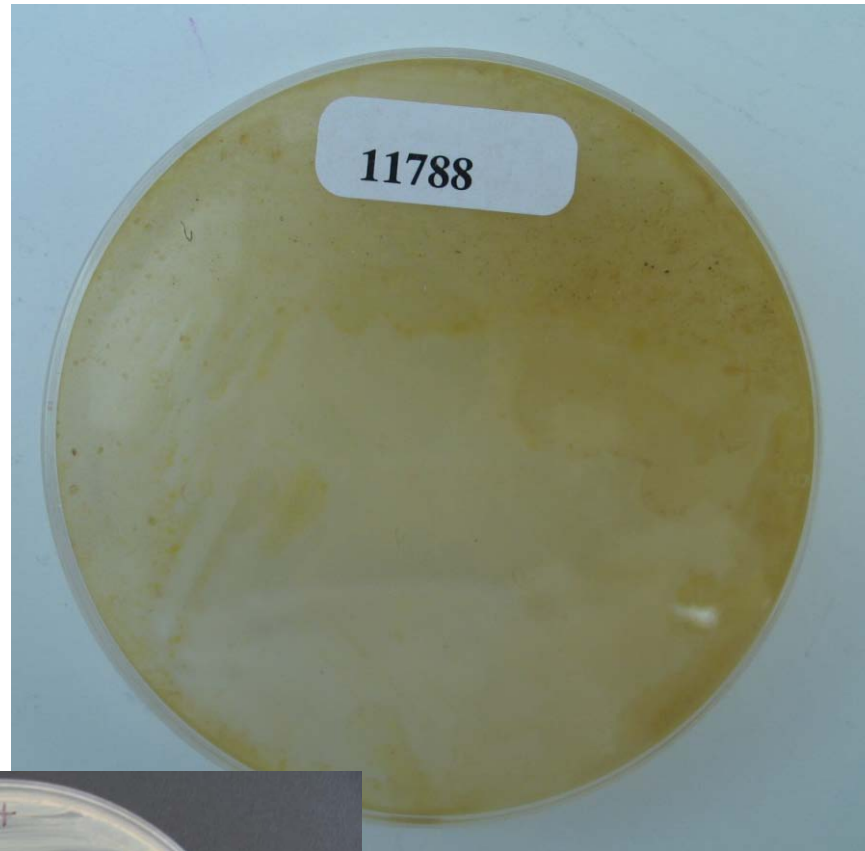
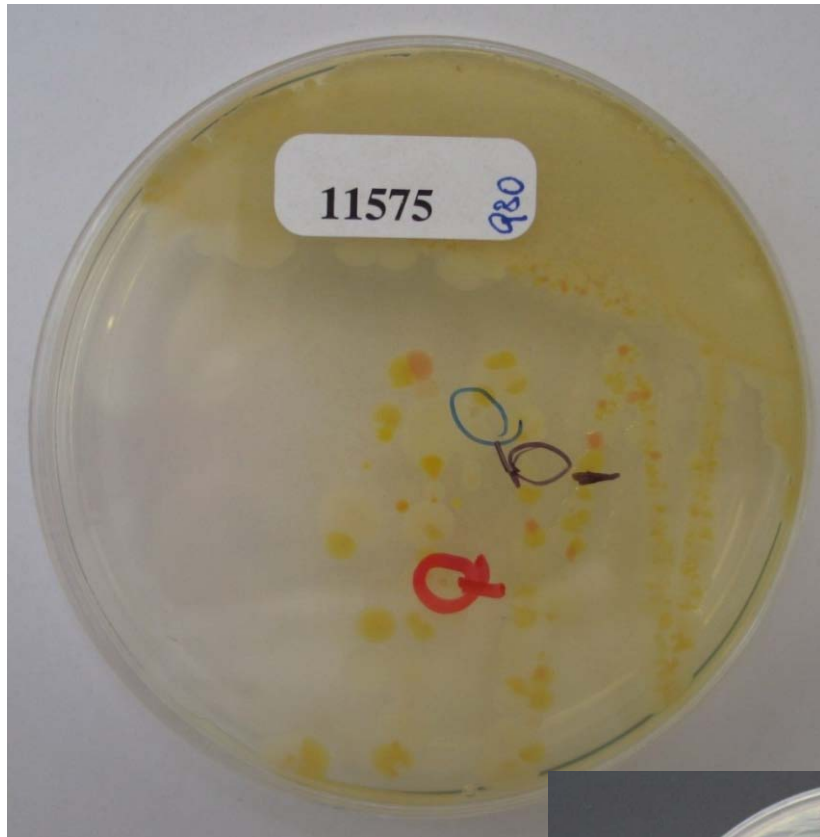
Different level of virulence of Psa



