



Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic Affairs



Evaluation of real-time PCR
platforms using TPS on
'*Ca. Liberibacter solanacearum*'

&

Validation/implementation of
detection and
identification of the bacterial
plant pathogen



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Evaluation of real-time PCR platforms



Applied Biosystems
7900 HT



Student Internship Project

Joyce van Assen

Sep-Dec 2013



Bio-Rad
CFX 96



Applied Biosystems
7500



Roche
LC 480



Roche
LC 96



- *Phytophthora ramorum*
 - *Clavibacter michiganensis subsp. michiganensis*
 - *Spodoptera* spp.
 - *Bursaphelenchus xylophilus*
 - Pospiviroid
-
- limit of detection
 - Analytical specificity
 - User friendly
 - Platform specifications



EUPHRESKO – IVIA - Test Performance Study
 detection of "*Candidatus Liberibacter solanacearum*"
 CaLsol/100 complete kit of Plant Print Diagnostics S.L.

TPS sample	7900HT (AB)		7500 (AB)		CFX96 (Bio-Rad)		LC480 (Roche)		LC96 (Roche)		Identity
	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2	
sample 01	-	35,8	36,0	36,7	35,0	35,1	36,0	-	33,8	34,7	+
sample 02	-	-	-	-	-	-	-	-	-	-	-
sample 03	-	-	-	-	-	-	-	-	-	-	-
sample 04	34,5	33,2	35,1	35,6	31,0	31,0	-	33,0	34,1	-	+
sample 05	33,5	31,9	34,6	36,3	31,1	31,0	33,5	33,0	33,5	-	+
sample 06	-	-	-	-	-	-	-	-	-	-	-
sample 07	35,0	35,6	37,8	35,3	33,4	33,3	-	33,8	35,8	-	+
sample 08	-	-	-	-	-	-	-	-	-	-	-
sample 09	34,1	34,2	35,2	36,0	40,0	33,5	-	35,9	34,7	36,1	+
sample 10	-	-	-	-	-	-	-	-	-	-	-



Since January 2013 Bio-Rad CFX 96





Future plans

- In-house analysis *Ca. Liberibacter solanacearum* survey
- Validate en implement (real-time) PCR detection assay



(Real-time) PCR detection assays

Author	Method	Target	internal control (plant)	Sensitivity	Theoretical LOD	Specificity
Liefting <i>et al.</i> 2009	PCR	16S rDNA	N.A.	unknown	100	Negative for 'Ca. L. asiaticus', 'Ca. L. africanus', or 'Ca. L. americanus'



(Real-time) PCR detection assays

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Li <i>et al.</i> 2009	PCR	16S rDNA	N.A.	10X PCR Liefting	10	18 Ca. L. solanacearum (positive Ct 20.6-35.2) 3 Ca. L. asiaticus, 3 Ca. L. africanus, 3 Ca. L. americanus (negative) Potato leaf roll virus, Clover proliferation phytoplasma, Candidatus Phytoplasma americanum, Xylella fastidiosa PD strain, Xylella fastidiosa CVC strain (negative)
	real-time PCR (TaqMan)	16S rDNA	COX	10X PCR Li	1	
Ravindran <i>et al.</i> 2011	PCR	16S-23S rDNA ITR	b-TUB	10X PCR Li	1	1 Ca. L. asiaticus (negative)
	PCR	adenylate kinase	b-TUB	10X PCR Li	1	
Beard <i>et al.</i> 2012	semi-nested real-time PCR (SYBR)	16S rDNA	EF-1a	50X cPCR Liefting	2	3 Ca. L. solanacearum (positive) 1 Ca. L. americanus, 1 Ca. L. africanus, 1 Ca. L. asiaticus, 1 Ca. L. europaeus (negative)
	semi nested PCR	16S rDNA	28S	1/10X semi-nested qPCR Beard	20	WX phytoplasma, PoiBI phytoplasma, WWB phytoplasma, Ca. Phytoplasma australiense, Pectobacterium atrosepticum, Pseudomonas fluorescens (1 each, negative)



Future plans

- In-house analysis *Ca. Liberibacter solanacearum* survey
- Validate en implement (real-time) PCR detection assay
- Implement haplotype identification assay



Haplotype identification assays

Haplotype	Host	vector	Distribution
A	Solanaceae	<i>Bactericera cockerelli</i>	Honduras, Guatemala, Mexico, USA, New Zealand
B	Solanaceae	<i>Bactericera cockerelli</i>	Mexico, USA
C	<i>Daucus carota</i>	<i>Trioza apicalis</i>	Finland, Sweden, Norway
D	<i>Daucus carota</i>	<i>Bactericera trigonica</i>	Spain, Canary Islands

- Discrimination based on SNPs in 16S, IRS-23S, 50S (rplJ and rplL)

Nelson *et al.* 2011

- DNA barcoding approach
- Real-time PCR with haplotype specific probes

- Discrimination based on microsatellite analysis

Lin *et al.*, 2011



Acknowledgement



Department of Molecular Biology