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PCR-based Assay for the Specific Detection of *Pseudomonas syringae* pv. *tagetis* using an AFLP-derived Marker

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A PCR method has been developed for the pathovar-specific detection of *Pseudomonas syringae* pv. *tagetis*, which is the causal agent of bacterial leaf spots and apical chlorosis of several species within the Compositae family. One primer set, PSTF and PSTR, was designed using a genomic locus derived from an amplified fragment length polymorphism (AFLP) fragment produced a 554-bp amplicon from 4 isolates of *P. syringae* pv. *tagetis*. In DNA dot-blot analysis with the PCR product as probe, a positive signal was identified in only 4 isolates of *P. syringae* pv. *tagetis*. These results suggest that this PCR-based assay will be a useful method for the detection and identification of *P. syringae* pv. *tagetis*.

Keywords : AFLP, Detection, PCR, *Pseudomonas syringae* pv. *tagetis*

Introduction

Pseudomonas syringae pv. *tagetis* was first described in Denmark as a pathogen that affects marigold production (Hellmers, 1955). It is now known as a phytopathogenic bacterium that is the causal agent of bacterial leaf spots and apical chlorosis in several species within the family Compositae: African marigold (*Tagetes erecta* L.), sunflower (*Helianthus annuus* L.), common ragweed (*Ambrosia artemisiifolia* L.), Jerusalem artichoke (*Helianthus. tuberosus* L.), dandelion (*Taraxacum officinale* Weber), compass plant (*Silphium perfoliatum* L.) and another sunflower species (*Helianthus salicifolius* A. Diter) (Gulya *et al*., 1981; Hellmers, 1955; Rhodehamel and Durbin, 1985; Rhodehamel and Durbin, 1989a; Shane and Baumer, 1984; Styer and Durbin, 1982).

P. syringae pv. *tagetis* produces a toxin (tagetitoxin) in host leaves that is then translocated to emerging leaves, where it inhibits RNA polymerase III, thereby preventing chloroplast biogenesis and resulting in apical chlorosis (Mathews and Durbin, 1990; Steinberg *et al*., 1990). The pathogens are divided into three classes based on their capability to produce tagetitoxin: class 1 and 2 strains produce tagetitoxin in plants; class 3 strains do not produce the toxin (Rhodehamel and Durbin, 1989b).

There are many reports that specific detection methods for phytotoxin-producing *P. syringae* pathovars have been developed based on genes required for their production (Bereswill *et al*., 1994; Lydon and Patterson, 2001; Schaad *et al*., 1995; Sorensen *et al*., 1998). Recently, a PCR protocol to distinguish *P. syringae* pv. *tagetis* from other *P. syringae* pathovars and closely related species was developed based on genes required for tagetitoxin production (Kong *et al*., 2004). However, this approach is unable to distinguish the bacterium from other *Pseudomonas* isolates at the pathovar level. Furthermore, *Pseudomonas* species other than *P. syringae* pv. *tagetis* have been reported to induce apical chlorosis in Canada thistle and pea (Suzuki *et al*., 2003; Zhang *et al*., 2002). Therefore, a PCR-based assay that is able to unambiguously distinguish *P. syringae* pv. *tagetis* from *P. syringae* pv. *helianthi* and other apical chlorosis-inducing *Pseudomonas* species is needed.

In this study, we report the development of a pathovar-specific marker derived from the AFLP technique for detecting and distinguishing *P. syringae* pv. *tagetis* from other pathovars and species of *Pseudomonas* and *Xanthomonas*. The specificity of the PCR-based assay using pathovar-specific primers was validated by testing 47 isolates collected from various geographical

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regions and host plants.

Material and Methods

Bacterial strains and DNA isolation. The bacterial strains that are listed in Table 1 were obtained from the Korean Agricultural Culture Collection (KACC) in Suwon, Korea, and the Belgian Co-ordinated Collections of Micro-organisms (BCCM) in Brussels, Belgium. The genomic DNA was isolated as described previously (Song *et al*., 2014).