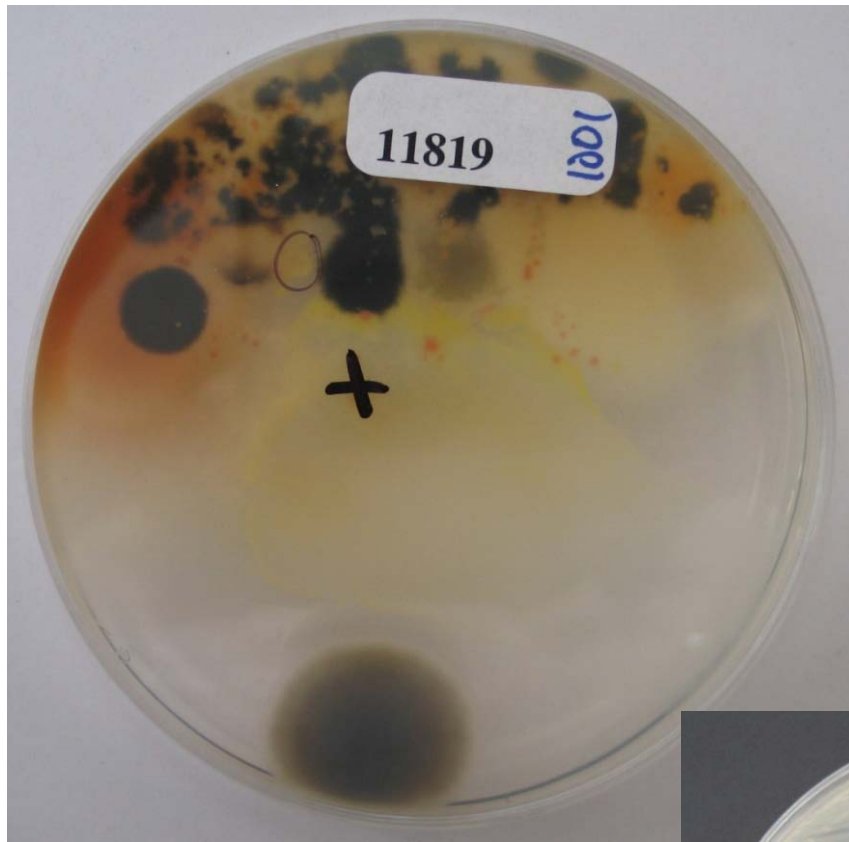
Pseudomonas syringae pv