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Genes responsible for coronatine synthesis in *Pseudomonas syringae* present in the genome of soft rot bacteria

Monika Slawiak · Ewa Lojkowska

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Abstract Primers for the PCR amplification of homologous genes encoding polyketide coronafacic acid and coronafacic ligase in the cells of *Pectobacterium atrosepticum* SCRI1043 (BX950851) were developed to study the presence of these genes in the genome of *Pectobacterium* sp. and *Dickeya* sp. Coronafacic ligase catalyses the formation of coronatine from polyketide coronafacic acid and coronamic acid. Coronatine is a toxin produced by *Pseudomonas syringae* and is one of the major virulence factors in this bacterium. This study using several strains of *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. isolated in different countries, indicated that all strains of *P. atrosepticum* possess genes coding coronafacic acid (*cfa* gene cluster) and coronafacic ligase (*cfl*). However, these genes were present only in the genome of five out of 50 tested *P. carotovorum* subsp. *carotovorum* strains and two out of 34 strains of *Dickeya* sp. tested. The PCR products homologous to the sequence of *cfa7* and *cfl* gene fragments were sequenced in order to check the level of homology between genes of *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. The sequences of the gene fragments amplified from all *P. atrosepticum* strains were almost identical (100% and 99.97%, respectively). The homology of the sequences obtained for *P. atrosepticum* and sequences of five *P. carotovorum* subsp. *carotovorum* and two *Dickeya* sp. was lower, between 89.69% to 95.00% for the *cfl* gene fragment, and about 94% for the *cfa7* gene fragment.

Keywords Coronafacic acid · Coronafacic ligase · *Dickeya* sp. · *Pectobacterium atrosepticum* · *Pectobacterium carotovorum* subsp. *carotovorum*

M. Slawiak · E. Lojkowska (✉)
Department of Biotechnology,
Intercollegiate Faculty of Biotechnology,
University of Gdansk and Medical University of Gdansk,
Kladki 24, 80-822 Gdansk, Poland
e-mail: lojkowsk@biotech.ug.gda.pl

The family *Enterobacteriaceae* includes several important plant pathogens such as *Pectobacterium atrosepticum* (formerly *Erwinia carotovora* subsp. *atroseptica*), *Pectobacterium carotovorum* subsp. *carotovorum* (previously *Erwinia carotovora* subsp. *carotovora*) and *Dickeya* sp. (earlier *Erwinia chrysanthemi*). *Pectobacterium atrosepticum* is an economically important pathogen restricted to potato in temperate regions, where it causes black leg in stems during the growing season and soft rot of the tubers during storage (Pérombelon and Kelman 1980; Sledz et al. 2000; Pérombelon 2002). The related soft rot bacteria *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. have broader host ranges, including potato, chrysanthemum, philodendron, dieffenbachia, cyclamen, corn, banana, carrot and cause disease in temperate and warm climates (Dickey 1979; Pérombelon 2002).

These three species of soft rot bacteria are responsible for crop losses worldwide. *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. are also able to live as epiphytes and endophytes on plants or as saprophytes in soil and ground water (Pérombelon and Kelman 1980; Pérombelon 2002), although much less is known about their non-pathogenic than pathogenic lifestyles. Moreover, the molecular basis of the differences in host range and the requirements for establishing disease symptoms are not fully understood. The pathogenesis of soft rot bacteria relies primarily on the prolific production of extracellular plant cell wall-degrading enzymes (PCWDEs) that cause extensive tissue maceration (Hugouvieux-Cotte-Pattat et al. 1996; Py et al. 1998). However, results of the study performed recently suggest that the process may be far more subtle and complex than previously thought (Toth et al. 2003). Sequencing of the genome of phytopathogens (da Silva et al. 2002; Salanoubat et al. 2002; Bell et al. 2004) has yielded a wealth of information on novel genes—candidates for phytopathogenicity determinants. Sequencing of the genome of *P. atrosepticum* strain SCRI1043 and assessing its similarity to other members of the *Enterobacteriaceae* and other plant pathogenic bacteria indicated a wide array of genes previously unknown in *P. atrosepticum*. This array of genes can be potentially involved in pathogenicity and metabolism.