AFLP PCR analysis. The AFLP assay was performed using a previously described method (Song *et al*., 2014), with minor modification. Genomic DNA (approximately 300 ng) was

Table 1. List of bacterial strains used in this study

No.	Species	Source[a]	Geographical origin	Hosts
1	*Pseudomonas syringae* pv. *tagetis*	LMG 5090	Zimbabwe	*Tagetes erecta*
2	*Pseudomonas syringae* pv. *tagetis*	LMG 5684	Australia	*Tagetes erecta*
3	*Pseudomonas syringae* pv. *tagetis*	LMG 5685	Australia	*Tagetes erecta*
4	*Pseudomonas syringae* pv. *tagetis*	LMG 5686	USA	*Tagetes* sp.
5	*Pseudomonas syringae* pv. *helianthi*	LMG 2198	Zambia	*Helianthus annuus*
6	*Pseudomonas syringae* pv. *helianthi*	LMG 5067	Mexico	*Helianthus annuus*
7	*Pseudomonas syringae* pv. *helianthi*	LMG 5556	Canada	*Helianthus annuus*
8	*Pseudomonas syringae* pv. *helianthi*	LMG 5557	Germany	*Helianthus annuus*
9	*Pseudomonas syringae* pv. *helianthi*	LMG 5558	New Zealand	*Helianthus annuus*
10	*Pseudomonas syringae* pv. *syringae*	LMG 1274	UK	–
11	*Pseudomonas syringae* pv. *syringae*	LMG 5082	UK	*Zea mays*
12	*Pseudomonas syringae* pv. *syringae*	LMG 5494	Hungary	*Prunus avium*
13	*Pseudomonas syringae* pv. *actinidiae*	KACC10772	–	–
14	*Pseudomonas syringae* pv. *aptata*	LMG 5059	USA	*Beta vulgaris*
15	*Pseudomonas syringae* pv. *atrofaciens*	LMG 5095	New Zealand	*Triticum aestivum*
16	*Pseudomonas syringae* pv. *atrofaciens*	LMG 5000	–	Thatcher wheat
17	*Pseudomonas syringae* pv. *japonica*	LMG 5068	Japan	*Hordeum vulgare*
18	*Pseudomonas syringae* pv. *tomato*	LMG 5093	UK	*Lycopersicon esculentum*
19	*Pseudomonas syringae* pv. *tabaci*	LMG 5393	Hungary	*Nicotiana tabacum*
20	*Pseudomonas syringae* pv. *mori*	LMG 5074	Hungary	*Morus alba*
21	*Pseudomonas syringae* pv. *antirrhini*	LMG 5057	UK	*Antirrhinum majus*
22	*Pseudomonas syringae* pv. *glycinea*	LMG 5066	New Zealand	–
23	*Pseudomonas syringae pv. delphinii*	LMG 5381	New Zealand	*Delphinium* sp.
24	*Pseudomonas syringae* pv. *eriobotryae*	LMG 2184	USA	*Eriobotrya japonica*
25	*Pseudomonas syringae* pv. *lachrymans*	LMG 5070	USA	*Cucumis sativus*
26	*Pseudomonas syringae* pv. *morsprunorum*	LMG 5075	–	*Prunus domestica*
27	*Pseudomonas syringae* pv. *morsprunorum*	LMG 2222	UK	*Prunus avium* cv. Napoleon
28	*Pseudomonas syringae* pv. *garcae*	LMG 5064	Brazil	*Coffea arabica*
29	*Pseudomonas syringae* pv. *delphinii*	LMG 2177	UK	*Delphinium elatum*
30	*Pseudomonas syringae* pv. *pisi*	LMG 5383	Canada	*Pisum sativum*
31	*Pseudomonas syringae* pv. *pisi*	LMG 5384	Italy	*Pisum sativum*
32	*Pseudomonas syringae* pv. *sesami*	LMG 2289	Yugoslavia	–
33	*Pseudomonas azotoformans*	KACC10302	–	–
34	*Pseudomonas fuscovaginae*	LMG 2158	Japan	*Oryza sativa*
35	*Pseudomonas coronafaciens*	LMG 5060	UK	*Avena sativa*
36	*Pseudomonas citronellolis*	LMG 18378	USA	soil collected under pine trees
37	*Pseudomonas oryzihabitans*	LMG 7040	Japan	rice paddy
38	*Pseudomonas mucidolens*	LMG 2223	USA	–
39	*Pseudomonas graminis*	LMG 21661	Germany	grasses
40	*Pseudomonas jessenii*	LMG 21605	France	–
41	*Pseudomonas libanensis*	LMG 21606	Lebanon	–
42	*Pseudomonas lundensis*	LMG 13517	–	–
43	*Pseudomonas taetrolens*	LMG 2336	–	–
44	*Xanthomonas oryzae* pv. *oryzae*	KACC10331	Korea	–
45	*Xanthomonas campestris* pv. *citri*	KACC10444	Korea	–
46	*Xanthomonas campestris* pv. *glycines*	KACC10445	Zambia	–
47	*Xanthomonas campestris* pv. *vesicatoria*	LMG 905	–	–

[a]KACC, Korean Agricultural Culture Collection, Korea (http://www.genebank.go.kr/); LMG, The Belgian Co-ordinated Collections of Microorganisms (BCCM), Belgium; '–' unknown.

