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Phenotypic and genotypic characteristics of Iranian soft rot bacterial isolates from different hosts

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Summary. During 2005–2006, 42 soft rot bacterial strains were isolated from the infected tubers of potato, roots of carrot, sugar beet and turnip, and the leaves of lettuce and cabbage with soft rot symptoms in Iran. The isolates were rod-shaped, motile with peritrichous flagella, gram negative, facultative anaerobe, oxidase and urease negative and they rotted potato tuber slices. Of the 42 isolates, 20 were identified as *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*), 6 as *P. carotovorum* subsp. *odoriferum* (*Pco*), 4 as *P. betavasculorum* (*Pb*) and 12 strains as *Dickeya dadantii* (*Dda*). PCR amplification of fingerprints of repetitive bacterial DNA elements using the REP, ERIC and BOX primers differentiated the soft rot bacteria to the species and subspecies level. Strains of *Pcc* and *Dda* were phenotypically and genotypically highly variable, but *Pb* and *Pco* strains had low variability. REP-PCR was found to be a promising genotypic tool for the rapid and reliable speciation and typing of soft rot bacteria..

Key words: *Pectobacterium, Dickeya,* Rep-PCR.

Introduction

The family of the *Enterobacteriaceae* groups various species of plant pathogenic bacteria that cause soft rot in a wide range of plants (Toth *et al*., 2003). Based on 16 ribosomal DNA (rDNA), Hauben *et al*. (1998), offered to re-establish the genus *Pectobacterium* which now comprises four species: *Pectobacterium carotovorum, P. atrosepticum*, *P. wasabiae* (*Pw*) and *P. betavasculorum (Pb)*. *P. carotovorum* was further divided into two subspecies: *P. carotovorum* subsp. *carotovorum* (*Pcc*) and *P. carotovorum* subsp. *odoriferum* (*Pco*) (Helias *et al*., 1998; Garden *et al*., 2003). Samson *et al*. (2005) transferred *Pectobacterium chrysanthemi* to the genus *Dickeya* as *Dickeya chrysanthemi* and defined four novel species: *D. dadantii (Dda), D. dianthicola*, *D. dieffenbachiae* and *D. zeae*. The biochemical characteristics of 6 species of *Dickeya* spp. have recently been described (Palacio-Bielsa *et al*., 2006). *Pcc*, *Pb* and *Dickeya* spp. are bacterial pathogens of important crops world-wide. However, the other species and subspecies are also important (Dickey, 1979; Pérombelon and Kelman 1980). Realizing the diversity within and the relationship between pathogenic taxa is a prerequisite to identifying, detecting and studying pathogens epidemiologiy (Avrova *et al*., 2002). Current identification techniques are based mainly on biochemical and phenotypic characteristics, with which rapid and precise identification is not always possible (Kwon *et al*., 1997). Biochemical tests are currently accepted as a standard to identify and characterize pathogenic bacteria, but they are very much time consuming (Toth *et al*., 2001). The commonly used approaches for the diagnosis and identification of pathogens include biochemical and physiological characteristics, pathogenicity tests, as well as serological techniques and techniques based on the polymerase chain reaction (PCR). Repetitive sequence based genomic fingerprinting (REP-PCR), which uses primers matching the endogenous interspersed repetitive sequences, is one

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of the PCR-based techniques used to identify and classify bacteria (Versalovic *et al*., 1991; Louws *et al*., 1995 and 1999). This technique is based on the PCR-mediated amplification of DNA sequences located between specific interspersed sequences of highly conserved elements in prokaryotic genomes. These repeated sequences are named BOX, REP, and ERIC elements (Versalovic *et al*., 1994). The method has been applied to members of diverse bacterial genera. Iranian soft rot bacteria have been reported to be diverse in studies using phenotypic characteristics and whole-cell protein electrophoresis (Ahmadvand and Rahimian, 2002; Soltani-Nejad *et al*., 2005; Mahmoudi *et al*., 2007; Zohour Paralak *et al*., 2007). The aim of this study was to characterize Iranian soft rot bacteria strains on the basis of biochemical, physiological and REP-PCR genomic fingerprinting, and to determine whether REP-PCR differentiated the current species and subspecies of *Pectobacterium* and *Dicheya* isolates in Iran.

Materials and methods

Bacterial strains and isolation

Forty-two isolates from diseased plants, including potato, cabbage, carrot, onion, sugar beet, pepper and lettuce, were recovered in 2006–2007 from different counties in the Fars Province of Iran (Table 1). A small piece of tissue from the periphery of decayed lesions of these plants was homogenized in sterile water, and a loopful of the suspension was streaked on Eosin methylen blue agar (EMB) and nutrient agar (NA) (Mahmoudi *et al*., 2007). Colonies were further purified on NA. Reference strains of *Pcc* (EccSCRI 193), *Dickeya* sp. (EchSCRI 3739) and *Pb* (SCRI 479) from the Scottish Crop Research Institute (SCRI, Dundee, UK) and the type strain of *Pco* (CFBP 1878) from the Collection Française de Bactéries Phytopathogènes (Beaucouzé, France) were also used in this study.

Physiological and biochemical tests

The 42 isolates were compared on the basis of the following biochemical, physiological and nutritional characteristics: gram reaction, catalase production, phosphate activity, oxidase production, growth at 37ºC, nitrate reduction, reducing substance from sucrose, indole production, erythromycin sensitivity, growth on 5% NaCl, urease and levan production, lecithinase, Tween 80 hydrolysis, utilization of inulin, citrate, malonate and α-methyl glucoside (De Boer and Kelman 2001; Schaad *at al*., 2001), fermentative metabolism, arginine dihydrolase, H_2S production from cystein, potato soft rot, gelatin liquefaction, acetoin production (VP), and gas from glucose (Schaad *et al*., 2001). Carbohydrate utilization was determined using the basal medium of Ayers *et al*., (1919) from glucose, fructose, rhamnose, arabianose, sorbitol, raffinose, melibiose, trehalose, galactose, cellobiose, lactose, maltose, mannose, and arabitol and the results were evaluated daily for 1 month (Fahy and Persley 1983).