Toxins are important components of the animal and human pathogenic enterobacteria. The presence of toxins is closely associated with differences in disease phenotypes and often with horizontal gene transfer (HGT). Plant pathogenic bacteria also produce toxins. The bacterial pathogen *Pseudomonas syringae* synthesises a range of toxins, including the nonribosomal peptide syringomycin and the type I polyketide coronatine (Bender et al. 1999). Coronatine (COR) produced by *P. syringae* (pvs *atropurpurea, glycinea, maculicola*, *morsprunorum* and *tomato*) causes diffuse chlorosis on a wide variety of plant species and is one of the major virulence factors. It is formed by the conjugation of the polyketide coronafacic acid (CFA) to coronamic acid (CMA). In addition, CFA may independently be coupled to other amino acids, forming a variety of coronafacoyl conjugates. CFA is synthesised by the *cfa* gene cluster and CMA by the *cma* operon. Conjugation of CFA to CMA is enabled by coronafacic ligase (Cfl) (Bender et al. 1999). *Pectobacterium atrosepticum* SCRI1043 (BX950851) possesses an entire *cfa* operon, which is similar in structure and organisation to that in *P. syringae.* This strain also has the *cfl* gene but lacks genes which are similar to those of the *cma* operon, suggesting that COR is not synthesised by *P. atrosepticum* (Toth et al. 2006). Mutations in the genes *cfa6* and *cfa7* significantly reduce *P. atrosepticum* pathogenicity in potato, and suggest that CFA does play an important role in the virulence of *P. atrosepticum* (Bell et al. 2004). However, to date, there is no information on whether *P. atrosepticum* synthesises other coronofacoyl conjugates as has been observed for *P. syringae* (Bender et al. 1999; Toth et al. 2006). The *cfa* and syringomycin genes are found in the *P. atrosepticum* genome on horizontally acquired islands (HAIs) 2 and 6, respectively, suggesting they have been acquired by HGT during the evolution of *P. atrosepticum* (Toth et al. 2006).

The aim of the present work was to study whether strains of *P. atrosepticum* from different origins and also other soft rot bacteria possess genes that are homologous to genes encoding CFA and Cfl.

For the detection of the genes from the *cfa* operon and also the *cfl* gene, PCR primers were designed on the basis of the sequences of *cfl*, *cfa6* and *cfa7* genes of *P. atrosepticum* SCRI1043 available in GenBank (www.ncbi.nlm.nih.gov). The sequences of the six 20-mer oligonucleotides cfl-1F, cfl-2R, cfa6-1F, cfa6-2R, cfa7-1F, and cfa7-2R are shown in Table 1. The primers amplified a 685 bp fragment of the *cfl* gene, 761 bp fragment of the *cfa6* gene and 683 bp fragment of the *cfa7* gene from the genome of *P. atrosepticum* SCRI1043.

In this study, 11 strains of *P. atrosepticum*, 11 strains of *P. carotovorum* subsp. *carotovorum* and 34 strains of *Dickeya* sp. isolated in different countries were tested for the presence of the above genes (Table 2). In addition, 39 strains of *P. carotovorum* subsp. *carotovorum* isolated in Poland were tested for the presence of *cfa* and *cfl* genes. *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. isolates were grown on trypticase soy agar (TSA) (Becton Dickinson) at 27°C for 48 h. Bacterial suspensions were prepared in sterile double distilled water at $OD_{600\ nm}$=0.1. PCR was performed in 25 μl of reaction mixture containing 0.6 μM of each primer, 250 μM of dNTPs, 2.5 mM of $MgCl_2$, 1 U of *Pfu* DNA polymerase (Fermentas, Life Science, Lithuania), and 1 μl of bacterial suspension