Table 2. Oligonucleotide adaptors and primers used for AFLP analysis

No.	Primer name		Sequence (5′ - 3′)	No.	Primer name		Sequence (5′ - 3′)
1	*Eco*RI-Adaptor	Forward	CTCGTAGACTGCGTACC	1	*Mse*I-Adaptor	Forward	TACTCAGGACTCAT
2		Reverse	AATTGGTACGCAGTCTAC	2		Reverse	GACGATGAGTCCTGAG
3	*EcoRI* + 0	Eco0	GACTGCGTACCAATTC	3	*Mse*I + 0	Ms0	GATGAGTCCTGAGTAA
4	*Eco*RI + 3	Eco1	GACTGCGTACCAATTCAGG	4	*Mse*I + 3	Ms1	GATGAGTCCTGAGTAACAT
5		Eco2	GACTGCGTACCAATTCACG	5		Ms2	GATGAGTCCTGAGTAACTT
6		Eco3	GACTGCGTACCAATTCAAC	6		Ms3	GATGAGTCCTGAGTAACAC
7		Eco4	GACTGCGTACCAATTCACA	7		Ms4	GATGAGTCCTGAGTAACTA
8		Eco5	GACTGCGTACCAATTCACC	8		Ms5	GATGAGTCCTGAGTAACAG
9		Eco6	GACTGCGTACCAATTCAGC	9		Ms6	GATGAGTCCTGAGTAACTC
10		Eco7	GACTGCGTACCAATTCACT	10		Ms7	GATGAGTCCTGAGTAACAA
11		Eco8	GACTGCGTACCAATTCAAG	11		Ms8	GATGAGTCCTGAGTAACTG
12		Eco9	GACTGCGTACCAATTCAGA	12		Ms9	GATGAGTCCTGAGTAACCA
13		Eco10	GACTGCGTACCAATTCAGT	13		Ms10	GATGAGTCCTGAGTAACGA

digested with *Eco*RI and *Mse*I enzymes and was then ligated to the ends of the restricted DNA fragments with *Eco*RI adaptor and *Mse*I adaptor (Table 2). A pre-selective PCR reaction was performed with the AccuPower PCR Premix (Bioneer, Daejeon, Korea) in a 25 μl reaction mixture containing 1 μl of DNA (50 ng/μl), 10 pmol of Eco0 (5′-GACTGCGTACCAATTC-3′), and 10 pmol of Ms0 (5′-GATGAGTCCTGAGTAA-3′). The pre-selective PCR and AFLP PCR amplification were conducted as described previously (Song *et al*., 2014). The amplified products were resolved in a 1.2% agarose gel with a 1-kb DNA ladder (TNT Research, Seoul, Korea) as a reference, stained with ethidium bromide, and visualized on a UV transilluminator.

Primer design and PCR amplification. The specific DNA fragment was eluted as described previously (Song *et al*., 2014). DNA was directly used in the ligation reaction with a pGEM-T Easy Vector (Promega, Madison, WI, USA) and was then transferred into competent DH5α (RBC Bioscience, Taipei, Taiwan) cells according to the supplier's instructions. The sequencing reaction was performed with an ABI Prism 3730 DNA Sequencer (Life Technologies, Carlsbad, CA, USA). After trimming the vector sequence, one pair of primers was designed based on the obtained sequence.

The specificity of the designed primers was evaluated against *P. syringae* pv. *tagetis* and other *Pseudomonas* and *Xanthomonas* species. The PCR reaction was performed with premixed polymerase (Taq PreMix; TNT Research, Seoul, Korea) in a 20 μl reaction mixture containing 1 μl of DNA (50 ng/μl), 10 pmol of PSTF (5′-AATGAGCTGAAATTCAACGG-3′), and 10 pmol of PSTR (5′-CGACCTGGATATAAGTTGCC-3′). The PCR amplification was performed with a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation (5 min at 96°C), 25 cycles (15 s at 96°C; 15 s at 62°C; and 30 s at 72°C), and a final extension (5 min at 72°C). Subsequently, 5 μl of each reaction mixture was resolved in a 1.2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator.

