SUMMARY

DETECTION, CHARACTERISATION AND SUPPRESSION OF *RALSTONIA SOLANACEARUM*

by

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The primary objective of this study was to develop an alternative method for the detection of *Ralstonia solanacearum* in soil systems. The pathogen could successfully be detected with this trapping technique. The technique was easy to apply and sensitive enough to detect pathogen concentrations of 10^1 cfu ml^{-1} soil suspension. Results could not be obtained fast enough for commercial application and the technique could therefore not replace traditional selective media and the ELISA-technique currently used in South Africa. The second objective of this study was to evaluate different molecular techniques for the identification and characterisation of different *R. solanacearum* isolates. The ERIC-PCR was used on eight biovar 2 and 3 isolates and could successfully distinguish between the two groups. The RISA-PCR and RFLP with *Sau*3A were used to characterise 44 *R. solanacearum* isolates. Although the techniques could distinguish between the two groups of biovars, it could not be used to draw a correlation between the isolates and the different regions from which they were isolated. The last objective of this study was to evaluate 13 herbal species for their potential use as biofumigation agents to suppress *R. solanacearum* in soil. Problems were experienced with soil inoculation and suppression of the pathogen could not be evaluated successfully.

OPSOMMING

OPSPORING, KARAKTERISERING EN ONDERDRUKKING VAN *RALSTONIA SOLANACEARUM*

deur

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Die primêre doel van hierdie studie was om 'n alternatiewe tegniek te ontwikkel vir die opsporing van *Ralstonia solanacearum* in grondmonsters. 'n Lokaastegniek is ontwikkel waarmee die patogeen suksesvol opgespoor kon word. Die tegniek was maklik toepasbaar en sensitief genoeg om patogeenkonsentrasies so laag as 10^1 kolonie-vormende eenhede ml^{-1} grondoplossing mee op te spoor. Dit kon egter nie vinnig genoeg resultate lewer om kommersieël aangewend te word nie en was dus nie geskik om tradisionele selektiewe media en die ELISA-tegniek te vervang wat tans in Suid-Afrika gebruik word nie. Die tweede doel van hierdie studie was om molekulêre tegnieke te ondersoek waarmee *R. solanacearum* isolate uitgeken en gekarakteriseer kon word. Die ERIC-PCR is gebruik om agt biovar 2 en 3 isolate mee te ondersoek en kon suksesvol tussen die twee groepe biovars onderskei. Die RISA-PCR en RFLP met *Sau*3A is gebruik om 44 *R. solanacearum* isolate mee te ondersoek. Hoewel die tegnieke onderskeid kon tref tussen die twee groepe biovars, kon geen korrelasie getref word tussen die isolate en die verskillende streke waaruit dit geïsoleer was nie. Die laaste doel van hierdie studie was om 13 kruiespesies te ondersoek vir moontlike gebruik in bioberoking vir die onderdrukking van *R. solanacearum* in grond. Probleme is ondervind met grondinokulasie en onderdrukking van die patogeen kon nie suksesvol ondersoek word nie.

APPENDIX 1

RALSTONIA SOLANACEARUM ISOLATES ISOLATED THROUGHOUT SOUTH AFRICA FROM INFECTED POTATOES

***Ralstonia solanacearum* isolates collected throughout the potato growing regions of South Africa**

No.	Isolate	Biovar	District	Supplier
1	SAP 1	2	Dendron	A.N. Hall + A.C. Hayward
2	SAP 6	3	Winterton	A.N. Hall + A.C. Hayward
3	SAP 111	2	Zimbabwe	A.N. Hall
4	SAP 117	3	Hlabisa	A.N. Hall
5	SAP 93	3	Ladysmith	A.N. Hall
6	SAP 23	2	Warmbad	A.N. Hall + A.C. Hayward
7	SAP 3	2	Vivo	A.N. Hall + A.C. Hayward
8	SAP 46	2	Badplaas	A.N. Hall
9	SAP 62	2	Rooiwal	A.N. Hall
10	SAP 19	2	Dendron	A.N. Hall + A.C. Hayward
11	SAP 2	2	Dendron	A.N. Hall + A.C. Hayward
12	SAP 22	2	Vivo	A.N. Hall + A.C. Hayward
13	SAP 60	2	Unknown	A.N. Hall
14	SAP 7	2	Winterton	A.N. Hall + A.C. Hayward
15	SAP 45	2	Barkly Wes	A.N. Hall
16	SAP 79	2	Douglas	A.N. Hall
17	SAP 16	2	Letsitele	A.N. Hall + A.C. Hayward
18	SAP 64	2	Unknown	A.N. Hall
19	SAP 92	2	Dendron	A.N. Hall

