COMPARISON OF PATHOGENICITY AND PCR TESTS FOR CONFIRMATION OF *XANTHOMONAS HORTORUM* PV. *CAROTAE*

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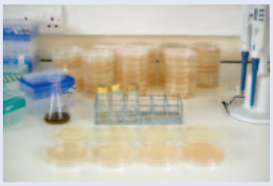
Background

- *Xanthomonas hortorum* pv. *carotae* (*Xhc*) causes bacterial blight of carrots and is seed-borne.
- A seed test method published on the ISHI web-site (van Bilsen 2002) suggests confirmation of the identity of *Xhc* by pathogenicity assay or by PCR.
- Many laboratories favour PCR due to the faster time to a result and apparently fewer ambiguous results
- We had problems with the *routine* application of both methods and therefore lacked confidence in the results obtained.
- Modifications were made to both methods and results compared for over one hundred isolates.

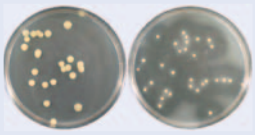
Test Method

Soak seeds overnight at 4°C

Shake flasks for 3 min



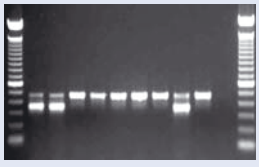
Dilute and plate on selective media



Incubate at 30°C for 5-6 d

Sub-culture to sectored plates

Confirm identity by pathogenicity



or by PCR

Pathogenicity

- Carrot seeds sown in pots and grown to 3-5 true leaves.
- Inoculum prepared by suspending growth from 24-48 h YDC plate to give 10^6 cfu/ml
- Plants enclosed in polythene bags for 24-48 h before inoculation - Petri dish bags are ideal and can be closed with a paperclip.
- Inoculum sprayed gently into the bag and onto foliage using deVilbiss atomiser.
- Bags opened 48 h after inoculation, but plants remain in bags to keep foliage separate and prevent cross-contamination.

PCR

- Reaction optimised so that both specific primers and universal primers included in the same tube.
- Expected results:
  - two bands (specific and universal) = positive identification
  - one band (universal) = negative identification
  - no bands = bacterial template absent (repeat reaction)

Results/Conclusions

- All PCR positive isolates were also pathogenic.
- Only 3% of path. positive isolates were PCR negative.
- Both methods can be considered to be reliable.
- No need for overnight liquid culture for pathogenicity test.
- In-tube validation of PCR gives confidence in results.

	No. of isolates
Suspects tested	130
Path. positive	100
PCR positive	97

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PCR AND PATHOGENICITY DETAILS

Primers:

Xhc specific (350 bp product; Meng *et al.* 2004):

3Sforw: 5' CAT.TCC.AAG.AAG.CAG.CCA 3'
3Srev: 5' TCG.CTC.TTA.ACA.CCG.TCA 3'

Universal (441 bp product; M. Asma, Bejo, pers. comm):

1052F: 5' GCA.TGG.TTG.TCG.TCA.GCT.CGT 3'
Bac R: 5' TAC.GGC.TAC.CTT.GTT.ACG.ACT.T 3'

10x Buffer:

Tris-HCl (pH 9.0)	750 mM
$(NH_4)_2SO_4$	200 mM
$MgCl_2$	15 mM
Tween 20	0.1% (v/v)

Reaction mix:

Component	Final concentration	Volume (µl) in 10 µl
H_2O		5.42
Buffer (10x)	1x	1.00
dNTP's (2.5 mM each) (10 mM total)	0.20 mM each (0.8 mM total)	0.80
Primer 3Sforw (20 pmol/µl)	0.50 µM	0.25
Primer 3Srev (20 pmol/µl)	0.50 µM	0.25
U Primer 1052 F (5 pmol/µl)	0.05 µM	0.10
U Primer Bac R (5 pmol/µl)	0.05 µM	0.10
Taq (5 U/µl)	0.04 U/µl	0.08
Bacterial suspension		2.00

PCR Conditions:

1 cycle: 95°C for 5 min
35 cycles: 94°C for 15 s
58°C for 15 s
72°C for 30 s

Pathogenicity:

Cultivar - Napoli

Recording
- 2 to 4 weeks after inoculation depending on temperature
- Comparison should be made with a known positive control isolate
- Symptoms of brown/dark necrotic spots/areas often surrounded by chlorotic halo are taken to indicate a positive response

References

Meng, X.Q., Umesh, K.C., Davis, R.M. and Gilbertson, R.L. (2004) Development of PCR-based assays for detecting *Xanthomonas campestris* pv. *carotae*, the carrot bacterial leaf blight pathogen, from different substrates. *Plant Disease* **88**, 1226-1234.

van Bilsen, J (2002) Detection of *Xanthomonas campestris* pv. *carotae* on Carrot (*Daucus carota*) . *http://www.worldseed.org/pdf/carrot%20Xcc.pdf*