DNA dot-blot analysis. A DNA dot-blot analysis was performed using a previously described method (Kang *et al*., 2007), with some modifications. A total volume of 5 μl of genomic

Fig. 1. Agarose gel electrophoresis of PCR amplicons amplified from *Pseudomonas syringae* pv. *tagetis* isolates using the pathovar-specific PSTF/PSTR primer set. Lane M: size marker (1-kb ladder); lanes 1–47: *Pseudomonas* and *Xanthomonas* isolates (numbers 1–47, respectively, in Table 1).

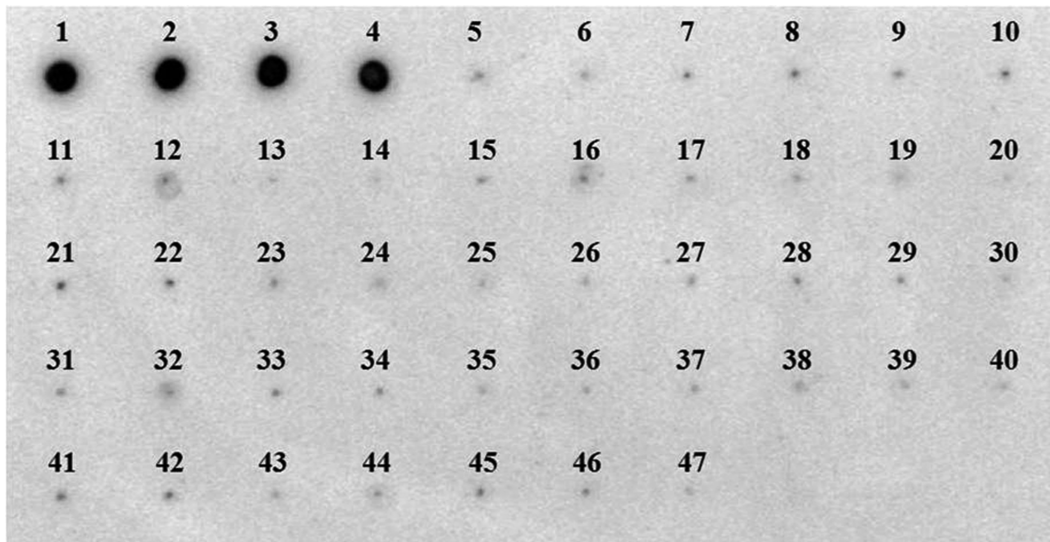

Fig. 2. DNA dot-blot analysis using PCR amplicon with PSTF and PSTR from *Pseudomonas syringae* pv. *tagetis* LMG 5090. Lanes 1–4: *P. syringae* pv. *tagetis*; lanes 5–47: corresponding to isolates numbered in Table 1.

DNA (approximately 250 ng) was spotted onto an Amersham Hybond-N+ nylon membrane (GE Healthcare, Little Chalfont, UK), which was then air-dried and baked at 80°C for 2 h. The PCR product from *P. syringae* pv. *tagetis* LMG 5090 was labeled with [α-^{32}P] dCTP using a random primer method according to the manufacturer's instructions (Ladderman Labeling Kit, Takara Bio, Otsu, Japan). The pre-hybridization and hybridization were conducted as described by Sambrook and Russell (2001). The membrane was exposed to an imaging screen (Fuji, Tokyo, Japan) for 3 h, and captured radiation was visualized using a Personal Molecular Imager system (Bio-Rad).

Results

Specificity of an AFLP-derived marker. In order to develop the pathovar-specific marker, 100 AFLP primer combinations (*Eco*RI + 3 / *Mse*I + 3) were tested with 4 isolates of *P. syringae* pv. *tagetis* and 12 isolates of *P. syringae* pathovars, including the closely related *P. syringae* pv. *helianthi* (data not shown). From this, a specific 594-bp amplicon for *P. syringae* pv. *tagetis* was cloned and sequenced. The sequence was analyzed for similarity with sequences in the National Center for Biotechnology Information (NCBI) GenBank database. BLASTN results showed that no significant similarity was found. Based on this sequence, the PSTF/PSTR primer set was designed to amplify a 554-bp amplicon.