DNA Extraction

The SDS-boiling method was used for DNA extraction (Mahmoudi *et al*., 2007). Total DNA was extracted from bacterial culture in a Luria Bertani (LB) medium (0.5% yeast extract, 1% tryptone, 1% NaCl). After 24 h, 3 ml of culture was removed and centrifuged at 14000 *g* for 3 min. The pellet was resuspended in 500 mL extraction buffer (0.2 M tris-HCl, pH 8.0; 0.2 M EDTA; 2% SDS) and boiled at 100ºC for 8 min. Then 250 ml phenol and 250 ml chloroform-isoamyl alcohol (24:1, v:v) were added to the tube and centrifuged at 13000 *g* for 5 min. The supernatant was transferred to a new tube. To eliminate the RNA, 3 ml of RNase-A was added to the tube, the nucleic acid solution was incubated at 37ºC for 1 h and then extracted with chloroform-isoamyl alcohol (24:1, v:v). The mixture was centrifuged at 13000 *g* for 5 min and the upper phase was transferred to a new tube. DNA was precipitated with an equal volume of cool isopropanol, washed with 70% ethanol:water (v:v), dried, resuspended in 50 ml of TAE (4% M Tris-HCl, 20 mM sodium acetate, 2 mM EDTA) buffer, and used for PCR amplification.

Species-specific PCR for identification of *Pcc*

The oligonucleotide primers EXPCCR (5'-CCGTAATTGCCTACCTGCTTAAG-3') and EXPCCF (5'-AACTTCGCACCGCCGACCTTCTA-3') were used in a standard PCR assay (Kang *et al*., 2003). The PCR reactions were performed in a Bio-Rad I-cycler (USA) in 25 mL PCR mixture containing 10 mM tris-HCL, 1.5 mM $MgCl_2$, 200 mm of each dNTPmix, 100 ng of primers, 1 unit of Taq poly-

Table 1. Characteristics of soft rot bacteria isolates and reference strains used in this study.

Bacterial strain	Bacterial species[a]	Host	Rep-Cluster[b]
Po_1	*Pcc*	Potato	1
Po_2	*Pcc*	Potato	1
Po_3	*Pcc*	Potato	1
Po_4	*Pcc*	Potato	1
Po_5	*Dda*	Potato	4
Po_6	*Dda*	Potato	4
Po_7	*Dda*	Potato	4
Po_8	*Pco*	Potato	3
Po_9	*Pco*	Potato	3
Po_{10}	*Pco*	Potato	3
Po_{11}	*Pco*	Potato	3
L_1	*Pcc*	Lettuce	1
L_2	*Pcc*	Lettuce	1
L_3	*Dda*	Lettuce	4
S_1	*Pcc*	Sugar beet	1
S_2	*Pcc*	Sugar beet	1
S_3	*Pcc*	Sugar beet	1
S_4	*Dda*	Sugar beet	4
S_5	*Pb*	Sugar beet	2
S_6	*Pb*	Sugar beet	2
S_7	*Pb*	Sugar beet	2
S_8	*Pb*	Sugar beet	2
Pe_1	*Pcc*	Pepper	1
Pe_2	*Pcc*	Pepper	1
Pe_3	*Dda*	Pepper	4
T_1	*Pcc*	Turnip	1
T_2	*Pcc*	Turnip	1
T_3	*Dda*	Turnip	4
T_4	*Dda*	Turnip	4
T_5	*Pco*	Turnip	3
Cr_1	*Pcc*	Carrot	1
Cr_2	*Pcc*	Carrot	1
Cr_3	*Dda*	Carrot	4
Cb_1	*Pcc*	Cabbage	1
Cb_2	*Pcc*	Cabbage	1
Cb_3	*Pcc*	Cabbage	1
Cb_4	*Dda*	Cabbage	4
Cb_5	*Pco*	Cabbage	3
O_1	*Pcc*	Onion	1
O_2	*Pcc*	Onion	1
O_3	*Dda*	Onion	4
O_4	*Dda*	Onion	4
Ecc SCRI 193	*Pcc*	Potato	1
Pb SCRI 479	*Pb*	Sugar beet	2
Pco CFBP 1878	*Pco*	*Cichorium intybus*	3
Ech SCRI 3739	*Dda*	Potato	4

[b] Classification on the basis of biochemical and physiological properties. See Table 2.
[a] Cluster analysis on the basis of REP-PCR with REP, ERIC and BOX primers. See Figure 4.
[c] *Pcc* EccSCRI 193.
[d] *Pco* CFBP 1878.
[e] *P. betavasculorum* SCRI 479
[f] *D.dadantii* EchSCRI 3739.

merase (Metabion, Martinsried, Germany) and 1 mL of DNA template. The PCR reaction was carried out as follows: 94ºC for 4 min, for initial denaturation; 30 cycles of 94ºC for 1 min; 60ºC for 1 min; and 72ºC for 2 min, followed by a final elongation step of 72ºC for 7 min. PCR products were analyzed on 1% agarose gel in TAE buffer and visualized by staining with ethidium bromide (Sambrook *et al*., 1989).