Table 1 Characteristics of the PCR primers designed and tested in this study

Gene	Primers	Sequence (5'-3')	Optimal annealing temperature (°C)	Position of forward and reverse primers[a]	Size of the amplicon
cfl	cfl-1F	AATCCAGCGAATAGCCACAG	55.0	105–770	685 bp
	cfl-2R	TGAAGGTGTTCCTGCAATCC			
	cfl-3F	ATCAGGAACGACAGGACACC	52.9	504–1100	616 bp
	cfl-4R	GTCATGCCCATTCCAAATTC			
	cfl-5F	ATGGATTGCAGGAACACCTT	53.3	768–1462	714 bp
	cfl-6R	ATTTTTCCCGTGGTGTTGAC			
cfa6	cfa6-1F	AACGGGCATAACCTCAACTG	61.5	4249–4990	761 bp
	cfa6-2R	TGCAGTACGGTATCGAGCAG			
cfa7	cfa7-1F	GCTGCCTACCTATCCCTTCC	53.6	2769–3432	683 bp
	cfa7-2R	CTCCCAGTTCCGCATGAC			
	cfa7-3F	TGTAGTGATATCCGGCGACA	58.0	2241–2920	699 bp
	cfa7-4R	TGTCTGGCTGCGTCTAATTG			
	cfa7-5F	GTGTATTCCCACGTCCATCC	57.3	3084–3825	761 bp
	cfa7-6R	ACCTGTTTTGCGGAACAATC			
	cfa7-7F	CAGCGAATATGCGTTACGAC	57.4	3648–4289	661 bp
	cfa7-8R	TCGCGGGGTGAGTATTTTAC			
	cfa7-9F	TGGAGAGAATGTCCCGAATC	58.0	4107–4792	705 bp
	cfa7-10R	CTGACCGCGGAAGAATAGAG			
	cfa7-11F	TGGAATCTGCATCAGCTCAC	57.4	4744–5331	607 bp
	cfa7-12R	CCATTTCCACCGAGGTAAGA			
	cfa7-13F	TCTTACCTCGGTGGAAATGG	58.2	5331–5990	679 bp
	cfa7-14R	CATTGTGGCTTCATTCATGG			

[a] Refer to *P. atrosepticum* SCRI1043 *cfl*, *cfa6* and *cfa7* genes respectively

as a template. The amplification programme consisted of an initial denaturation at 95°C for 3 min, followed by 32 cycles of denaturation at 94°C for 1 min, annealing for 1 min at the temperature given for each pair of primers in Table 1 and extension at 72°C for 2 min, with a final extension at 72°C for 5 min, and stored at 4°C until used. Amplicons were visualised by ethidium bromide staining after electrophoresis in a standard 2% agarose (Prona Agarose, Spain) gel. A 100 (Fermentas, Life Science, Lithuania) molecular marker was used.

Amplicons of 685 bp of the *cfl* gene, 761 bp of the *cfa6* gene and 683 bp of the *cfa7* gene were obtained from all *P. atrosepticum* strains tested, five *P. carotovorum* subsp. *carotovorum* as well as two *Dickeya* sp. strains. No amplicons were obtained from the other tested isolates of *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. (Table 2).

Two genes, *cfl* and *cfa7,* were chosen to study homology among sequences of the genes amplified from *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. Therefore, to obtain longer fragments of these genes two additional pairs of primers for the *cfl* gene and six additional pairs of primers for the *cfa7* gene were designed (Table 1). The new primer oligonucleotides amplified PCR products from all *P. atrosepticum* strains tested and also from five *P. carotovorum* subsp. *carotovorum* and two *Dickeya* sp. strains. PCR conditions were identical to those previously described except for annealing temperatures shown in Table 1. Amplicons obtained from five *P. atrosepticum* strains (UG46A1, UG1055, UG1076, UG1092, UGCA16), three *P. carotovorum* subsp. *carotovorum* strains (UGC30, UGC31, UGC33) and two *Dickeya* sp. strains (UGCH20, UGCH24) were subsequently sequenced (PPNT, Gdynia, Poland).

All strains homologous to *cfa* and *cfl* genes were amplified by PCR and confirmed as belonging to *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* or *Dickeya* sp. by double checking their identity with biochemical methods (Hyman et al. 1998), PCR amplification with earlier described primers (Darrasse et al. 1994; DeBoer and Ward 1995; Nassar et al.

Table 2 Strains used in the present study and the presence of *cfl, cfa6* and *cfa7* genes in their genomes