The specificity of the designed primers was evaluated by testing all isolates shown in Table 1. The PCR product was amplified from only 4 isolates of *P. syringae* pv. *tagetis* from among 47 isolates of other pathovars and species of *Pseudomonas* and *Xanthomonas* (Fig. 1). These results indicate that the pathovar-specific primers are highly specific for detecting this pathogen.

DNA dot-blot analysis. To confirm whether the entire 554-bp amplicon was unique to *P. syringae* pv. *tagetis*, the amplicon was used as a probe against genomic DNA extracted from *P. syringae* pv. *tagetis* and other *Pseudomonas* and *Xanthomonas* isolates shown in Table 1. Positive signals were found in only 4 isolates of *P. syringae* pv. *tagetis* from among 47 isolates of other pathovars and species of *Pseudomonas* and *Xanthomonas*, including the closely related *P. syringae* pv. *helianthi* and other apical chlorosis-inducing *Pseudomonas* species (Fig. 2). This result revealed that this amplicon is highly conserved in *P. syringae* pv. *tagetis* and does not share considerable homology with other bacteria.

Discussion

The plant pathogen *P. syringae* pv. *tagetis* causes apical chlorosis and bacterial leaf spots in various Asteraceae, including the weeds common ragweed and dandelion (Gulya *et al*., 1981; Hellmers, 1955; Rhodehamel and Durbin, 1985; Rhodehamel and Durbin, 1989a; Shane and Baumer, 1984; Styer and Durbin, 1982). Since the isolation of this pathogen from weeds displaying apical chlorosis, it has been evaluated as a biological agent to control Canada thistle in soybean and woollyleaf bursage in cotton (Gronwald *et al*., 2002; Sheikh *et al*., 2001). Apical chlorosis-inducing *Pseudomonas* species other than this pathogen have also been reported in Canada thistle and pea (Suzuki *et al*., 2003; Zhang *et al*., 2002). However, pathovar-specific primers, which could be used for identifying a particular *P. syringae* pv. *tagetis*, are still lacking. Therefore, we utilized the AFLP technique to identify a specific polymorphic amplicons for *P. syringae* pv. *tagetis*. Polymorphic band produced only from this pathogen was cloned and sequenced. The sequence was used

to design pathovar-specific primers that precisely distinguished *P. syringae* pv. *tagetis* from other pathovars and species of *Pseudomonas* and *Xanthomonas* (Fig. 1).

Previously, Kong *et al*. (2004) described a PCR method for the identification of *P. syringae* pv. *tagetis* based on genes required for tagetitoxin production, but was unable to differentiate between *P. syringae* pv. *tagetis* and *P. syringae* pv. *helianthi* or *P. syringae* pv. *atrofaciens*. In contrast, the PCR technique described in this study was able to unambiguously differentiated 4 isolates of *P. syringae* pv. *tagetis* from among other *Pseudomonas* and *Xanthomonas* isolates, including both *P. syringae* pathovars (Fig. 1). Furthermore, a DNA dot-blot analysis using the PCR product as a probe showed a positive signal for all the *P. syringae* pv. *tagetis* (Fig. 2), confirming that the entire 554-bp amplicon was highly conserved in this pathogen. This fragment was analyzed by a BLASTN search and showed no significant matches with known nucleotide sequences. BLASTX results revealed that the sequences showed relatively low similarity (32%) to the hypothetical protein from *Paenibacillus* sp. WLY78. These results suggest that the specificity of the primers for *P. syringae* pv. *tagetis* described in the present study is due to the uniqueness of the DNA sequence within the amplified region.

In conclusion, the results presented herein indicate that this PCR-based assay could be a reliable and useful method for the specific detection of *P. syringae* pv. *tagetis* strains.

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References

Bereswill, S., Bugert, P., Volksch, B., Ullrich, M., Bender, C. L. and Geider, K. 1994. Identification and relatedness of coronatine-producing *Pseudomonas syringae* pathovars by PCR analysis and sequence determination of amplification products. *Appl. Environ. Microbiol.* 60: 2924–2930.

Gronwald, J. W., Plaisance, K. L., Ide, D. A. and Wyse, D. L. 2002. Assessment of *Pseudomonas syringae* pv. *tagetis* as a biocontrol agent for Canada thistle. *Weed Sci.* 50: 397–404.