REP-PCR

Repetitive PCR (REP-PCR) was used with ERIC, BOX and REP primers as described by Louws *et al*. (1995). The ERIC, BOX, and REP primer sets were synthesized by Metabion. Amplification was performed in a Bio-Rad I-cycler (Hercules, CA, USA) in 25 ml volumes containing 200 mM of each dNTPmix, 2 mM $MgCl_2$, 1.5 pM primers, 1U of Taq polymerase and 4 mL of DNA template. Thermal cycling was carried out as described by Louws *et al*. (1994): an initial denaturation cycle at 95ºC for 7 min, 30 cycles of denaturation at 94ºC for 1 min, annealing at 52ºC (ERIC), 53ºC (BOX) and 44ºC (REP) for 1 min, extension cycle at 65ºC for 8 min, and a final extension cycle at 65ºC for 15 min. The PCR products were separated on 1.5% gel electrophoresis agarose in TAE buffer (Sambrook *et al*., 1989). After staining with ethidium bromide, the gels were viewed and photographed under UV illumination.

Data analysis

Data were statistically analyzed using the numerical taxonomy and multivariate analysis system (NTSYSPC) software package version 2.1. Forty phenotypic characters were included in the analysis. Genetic Relationships within and between strains were determined by cluster analysis performed by UPGMA on distance matrices calculated with the jaccard coefficient (Rohlf, 2000).

Results

Phenotypic characteristics

The biochemical and physiological properties of the bacterial isolates are shown in Table 2. The 42 strains isolated from various hosts in Iran were identified as *Pcc* (20 isolates), *Pco* (6 isolates), *Pb* (4 isolates) and *Dda* (12 isolates).

Species-specific PCR for *Pcc*

The oligonucleotide primers EXPCCF and EXPCCR were used to identify the Iranian *Pcc* strains from the various hosts. All strains of *Pcc* produced the expected 550 bp product after amplification with the primers (Figure 1). No PCR product was amplified with strains from the other group, *Pco*, *Pb*, or *Dda*.

REP-PCR

Primers corresponding to conserved sequences of the REP, ERIC and BOX elements annealed to genomic DNA and generated unique genomic fingerprints for the strains of *Pcc, Pb*, *Pco* and *Dda* tested. The fingerprint of strains of *Pcc*, and *Dda* are shown in Figures 2 and 3. REP, BOX, and

Table 2. Phenotypic and biochemical characteristics of Iranian soft rot bacteria isolates from different hosts.

Characteristic	Identified species				Reference strain[b]			
	Pcc (20)[a]	*Pco* (6)	*Pb* (4)	*Dda* (12)	*Pcc*	*Pco*	*Pb*	*Dda*
Gram negative reaction	100[d]	100	100	100	_[c]	_	_	_
Catalase production	100	100	100	100	+	+	+	+
Phosphatase activity	0	0	0	100	_	_	_	+
Oxidase production	0	0	0	0	_	_	_	_
Growth at 37°C	100	100	100	100	+	+	+	+
Nitrate reduction	100	33	100	100	+	+	+	+
Fermentative metabolism	100	100	100	100	+	+	+	+

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(Table 2 continued)

Characteristic	Identified species[a]				Reference strain[b]			
	Pcc (20)	*Pco* (6)	*Pb* (4)	*Dda* (12)	*Pcc*	*Pco*	*Pb*	*Dda*
Arginine dihydrolase	0	0	0	66	–	–	–	–
H_2S production from cystein	100	100	100	100	+	+	+	+
Potato soft rot	100	100	100	100	+	+	+	+
Gelatin liquefaction	65	50	50	100	+	+	+	+
Acetoin production (VP)	100	100	100	100	+	+	+	+
Gas from glucose	0	0	0	91	–	–	–	+
Reducing substrate from sucrose	0	100	100	0	–	+	+	–
Indole production	0	0	0	100	–	–	–	+
Erythromycine sensitivity	10	0	0	100	–	–	–	+
Growth on 5% NaCl	90	100	100	0	+	+	+	–
Urease production	0	0	0	0	–	–	–	–
Levan production	5	0	0	58	–	–	–	–
Lecithinase	4	0	0	83	–	–	–	+
Tween 80 hydrolysis	0	0	0	83	+	–	+	–
Acid production from :								
Glucose	100	100	100	100	+	+	+	+
Fructose	100	100	100	100	+	+	+	+
Ramnose	91	100	100	91	+	+	+	+
Arabinose	100	100	100	100	+	+	+	+
Sorbitol	25	100	0	0	–	+	–	–
Raffinose	100	100	33	66	+	+	+	+
Melibiose	75	100	50	100	+	+	–	+
Trehalose	100	100	50	0	+	+	+	–
Galactose	91	100	100	100	+	+	+	+
Cellobiose	100	100	0	100	+	+	–	+
Lactose	100	100	100	0	+	+	+	+
Maltose	9	75	75	0	–	V	+	–
Mannose	100	75	100	91	+	+	+	+
Arabitol	0	100	0	0	–	+	–	–
Utilization of :								
Inulin	0	0	100	0	–	–	+	–
Citrate	90	100	0	100	+	+	–	+
Malonate	0	0	0	100	–	–	–	+
α-methyl glucoside	0	100	100	0	–	+	+	–

[a] In parenthesis No. of strains tested.
[b] *Pcc*, *Pectobacterium carotovorum* subsp. *carotovorum* EccSCRI 193; *Pco*, *P. carotovorum* subsp. *odoriferum* Strain CFBP 1878; *Pb*, *P. betavasculorum* SCRI 479; *Dda*, *Dickeya dadantii* EchSCRI 3739.
[c] +, positive reaction; -, negative reaction; V, variable.
[d] Percentage of strains with positive reaction.