Isolate designation	Isolate orgin	PCR product			*recA* PCR-RFLP profile (Waleron et al. 2002)
		cfl	*cfa6*	*cfa7*	
P. atrosepticum					
UG46A/1	Poland [f]	+	+	+	1
UG1039	UK, 1039 [e]	+	+	+	1
UG1054	Israel, 1054 [e]	+	+	+	1
UG1055	UK, 1055 [e]	+	+	+	1
UG1056	UK, 1056 [e]	+	+	+	1
UG1076	Poland [f]	+	+	+	1
UG1088	UK, 1088 [e]	+	+	+	2
UG1091	UK, 1091 [e]	+	+	+	2
UG1092	UK, 1092 [e]	+	+	+	1
UGCA12	UK, 1043 [e]	+	+	+	2
UGCA16	Canada, 1086 [e]	+	+	+	2
P. carotovorum subsp. *carotovorum*					
UGC1	UK, 103 [e]	-	-	-	3
UGC13	Tasmania, 147 [e]	-	-	-	4
UGC25	Peru, 171 [e]	+	+	+	7
UGC28	Peru, 174 [e]	-	-	-	8
UGC30	Peru, 176 [e]	+	+	+	9
UGC31	Peru, 178 [e]	+	+	+	10
UGC32	Peru, 179 [e]	+	+	+	11
UGC33	Peru, 180 [e]	+	+	+	7
UGC41	UK, 249 [e]	-	-	-	12
UGC63	Tasmania, 159 [e]	-	-	-	5
UGC88	Finland, SCC3193 [g]	-	-	-	3
UG1a/1	Poland [f]	-	-	-	3
UG1a/3	Poland [f]	-	-	-	3
UG1a/4	Poland [f]	-	-	-	3
UG15a/3	Poland [f]	-	-	-	3
UG17b/3	Poland [f]	-	-	-	3
UG36a/1	Poland [f]	-	-	-	3
UG36a/2	Poland [f]	-	-	-	3
UG36a/6	Poland [f]	-	-	-	3
UG25a/1	Poland [f]	-	-	-	nt
UG25a/2	Poland [f]	-	-	-	nt
UG25a/7	Poland [f]	-	-	-	nt
UG25a/9	Poland [f]	-	-	-	nt
UG42a/1	Poland [f]	-	-	-	nt
UG42a/2	Poland [f]	-	-	-	nt
UG43b/1	Poland [f]	-	-	-	18
UG47a/1	Poland [f]	-	-	-	nt
UG56a/1	Poland [f]	-	-	-	nt
UG56a/2	Poland [f]	-	-	-	nt
UG57a/2	Poland [f]	-	-	-	nt
UG64a/5	Poland [f]	-	-	-	nt
UG64a/12	Poland [f]	-	-	-	nt
UG65a/1	Poland [f]	-	-	-	nt
UG65a/9	Poland [f]	-	-	-	nt

Table 2 (continued)

Isolate designation	Isolate orgin	PCR product			*recA* PCR-RFLP profile (Waleron et al. 2002)
		cfl	*cfa6*	*cfa7*	
UG91b/1	Poland [f]	-	-	-	nt
UGC93bIII/1	Poland [f]	-	-	-	nt
UGC93bIII/2	Poland [f]	-	-	-	nt
UG93bIV/1	Poland [f]	-	-	-	nt
UG93bIV/2	Poland [f]	-	-	-	nt
UG104b/1	Poland [f]	-	-	-	nt
UG128a/1	Poland [f]	-	-	-	nt
UG128a/2	Poland [f]	-	-	-	nt
UG132b/1	Poland [f]	-	-	-	nt
UG132b/2	Poland [f]	-	-	-	nt
UG139a/1	Poland [f]	-	-	-	nt
UG139a/2	Poland [f]	-	-	-	nt
UG139b/1	Poland [f]	-	-	-	nt
UG139b/2	Poland [f]	-	-	-	nt
UG147a/2	Poland [f]	-	-	-	nt
UG152b/1	Poland [f]	-	-	-	nt
Dickeya sp.					
UGCH1	Martinique, ET3 [b]	-	-	-	34
UGCH4	Denmark, 1240 [b]	-	-	-	34
UGCH5	France, SF109-1 [b]	-	-	-	38
UGCH6	USA, EC16 [a]	-	-	-	38
UGCH7	India, 4072 [e]	-	-	-	36
UGCH8	USA, 1248 [b]	-	-	-	33
UGCH10	Comoro Islands, 1269 [e]	-	-	-	37
UGCH11	Peru, 4040 [e]	-	-	-	34
UGCH15	UK, 4067 [e]	-	-	-	38
UGCH16	France, 3937 [a]	-	-	-	34
UGCH18	St. Lucia, 4062 [e]	-	-	-	46
UGCH20	White Russia, ENA49 [a]	+	+	+	43
UGCH23	USA, B374 [a]	-	-	-	35
UGCH24	Germany, 4074 [e]	+	+	+	37
UGCH25	Italy, 1346 [b]	-	-	-	38
UGCH26	France, 1888 [b]	-	-	-	33
UGCH27	USA, 1891 [b]	-	-	-	39
UGCH28	The Netherlands, 502 [d]	-	-	-	45
UGCH32	The Netherlands, 976 [d]	-	-	-	45
UGCH35	The Netherlands, 998 [d]	-	-	-	45
UGCH39	Salomon Islands, 4061 [e]	-	-	-	34
UGCh40	Colombia, 1065 [e]	-	-	-	36
UGCH44	France, 4063	-	-	-	34
UGCH51	Peru, CIP366 [b]	-	-	-	34
UGCH54	USA, 260 [c]	-	-	-	36
UGCH59	Spain, 1483 [e]	-	-	-	35
UGCH64	Peru, 598 [d]	-	-	-	34
UGCH66	Peru, 597 [d]	-	-	-	47
UGCH71	Germany, 1260 [e]	-	-	-	34
UGCH74	The Netherlands, 996 [d]	-	-	-	42