20	SAP 66	2	Unknown	A.N. Hall
21	SAP 27	2	Pietersburg	A.N. Hall + A.C. Hayward
22	SAP 26	2	Dendron	A.N. Hall + A.C. Hayward
23	SAP 76	2	Plooysburg	A.N. Hall
24	SAP 17	2	Piketberg	A.N. Hall + A.C. Hayward
25	SAP 25	2	Vivo	A.N. Hall + A.C. Hayward
26	SA 1	2	Clanwilliam	A.E. Swanepoel
27	SA 3	2	Worcester	A.E. Swanepoel
28	SA 20	2	Piketberg	A.E. Swanepoel
29	SA 2	2	Worcester	A.E. Swanepoel
30	SA 6	2	Piketberg	A.E. Swanepoel
31	SA 7	2	Piketberg	A.E. Swanepoel
32	SA 8	2	Piketberg	A.E. Swanepoel
33	SB 8	2	Sandveld	A.E. Swanepoel
34	SB 292(b)	?	Unknown	A.E. Swanepoel
35	SB 292	?	Unknown	A.E. Swanepoel
36	SB 20	2	Piketberg	A.E. Swanepoel
37	Z 23	2	Dendron	A.E. Swanepoel
38	NB 346	3	Bergville	A.E. Swanepoel
39	NK 15	2	Itala Valley	A.E. Swanepoel
40	D 109	2	North Cape	A.E. Swanepoel
41	PB 1-2	2	Warmbad	A.E. Swanepoel
42	TB 40	2	Davel (Ermelo)	A.E. Swanepoel
43	TB 32	2	Alberton	A.E. Swanepoel
44	TB 16	2	Middelburg	A.E. Swanepoel

APPENDIX 2

2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE MEDIUM (TZC-1 *l*)

Peptone	10g
Casein hydrolysate	1g
Glycerol	5 ml
Distilled water	975 ml
Agar bacteriological	18g
2,3,5-Triphenyltetrazolium chloride	0.05g in 25 ml distilled water

Peptone, casein and glycerol are added to the distilled water and thoroughly mixed. The agar is added separately to the bottles and autoclaved for 15 min at 121°C. The tetrazolium chloride is autoclaved for 7 min at 121°C and added to the autoclaved peptone mixture while still hot. The mixture is shaken gently and poured into Petri dishes.

MODIFIED TZC MEDIUM CONTAINING CRYSTAL VIOLET (M-TZC-1 *l*)

Peptone	10g
Casein hydrolysate	1g
Glycerol	5 ml
Crystal violet	5 mg (1 ml stock solution: 0.25 g 50 ml^{-1} distilled water)
Distilled water	975 ml
Agar bacteriological	18g
2,3,5-Triphenyltetrazolium chloride	0.05g in 25 ml distilled water

Peptone, casein, glycerol and crystal violet are added to the distilled water and thoroughly mixed. The agar is added separately to the bottles and autoclaved for 15 min at 121°C. The tetrazolium chloride is autoclaved for 7 min at 121°C and added to the autoclaved peptone mixture while still hot.

The following antibiotics are added (using filter sterilization) to the medium just before pouring (Stock solutions can also be prepared and 1 ml added to the medium):

Polymixin B sulphate	100 mg	(1 g in 10 ml distilled water)
Bacitracin	25 mg	(0.25 g in 10 ml distilled water)
Chloramphenicol	5 mg	(0.25 g in 50 ml methanol)
Penicillin G potassium salt	0.5 mg	(0.05 g in 100 ml distilled water)

SELECTIVE MEDIA FROM SOUTH AFRICA (SMSA-1 *l*)

This medium is prepared in the same way as the M-TZC medium except that no crystal violet is added.