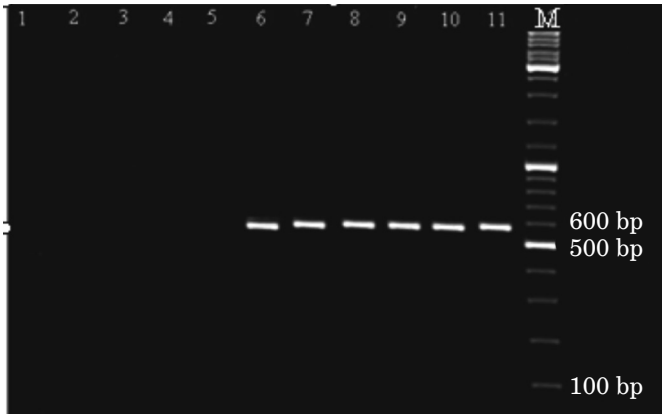
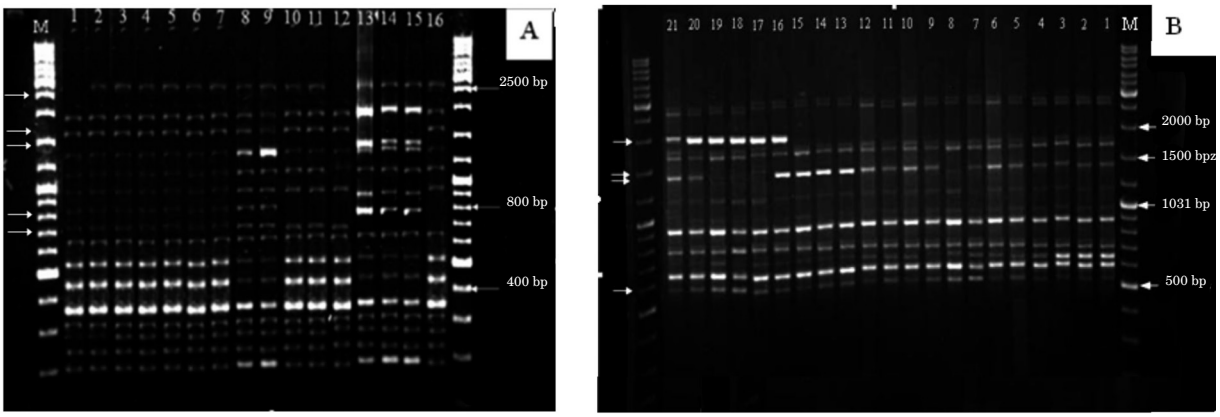


Figure 1. Agarose gel electrophoresis of PCR-products from *Pcc* strains with primers EXPCCR and EXPCCRF. Lanes: M, DNA molecular marker; 1, S7; 2, S8; 3, Po7; 4, Po11; 5, O4; 6, Ecc SCRI 193; 7, S1; 8, S3; 9, Po1; 10, L2; 11, Cb2.



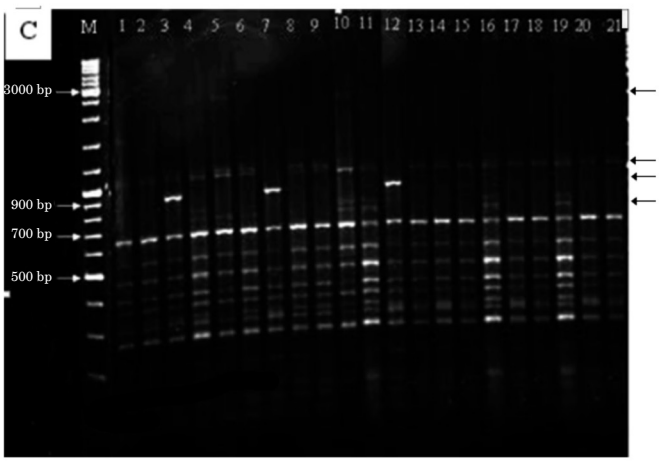


Figure 2. rep-PCR fingerprint patterns of Pcc strains isolated from different hosts.
A. REP-primers: 1, EccSCRI 193; 2, Pe2; 3, L1; 4, O2; 5, Po2; 6, Po3; 7, O1; 8, T2; 9, Cb1; 10, Po1; 11, Cr2; 12, Cr1; 13, T1; 14, S3; 15, Pe1; 16, Cb3.
B. BOX A1R: 1, Cb3; 2, Po; 3, Cr1; 4, S1; 5, Po3; 6, O1; 7, Po2; 8, L1; 9, O2; 10, Cb2; 11, S3; 12, S2; 13, Po1; 14, Cr2; 15, T2; 16, T1; 17, Pe2; 18, L2; 19, Cb1; 20, Pe1; 21, EccSCRI 193.
C. ERIC-primers: 1, Cb3; 2, Po4; 3, Cr1; 4, L2; 5, Cb1; 6, Pe1; 7, Pe2; 8, L1; 9, O2; 10, Cb2; 11, S3; 12, T1; 13, Po1; 14, Cr2; 15, T2; 16, S2; 17, Po2; 18, S1; 19, Po3; 20, O1; 21, EccSCRI 193; M, 1 Kb DNA molecular marker.
Differences in banding patterns between strains are highlighted by arrowheads.