Table 2 (continued)

Isolate designation	Isolate orgin	PCR product			*recA* PCR-RFLP profile (Waleron et al. 2002)
		cfl	*cfa6*	*cfa7*	
UGCH77	The Netherlands, 1651 [d]	-	-	-	34
UGCH85	Germany, 175 [d]	-	-	-	33
UGCH86	Taiwan, 655 [d]	-	-	-	39
UGCH87	Australia, 647 [d]	-	-	-	35

nt, not tested

+ Presence of the PCR product

− Absence of the PCR product

[a] Institut National des Sciences Appliquées de Lyon, Lyon, France

[b] Y. Bertheau, Institut National de la Recherche Agronomique, Paris, France

[c] K. Geider, Max-Planck-Institut für Zellbiologie, Rosenhof, Germany

[d] J. M. van der Wolf, Plant Research International, Wageningen, The Netherlands

[e] I. Toth, Scottish Crop Research Institute, Invergowie, Dundee, UK

[f] Department of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, Gdansk, Poland

[g] H. Saarilahti, University of Helsinki, Helsinki, Finland

1996; Jafra et al. 1998), and restriction analysis of the *recA* gene (Waleron et al. 2002). PCR-RFLP *recA* gene analysis showed that the *P. atrosepticum* strains belonged to two different profiles (Table 2). In addition, the tested strains represent different serogroups (Toth, personal information). All *P. atrosepticum* strains were isolated from potatoes in different countries (UK, Poland, Israel, and Canada). *Pectobacterium carotovorum* subsp. *carotovorum* strains containing *cfl* and *cfa* genes were isolated in Peru from potato plants with black leg symptoms. The two strains of *Dickeya* sp. were isolated in White Russia and Germany from potato and dieffenbachia, respectively.

The pathogenicity test performed on potato slices showed that strains of *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. with genomic copies of *cfl* and *cfa* genes caused more severe disease symptoms in comparison with those for which the predicted product was not amplified in PCR using the specified primers (Table 3). Bell et al. (2004) showed that disruption of CFA biosynthesis resulted in significantly reduced black leg symptoms caused by *P. atrosepticum*. Also work of Pitman et al. (2008) showed that the closely related to *P. atrosepticum* strain NZEC20 lacked the *cfa* biosynthetic cluster and did not induce black leg. These data together with our results indicate that the *cfa* gene

Table 3 Comparison of the width of rotting tissue following inoculation with *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, and *Dickeya* sp. strains possessing or not *cfa* operon and *cfl* gene

Soft rot bacteria	Number of tested strains	Presence of *cfa* operon and *cfl* gene	Diameter of rotting tissue (mm)[a]	
			10^7cfu ml^{-1}	10^9cfu ml^{-1}
P. atrosepticum	6	+	7.76±4.41	10.93±3.23
P. carotovorum subsp. *carotovorum*	3	+	14.15±1.54	16.19±1.47
P. carotovorum subsp. *carotovorum*	3	−	6.18±3.50	11.40±1.12
Dickeya sp.	2	+	10.22±0.94	12.67±3.38
Dickeya sp.	2	−	5.13±2.3	6.39±0.61

[a] Potato slices were inoculated with 25 µl of bacterial suspension (10^7, 10^9 cfu ml^{-1}) and maintained at 27°C and a relative humidity of 92%. Diameter of rotting tissue was measured within 72 h. Presented values are means for all tested strains; experiments were repeated three times

Table 4 Percent of differences between the nucleotide sequences of the *cfl* and *cfa7* gene fragments DNA amplified from tested isolates