Gulya, T. J., Urs, R. and Banttari, E. E. 1981. Apical chlorosis of sunflower caused by *Pseudomonas syringae* pv. *tagetis*. *Plant Dis.* 66: 598–600.

Hellmers, E. 1955. Bacterial leaf spot of African marigold (*Tagetes erecta*) caused by *Pseudomonas tagetis* sp. n. *Acta Agric. Scand.* 5: 185–200.

Kang, M. J., Lee, M. H., Shim, J. K., Seo, S. T., Shrestha, R., Cho, M. S., Hahn, J. H. and Park, D. S. 2007. PCR-based specific detection of *Ralstonia solanacearum* by amplification of cytochrome c1 signal peptide sequences. *J. Microbiol. Biotechnol.* 17: 1765–1771.

Kong, H., Patterson, C. D., Zhang, W., Takikawa, Y., Suzuki, A. and Lydon, J. 2004. A PCR protocol for the identification of *Pseudomonas syringae* pv. *tagetis* based on genes required for tagetitoxin production. *Biol. Control.* 30: 83–89.

Lydon, J. and Patterson, C. D. 2001. Detection of tabtoxin-producing strains of *Pseudomonas syringae* by PCR. *Lett. Appl. Microbiol.* 32: 166–170.

Mathews, D. E. and Durbin, R. D. 1990. Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and *Escherichia coli*. *J. Biol. Chem.* 265: 493–498.

Rhodehamel, N. H. and Durbin, R. D. 1985. Host range of strains of *Pseudomonas syrinage* pv. *tagetis*. *Plant Dis.* 69: 589–591.

Rhodehamel, N. H. and Durbin, R. D. 1989a. Two new hosts of *Pseudomonas syringae* pv. *tagetis*. *Plant Dis.* 73: 368. (Abstract)

Rhodehamel, N. H. and Durbin, R. D. 1989b. Toxin production by strains of *Pseudomonas syringae* pv. *tagetis*. *Physiol. Mol. Plant Pathol.* 35: 301–311.

Sambrook, J. and Russell, D. W. 2001. *Molecular cloning: a laboratory manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schaad, N. W., Cheong, S. S., Tamaki, S., Hatziloukas, E. and Panopoulos, N. J. 1995. A combined biological and enzymatic amplification (BIO-PCR) technique to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seed extracts. *Phytopathology* 85: 243–248.

Shane, W. W. and Baumer, J. S. 1984. Apical chlorosis and leaf spot of Jerusalem artichoke incited by *Pseudomonas syringae* pv. *tagetis*. *Plant Dis.* 68: 257–260.

Sheikh, T., Wheeler, T. A., Dotray, P. A. and Zak, J. C. 2001. Biological control of woollyleaf bursage (*Ambrosia grayi*) with *Pseudomonas syringae* pv. *tagetis*. *Weed Technol.* 15: 375–381.

Song, E. S., Kim, S. Y., Noh, T. H., Cho, H. J., Chae, S. C. and Lee, B. M. 2014. PCR-based assay for rapid and specific detection of the new *Xanthomonas oryzae* pv. *oryzae* K3a race using an AFLP-derived marker. *J. Microbiol. Biotechnol.* 24: 732–739.

Sorensen, K. N., Kim, K. H. and Takemoto, J. Y. 1998. PCR detection of cyclic lipodepsinonapeptide-producing *Pseudomonas syringae* pv. *syringae* and similarity of strains. *Appl. Environ. Microbiol.* 64: 226–230.

Steinberg, T. H., Mathews, D. E., Durbin, R. D. and Burgess, R. R. 1990. Tagetitoxin: a new inhibitor of eukaryotic transcription by RNA polymerase III. *J. Biol. Chem.* 265: 499–505.

Styer, D. J. and Durbin, R. D. 1982. Common ragweed: a new host of *Pseudomonas syringae* pv. *tagetis*. *Plant Dis.* 66: 71. (Abstract)

Suzuki, A., Togawa, M., Ohta, K. and Takikawa, Y. 2003. Occurrence of white top of pea caused by a new strain of *Pseudomonas syringae* pv. *pisi*. *Plant Dis.* 87: 1404–1410.

Zhang, W., Sulz, M., Mykietiak, T., Cole, D., Andreiuk, R., Tewari, J. P. and Harker, N. 2002. First report on Canada thistle disease caused by a bacterium in Alberta. *Can. J. Plant Pathol.* 24: 507. (Abstract)