ERIC-PCR clearly differentiated the Iranian soft rot bacteria strains isolated from the different hosts. The PCR bands were compared based on the presence or absence of fragments at a specific position, and the similarity coefficients of pairs of isolates were calculated to determine the genetic relationship among bacterial isolates. There were differences in the intensity of some amplified fragments as well as in the presence/absence of a number of polymorphic bands. When the data were analyzed by combining each set of fingerprints, four groups were clearly separated (Figure 4). Group I comprised all *Pcc* strains and generated unique patterns that were characteristic of this subspecies (Figure 2, A,B,C). Group II comprised all *Pb* strains and generated the unique patterns characteristic of this species. Group III comprised *Pco,* and group IV *Dda*. The Eric-PCR patterns of isolates within a species and subspecies were similar. *Pcc*, *Pco*, *Pb* and *Dda* each had a unique banding pattern that clearly distinguished it from all the others (Figure 5). Complex fingerprint patterns were obtained for all the isolates studied with these primers. Reproducible genomic PCR profiles consisted of bands ranging in size from 500–3000 bp for BOX primers, 250–3000 bp for ERIC, and 200–2500 bp for PCR primers. The 42 strains from different hosts that had been identified as *Pcc*, *Pb*, *Pco* or *Dda* by their phenotypic characters were clearly distinguished by REP-PCR with the REP, ERIC and BOX primers.

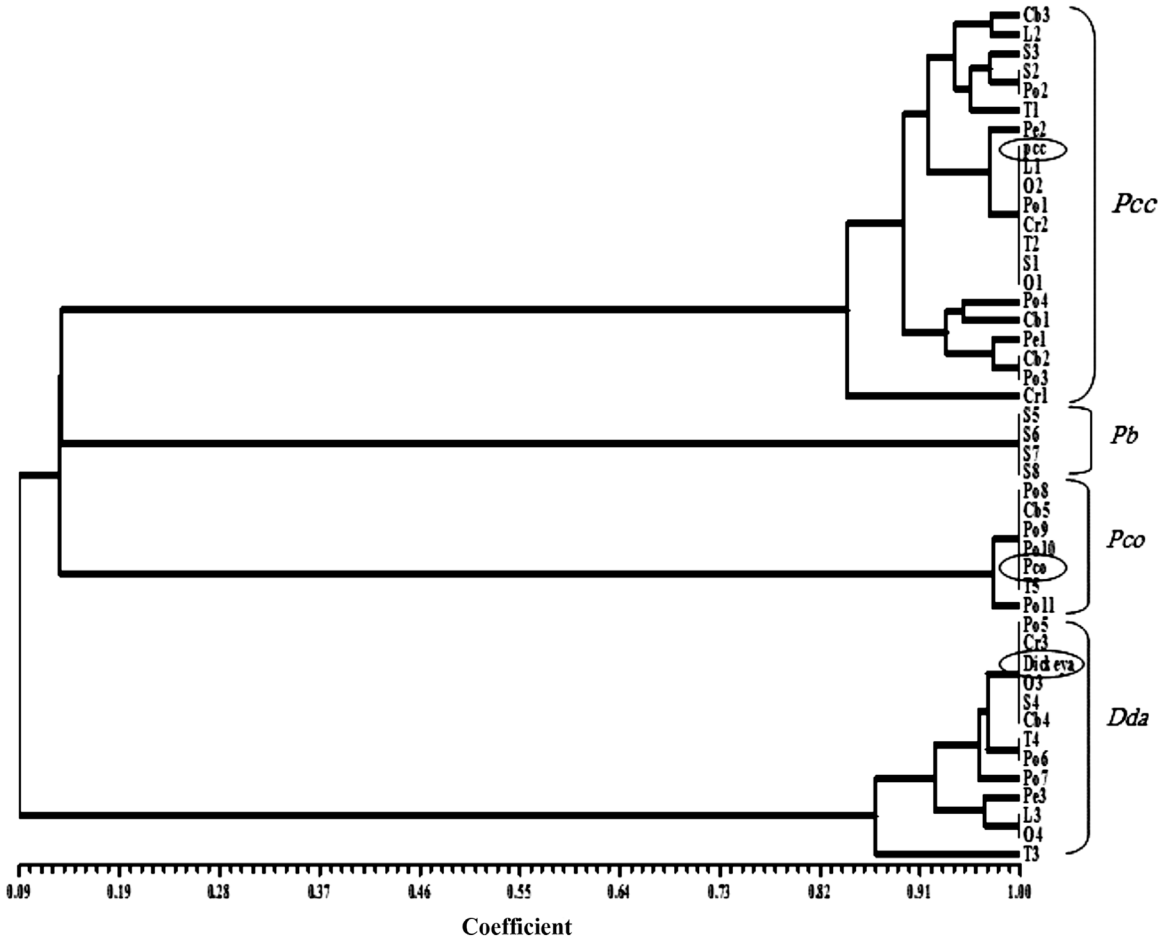


Figure 4. Dendrogram based on rep-PCR of soft rot bacteria isolates with REP, ERIC and BOX primers. Characteristics of the isolates were shown in Table 1. *Dickeya*, EchSCRI 3739; *Pco*, PcoCFBP 1878 and Figure 5. Agarose gel electrophoresis of PCR products obtained from representative strains of four distinct groups of Iranian soft rot bacteria with ERIC- primers.