actinidiae



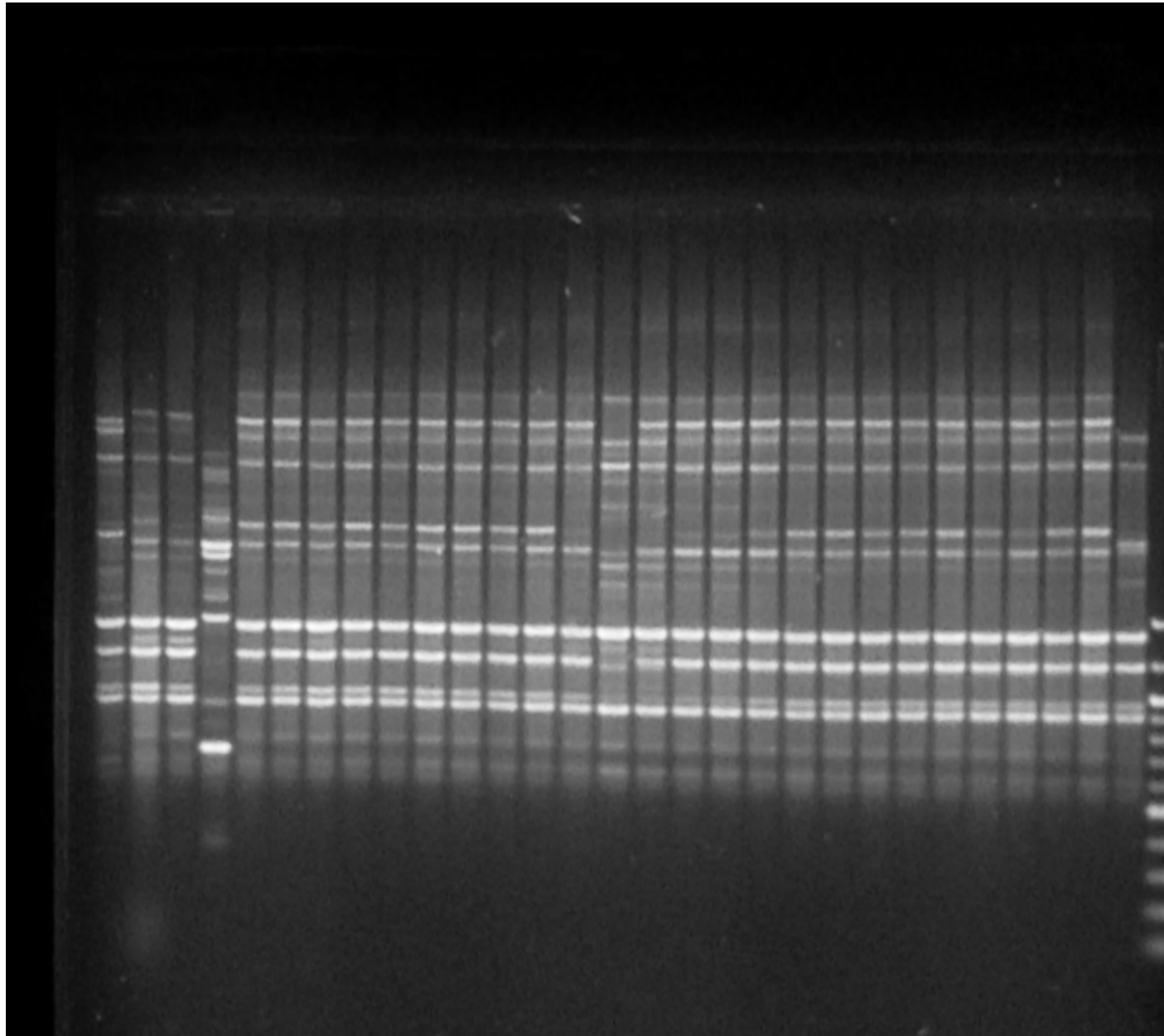
Where is Psa?



