PLANT & FOOD RESEARCH Pseudomonas syringae pv actinidiae (Psa) RESEARCH NOTE

PROJECT DETAILS

Project Title	7.5 Testing Kumeu Research Orchard historic collection for presence of Psa
Project Protocol No./ Objective No.	
Project Leader	Kerry Everett
Research Requested / Contracted by	
Date (Month, Year)	November 2010

KEY QUESTION AND AIM

Is Psa present in an historic collection of bacteria isolated from macerated kiwifruit buds, flowers and fruitlets from the Kumeu Research Orchard during spring 1991 (Everett & Henshall 1994)?

METHODOLOGY

1) Experimental Design

Records of tests on bacteria isolated from macerated kiwifruit buds, flowers and fruitlets during spring 1991 (Everett & Henshall 1994) were accessed. A total of 52 isolates of bacteria that were not fluorescent on King's medium B (King et al. 1954), and were not able to degrade pectin (Lelliott et al. 1966) were located in our culture collection. These isolates were tested by PCR to determine whether they were isolates of Psa.

2) Methods and/or Protocols

These bacteria had been stored in 1.5-ml Eppendorf tubes in 50% glycerol at -20°C. An aliquot of 100 µl of each isolate was placed on Kings' medium B in a Petri plate and spread with a sterilised bent glass rod. Cultures were incubated at 28^{o}C for 24-48 hours. Petri plates were then washed with 1 ml of bacterial saline (BS) then transferred to sterile 1.5 ml Eppendorf tubes and the total volume of each suspension adjusted to 1 ml with BS . Each bacterial suspension was vortexed and a 100 µl aliquot was added to 900 µl of BS. Following centrifugation for 5 min. at 8500 rpm, the resultant pellet was resuspended in 1 ml BS, centrifuged again and then resuspended in 1 ml 1 mM EDTA. A 200-µl aliquot of this suspension was then placed at 100^{o}C for 5 min., then placed immediately on ice to extract DNA. .A 1-µl aliquot of the remaining suspension was used as a template in PCR reactions.

The PCR primers F1/R2 and F3/R4, and the reaction conditions described in Rees-George et al. (2010) were used, except that there were 35 cycles. DNA previously extracted from two isolates of Psa obtained from the International Collection of Microorganisms from Plants (Landcare, Tamaki Campus) (ICMP 9617, ICMP 9855) was used as a positive PCR control, and there was also a water control.

For two isolates that produced faint bands, the PCR test described in Rees-George et al. (2010) was repeated, except that the originally recommended protocol of 30 cycles was used. In addition, the PCR primers and protocol of Koh & Nou (2002) were also used for testing these two isolates.

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

1) There were two faint positive bands from isolate 848 and isolate 872 from the Kumeu Research Orchard using the protocol of Rees-George et al. (2010) with the modification of 35 cycles. All other isolates from Kumeu Research Orchard (HortResearch), New Zealand were negative, as was the water control. The expected band was produced from DNA amplified from the two known Psa isolates used as controls.

2) When the PCR protocol and primers of Rees-George et al. (2010), using the 30 recommended cycles, was used then no product was amplified from DNA extracted from isolates 848 and 872.

3) No product was amplified from DNA extracted from isolates 848 and 872 when the primers and protocol of Koh & Nou (2002) were used.

CONCLUSIONS

Because the bacterial samples from isolates 848 and 872 were derived from single cell cultures, it is unlikely that the faint positive bands obtained using 35 cycles were from positive Psa isolates. When they were retested using the protocol of Rees-George et al. (2010) and 30 cycles no bands were produced. However, the Rees-George et al. (2010) primers are likely to be more sensitive than the Koh & Nou (2002) primers because the region that these primers amplify has fivecopies, whereas the region that the Koh and Nou (2002) primers amplify has only one copy. Both the higher copy number (5), and the higher number of cycles (35) mean that the Rees-George et al. (2010) primers are more sensitive than the low copy number (1) and lower number of cycles (30) of the primers designed by Koh and Nou (2002). Consequently, it is possible that Psa was present but only in small quantities as a contaminant. However, this is considered to be unlikely. On this basis it is concluded that Psa was not present on *Actinidia deliciosa* buds, flowers and fruitlets on Kumeu Research Orchard in 1991.

FUTURE RESEARCH STEPS

1) To be certain that Psa was not present on tissue from *Actinidia deliciosa* on Kumeu Research Ochard in 1991, DNA extracted from isolates 848 and 872 should be re-tested using 40 cycles and the primers of both Koh & Nou (2002) and Rees-George et al. (2010), and any products should be sequenced. This will establish if the faint positive bands are Psa, or not.

2) The rpoD gene region also needs to be sequenced to compare *P.s.* pv. *actinidiae* with other pathovars of *Pseudomonas syringae* that cannot be distinguished on the basis of the ITS sequence (*P.s.* pv. *passiflorae*), or are very similar (*P.s.* pv. *morsprunorum*). Downloading rpoD sequence of a New Zealand isolate of *P.s.* pv. *passiflorae* and overseas isolates of *P.s.* pv. *morsprunorum* from GenBank showed that in this region there are 9/472 differences in DNA sequence to *P.s*. pv. *passiflorae*, and 7/472 differences to *P.s*. pv. *morsprunorum*. If the rpoD gene region of isolates 848 and 872 are sequenced and compared to these pathovars, we expect that it will provide further evidence that these isolates are not Psa.

RECOMMENDATIONS FOR INDUSTRY

Our results suggest that it is unlikely that Psa is present in this historic culture collection from the Kumeu Research Orchard However, DNA sequencing confirmation is required. Isolates from this study (Everett & Henshall 1994) that were deposited in ICMP were also negative for Psa when tested with the Rees-George et al. (2010) primers, confirming the results of our study.

REFERENCES

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