Discussion

The main aim of this study was to investigate whether the REP-PCR technique discriminated the soft rot bacteria to the species and subspecies level in Iran. Highly conserved repetitive DNA elements, such as the repetitive extragenic palindromic (REP) elements, the enterobacterial repetitive intragenic consensus (ERIC) elements, and the BOX elements, seem to be widespread in the genomes of various bacterial groups (Versalovic *et al*., 1991). Amplification of the sequences between each of these repetitive elements has been used to generate DNA fingerprints of several gram-negative and gram-positive bacterial species. The soft rot bacteria *Pcc*, *Pb*, *Pco* and *D. dadantii* must be characterized if rapid diagnostic methods are to be developed. In Iran, *Pcc*, *Pb*, and *Dda* cause severe damage to various plants. In this study, we described 42 isolates of pectolytic bacteria that belong to *Pectobacterium* spp., and a *Dickeya* sp., obtained from diseased crops, including potato, cabbage, onion, turnip, sugar beet, pepper, lettuce and carrot. Clustering based on REP-PCR with BOX, ERIC and REP primers confirmed clustering based on phenotypic features. *Pcc* strains were phenotypically and genetically heterogeneous (Avrova *et al*., 2002; Waleron *et al*., 2002). Polymorphism among the REP-PCR products of the *Pcc* strains indicated that these strains were genetically variable. *Pcc* was the most diverse subspecies, but *Pco* and *Pb* were much more homogeneous. Such relatively low genetic diversity may be due to a subspecies having a more recent origin, to limited population

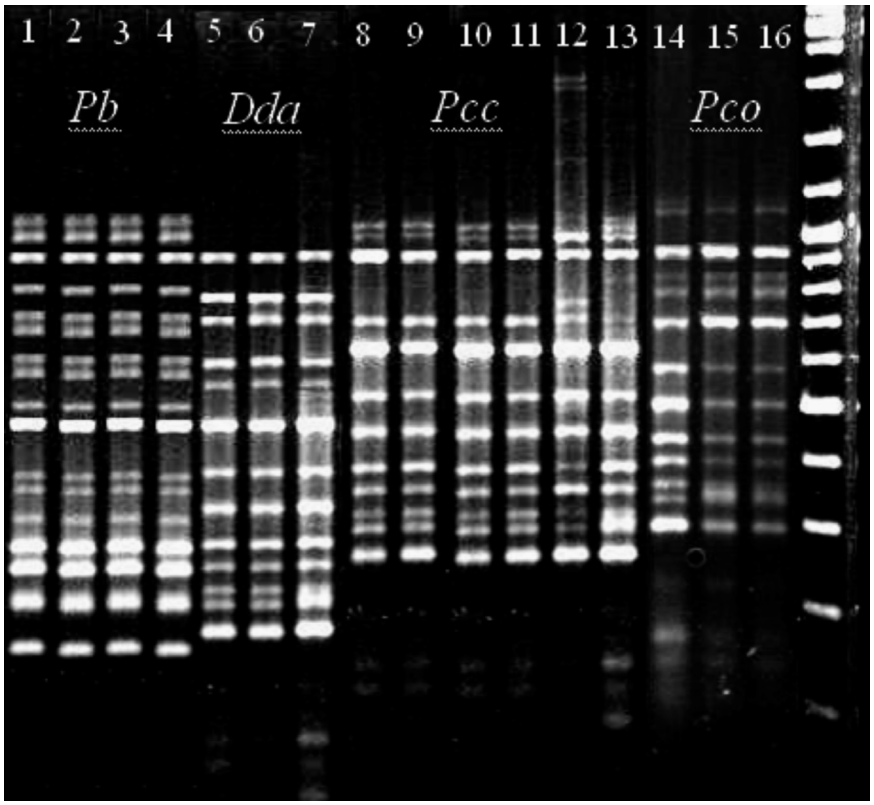


Figure 5. Agarose gel electrophoresis of PCR products obtained from representative strains of four distinct groups of Iranian soft rot bacteria with ERIC- primers.
1, S5; 2, S6; 3, S7; 4, S8 identified as *Pectobacterium betavasculorum* based on phenotypic characteristics. 5, EchSCRI 3739; 6, Pe3; 7, T4 identified as *Dickeya dadantii* based on phenotypic characteristics. 8, EccSCRI 193; 9, O2; 10, Cb2; 11, S3; 12, T1; 13, O1 identified as *Pectobacterium carotovorum* subsp. *carotovorum* based on phenotypic characteristics. 14, Po2; 15, T5; 16, Cb5 identified as *Pectobacterium carotovorum* subsp. *odoriferum* based on phenotypic characteristics. M, 1 Kb DNA molecular marker.

divergence or to a limited host range. *Pcc* has a wider host range, which may explain the genetic diversity of this subspecies (Avrova *et al*., 2002).

Pectobacterium betavasculorum causes soft rot of sugar beet and was also isolated from sunflower, artichoke, and potato (Avrova *et al*., 2002). All the *Pb* isolates recovered in this study were also recovered from sugar beet. Phenotypic tests showed that the *Pb* strains were distinct from the other species and subspecies. Based on REP-PCR with REP, ERIC and BOX primers, there was no difference between these strains (S_5, S_6, S_7, S_8) (Figures 4 and 5). The primers EXPCCF and EXPCCR were used to detect the isolated bacteria from sugar beet. But only three sugar beet isolates (S_1–S_3) produced the expected 550 bp product following PCR with these primers and they were identified as *Pcc*. The remaining four strains of sugar beet were identified as *Pb* by their phenotypic characters.

Pco, previously designated as atypical *P. atrosepticum* (Samson *et al*., 1980), was isolated from witloof-chicory, leek, allium and celery (Gallois *et al*., 1992). *Pco* strains were isolated from potato, cabbage, and turnip and it seems that this subspecies has a broad host range. The *Pco* isolates were clearly differentiated from the isolates of other species and subspecies of soft rot bacteria based on REP-PCR (Figures 4 and 5).