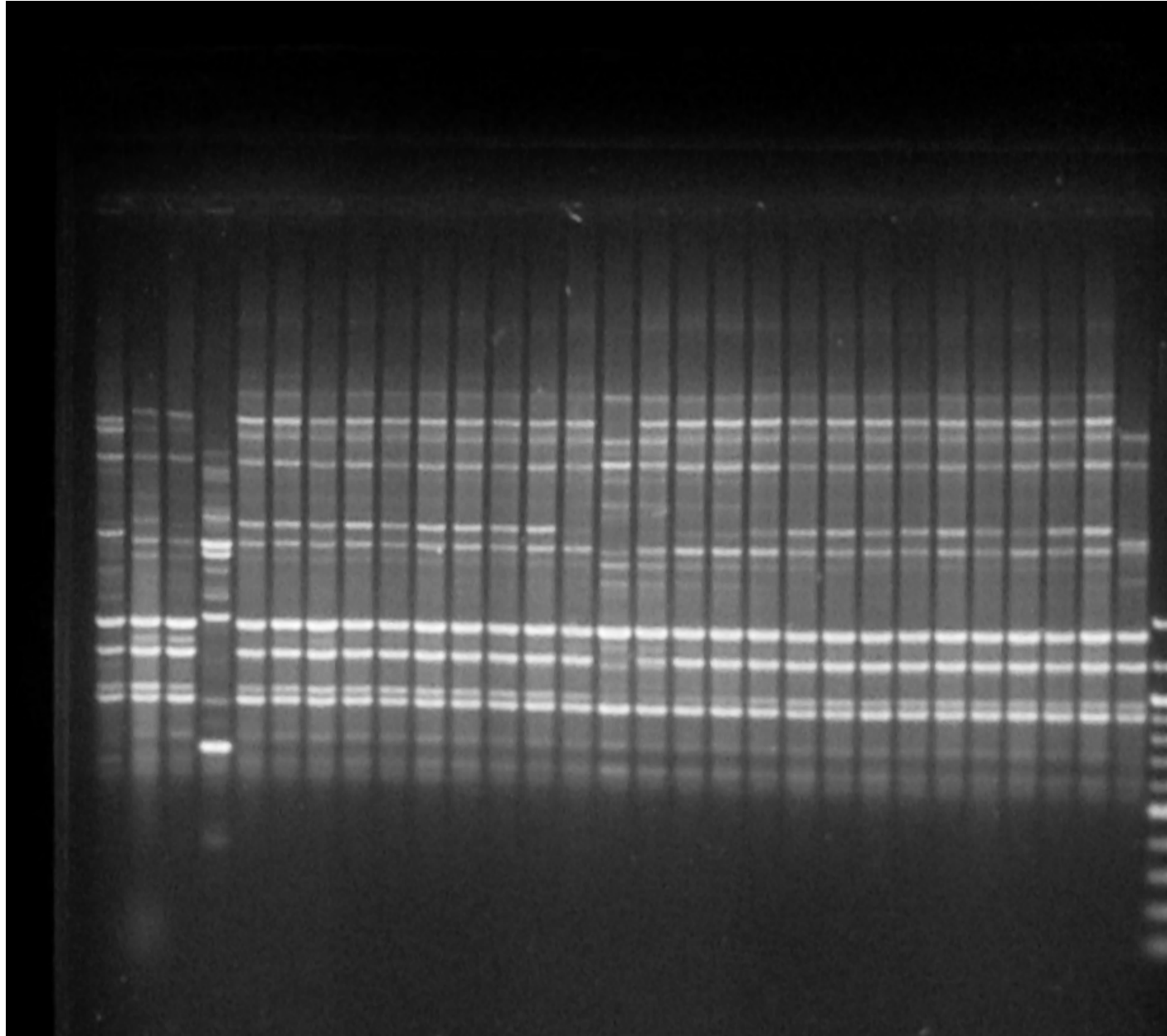
Where is Psa?



Typing: BOX PCR of Psa isolates



BOX PCR of Psa isolates: Critical Urgent versus important



Conclusions

Ability to control Psa is limited by our knowledge of the pathogen and the disease

Ability to gather that knowledge is limited by the tools developed for Psa

Over the last 2 years a series of coordinated research programmes have been put in place to address those limitations

Difficulties are in the constant balancing act between urgent and important, and between short term and long term needs

Our understanding of Psa and how to control it has increased tremendously over the last two years



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The basis of current research programmes

All the research programmes in New Zealand and in Italy aim at providing tools and increase our knowledge of the disease such as to control Psa

Three research programmes which started before November 2010 :

ZESPRI led programme in Italy
Bologna Uni. ZESPRI. PFR
KRIP programme

Since November 2010

ZESPRI-PFR research programmes are being developed to support and strengthen programmes previously developed