Gene	Size of the sequenced DNA fragment	Isolate	% of difference between compared gene fragments isolated from tested isolates									
			UG46A/1	UG1055	UG1076	UG1092	UGCA12	UGCA16	UGC30	UGC31	UGC33	UGCH20	UGCH24
cfl	1.36-kb	UG46A/1		0.00	0.00	0.00	0.00	0.00	5.73	5.73	5.73	10.31	6.07
		UG1055			0.00	0.00	0.00	0.00	5.73	5.73	5.73	10.31	6.07
		UG1076				0.00	0.00	0.00	5.73	5.73	5.73	10.31	6.07
		UG1092					0.00	0.00	5.73	5.73	5.73	10.31	6.07
		UGCA12						0.00	5.73	5.73	5.73	10.31	6.07
		UGCA16							5.73	5.73	5.73	10.31	6.07
		UGC30								0.00	0.00	12.04	7.04
		UGC31									0.00	12.04	7.04
		UGC33										12.04	7.04
		UGCH20											10.97
		UGCH24											
cfa7	3.67-kb	UG46A/1		0.03	0.03	0.00	0.03	0.03	4.27	4.27	4.27	5.82	5.28
		UG1055			0.00	0.03	0.00	0.00	4.24	4.24	4.24	5.79	5.25
		UG1076				0.03	0.00	0.00	4.24	4.24	4.24	5.79	5.25
		UG1092					0.03	0.03	4.27	4.27	4.27	5.82	5.28
		UGCA12						0.00	4.24	4.24	4.24	5.79	5.25
		UGCA16							4.24	4.24	4.24	5.79	5.25
		UGC30								0.00	0.00	7.21	3.24
		UGC31									0.00	7.21	3.24
		UGC33										7.21	3.24
		UGCH20											7.34
		UGCH24											

cluster could be a pathogenicity determinant of the black leg and soft rot diseases caused by *P. atrosepticum*. The next step to explain the role of the *cfa* gene cluster in *P. atrosepticum*, *P. carotovorum* and *Dickeya* sp. would be pathogenicity testing of mutants with disarmed *cfa* or *cfl* genes.

The PCR amplicons (*cfa* and *cfl* gene fragments) were sequenced twice with forward and reverse primers after purification using clean-up columns (A&A Biotechnology, Poland). Three pairs of primers were used to obtain the sequence of a 1,367 bp fragment of the *cfl* gene and seven pairs of primers were used to obtain a 3,677 bp fragment of the *cfa7* gene.

The similarities between the sequences of *P. atrosepticum* SCRI1043 and the sequences obtained from five isolates of *P. atrosepticum* were 100% for the *cfl* gene and 99.97% for the *cfa7* gene. This suggests a high degree of relatedness among the tested *P. atrosepticum* strains (Table 4). The analysis of the sequences coding CFA and Cfl proteins performed for the three isolates of *P. carotovorum* subsp. *carotovorum* indicated 100% homology between three of them, and 94.27% (*cfl* gene fragment) and 95.76% (*cfa7* gene fragment) homology with *P. atrosepticum* SCRI1043 (Table 4). However the relatedness between strains of *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. are distant, since the homology of the *cfl* and *cfa* gene sequences is, respectively, high. The conservation of the COR gene cluster in *P. syringae* pathovars, which produce the phytotoxin, has also previously been demonstrated (Bender et al. 1991; Bereswill et al. 1994). The region amplified by PCR and described by Bereswill et al. (1994) was highly effective for the detection of COR-producing *P. syringae* strains, and the sequence analysis of PCR products proved valuable in showing relatedness between strains and pathovars.

Although only one of the two gene clusters required for COR synthesis in *P. syringae* is present in *P. atrosepticum* strains, this cluster may play an important role in disease development, possibly through the production of an alternative polyketide phytotoxin (Bell et al. 2004). Polyketide phytotoxins have not previously been identified in enterobacterial plant pathogens but are important pathogenicity determinants in *P. syringae*. The present study indicates that not only do all tested strains of *P. atrosepticum* contain genes coding CFA and Cfl, but so do some of the strains of *P. carotovorum* subsp. *carotovorum* and *Dickeya* sp. The results suggest that *P. carotovorum* and *Dickeya* sp. *cfa* and *cfl* genes may have been horizontally acquired as previously proposed for *P. atrosepticum* by Bell et al. (2004).

The discovery that a wide spectrum of highly virulent strains of *P. atrosepticum* possess *cfa* and *cfl* genes may indicate the importance of these genes for the development of disease symptoms. Additional work will determine whether the *cfa* gene cluster can be used as a molecular marker for the detection of *Pectobacterium* and *Dickeya* strains with the ability to cause black leg symptoms in potato.

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