Different *Dickeya* spp. cause disease on different hosts under different climatic condition. *Dickeya* spp. are recognized as important pathogens in many crops (Pérombelon, 2002). Twelve strains, isolated from various hosts, were identified as *Dda* by phenotypic analysis and by REP-PCR. The *Dda* strains isolated from potato, cabbage, onion, turnip, sugar beet, pepper, lettuce and carrot in Iran were different from the *Dda* strains typically isolated in the Netherlands from blacking-diseased plants (Janse and Ruissen, 1988). Polymorphisms generated with ERIC primers were mainly seen in strains Po_4, T_4, Pe_3 and S_4 of *Dda,* characterized by the presence of a 320 bp and 380 bp fragment (Figure 3, A).

Phenotypic tests and serological methods have been developed to detect and characterize soft rot bacteria; however, not all these tests specifically detected the species and subspecies. Biochemical tests differentiate all species and subspecies, but they are time-consuming and not sensitive enough for testing purposes (Toth *et al*., 2001). Serologi-

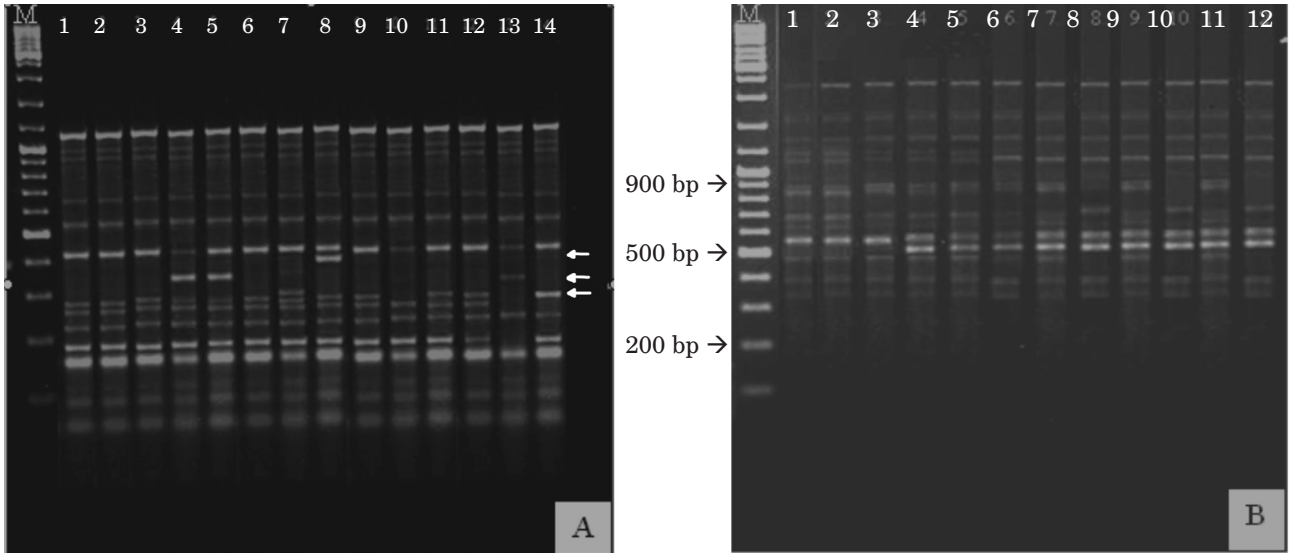


Figure 3. rep-PCR fingerprint patterns of *Dickeya dadantii* strains isolated from different hosts.
A. ERIC-primers: 1, EchSCRI3739; 2, EchSCRI3739; 3, Po5; 4, Po6; 5, T; 6, Cr3; 7, Po7; 8, S4; 9, L3; 10, O3; 11, O4; 12, T3; 13, Pe3; 14, Cb4; M, 1 Kb DNA molecular marker.
B. BOX A1R: 1, EchSCRI3739; 2, T3; 3, O3; 4, Po6; 5, T4; 6, Cr3; 7, Po5; 8, S4; 9, Cb4; 10, Pe3; 11, O4; 12, L3; M, 1 Kb DNA molecular marker. Differences in banding patterns between strains are highlighted by arrowheads.

cal techniques too are not sensitive enough to detect low but epidemiologically significant bacterial populations. A number of other methods have been used to identify the soft rot bacteria but all of them have their limitations. REP-PCR was considerably faster, more suitable and more accurate than the other identification methods (Toth *et al*., 2001). This was especially true when large numbers of isolates had to be tested. This study provides evidence for the belief that REP-PCR fingerprinting is an important tool to identify the various soft rot bacteria from different hosts and monitor them. Moreover, the differentiation capacity of this technique increased significantly if the band polymorphisms were analyzed together, and this is the first report of a combined analysis of soft rot bacteria in Iran.

Literature cited

Ahmadvand R. and H. Rahimian, 2002. Study on diversity of the pectolytic erwinias infecting potato in hamedan province. *Proceedings of the 15th Iranian Plant Protection*, 7–11 September 2002, Kermanshah, Iran, 115 (abstract).

Avrova A.O. J. Hyman, R.L. Toth and I.K. Toth, 2002. Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. *Applied and Environmental Microbiology* 68, 1499–1508.

Ayers S.H., P. Rupp and W.T. Johnson, 1919. A study of the alkali-forming bacteria in milk. *United States Department of Agriculture Bulletin*. 787, 1–39.

De Boer S.H. and A. Kelman, 2001. *Erwinia* soft rot group. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 3rd ed., (Schaad N.W., Jones J.B., Chun W. ed.), American Phytopathological Society, St. Paul, MN, USA, 56–72.

Dickey R.S., 1979. *Erwinia chrysanthemi*: a comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopathology* 69, 324–329.

Fahy P.C. and G.J. Persley, 1983. *Plant Bacterial Diseases: A Diagnostic Guide*, 1st ed. Academic Press, Sydney, Australia, 393 pp.

Gallois A., R. Samson, E. Ageron and P.A.D. Grimont, 1992. *Erwinia carotovora* subsp. *odorifera subsp*. nov., associated with odorous soft rot of chicory (*Cichorium intybus* L.). *International Journal of Systematic Bacteriology* 42, 582–588.

Garden L., C. Gouye, R. Christen and R. Samson, 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrospecticum* sp. nov., *Pectobacterium betavascularum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *International Journal of Systematic Bacteriology* 53, 381–391.

Hauben L., E.R.B. Moore, L. Vauterin, M. Steenackers, J. Mergaert, L. Verdonck. and J. Swing, 1998. Phylogenic position of phytopathogens within the *Enterobacteriaceae*. *Systematic and Applied Microbiology* 21, 384–397.

Helias V., L.A. Le Roux, Y. Bertheau, D. Andrivon, J. Gauthier. and B. Jouan, 1998. Characterisation of *Erwinia carotovora* subspecies and detection of *Erwinia carotovora* subsp. *atroseptica* in potato plants, soil and water extracts with PCR-based methods. *European Journal of Plant Pathology* 104, 685–699.

Janse L.D. and M.A. Ruissen, 1998. Characterization and classification of *Erwinia chrysanthemi* strains from several hosts in the Netherlands. *Phytopathology* 78, 800–807.

Kang H.W., S.W. Kown and S.J. Go, 2003. PCR-based specific and sensitive detection of *Pectobacterium carotovorum* ssp. *carotovorum* by primers generated from a URP-PCR fingerprinting-derived polymorphic band. *Plant Pathology* 52, 127–133.

Kwon S.W., S.J. Go, H.W Kang, J.C Rya and T.K. Jo, 1997. Phylogenetic analysis of *Erwinia* species based on 16S rDNA gene squences. *International Journal of Systematic Bacteriology* 47, 1061–1067.

Louws F.J., D.W. Fulbright, C.T. Stephens and D.J. De Bruijn, 1995. Differentiation of genomic structure by rep-PCR fingerprinting to rapidly classify *Xanthomonas campesteris* pv. *vesicatoria*. *Phytopathology* 85, 528–536.

Louws F.J., J.L.W. Rademaker and F.J. de Bruijn, 1999. The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. *Annual Review of Phytopathology* 37, 81–125.

Mahmoudi E., M.J. Soleimani and S.M. Taghavi, 2007. Detection of bacterial soft-rot of crown imperial caused by *Pectobacterium carotovorum* subsp. *carotovorum* using specific PCR primers. *Phytopathologia Mediterranea* 46, 1–8.

Palacio-Bielsa A., M.A. Cambra and M.M. López, 2006. Characterization of potato isolates of *Dickeya chrysanthemi* in Spain by a microtitre system for biovar determination. *Annals and Applied Biology* 148, 157–164.

Pérombelon M.C.M., 2002. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. *Plant Pathology* 51, 1–12.

Pérombelon M.C.M. and A. Kelman, 1980. Ecology of the soft rot Erwinias. *Annual Review of Phytopathology* 18, 361–387.

Rohlf F.J., 2000. NTSYS-PC, Numerical taxonomy and Multivariate analysis system, Version 2.11j. Exeter Software, Setauket, NY, USA.

Sambrook J., Fritsch E.F., Maniatis T., 1989. *Molecular Cloning; A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY, USA.

Samson R., J.B. Legendre, R. Christen, W. Achuak and L. Garden, 2005. Transfer of *Pectobacterium chrysanthemi* (Brenner *et al*. 1973) and *Brenneria paradisiaca* (Hauben *et al*. 1998) to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species:

Dickeya dadantii sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *International Journal of Systematic and evolutionary Microbiology* 55, 1415–1427.

Samson R., F. Poutier, M. Sailly, L. Hingand and B. Jouan, 1980. Bacteria associated with soft-rot of witloof-chicory. *Annual Review of Phytopathology* 12, 3–11.

Schaad N.W., J.B. Jones and W. Chun, 2001. *Laboratory Guide for identification of Plant Pathogenic bacteria*. 3rd ed. American Phytopathological Society St. Paul, MN, USA, 378 pp.

Soltani-Nejad S., S.M. Taghavi, J. Hyati and R. Mostowfizadeh Ghalamfarsa, 2005. Study of phenotypic features and pathogenicity of soft rot pectobacteria in Khuzestan Province. *Iranian Journal of Plant Pathology* 41, 585–612.

Toth I.K., A.O. Avrova and L.J. Hyman, 2001. Rapid identification and differentiation of the soft rot erwinias using 16S-23S intergenic transcribed spacer (ITS)- PCR and RFLP analyses. *Applied and Environmental Microbiology* 67, 4070–4076.

Toth I.K., K.S. Bell, C.M. Holeva and J.R. Birch, 2003. Soft rot erwiniae: from genus to genomes. *Molecular Plant Pathology* 4, 17–30.

Versalovic J., T. Koeuth and J.R. Lupski, 1991. Distribution of repetitive DNA-sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* 19, 6823–6831.

Versalovic J., M. Schneider, F.J. de Bruijn and J.R. Lupski, 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods in Molecular and Cellular Biology* 5, 25–40.

Waleron M., K. Waleron and A.J. Podhajska, 2002. Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiology* 148, 583–595.

Zohour Paralak E., H. Rahimian and Z. Banihashemi , 2007. A comparative study on phenotypic erwinias isolated from potato in Fars province. *Iranian Journal of Plant Pathology* 43, 121–144.

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