

Full Length Research Paper

Genetic diversity of Iranian potato soft rot bacteria based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis

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Bacterial soft rot diseases caused by *Pectobacterium-Dickeya* complex are the most important and yield losses diseases of potato crop worldwide. Loss due to these diseases in some years/fields under Iran condition is huge and destructive. To screen and characterize the causal agents, thirty bacterial soft rot isolates including 10 authentic pectolytic strains were investigated. Based on biochemical and physiological tests, the Iranian strains were identified as either *Pectobacterium carotovorum* subsp. *carotovorum* or *P. carotovorum* subsp. *wasabiae*. Sequence analysis of *recA* gene revealed that the strains closely related to *Pectobacterium* spp. To assess the genomic diversity, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed. PCR amplification of the *recA* gene followed by RFLP revealed 14 distinct RFLP groups. Here also, the same results were obtained and all 19 Iranian strains were assigned as either *Pectobacterium carotovorum* subsp. *carotovorum* or *P. carotovorum* subsp. *wasabiae*.

Key words: Genetic diversity, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), *recA* gene, *Pectobacterium* spp.

INTRODUCTION

Soft rot bacteria are global pathogens and amongst the most prevalent and destructive bacterial diseases that affect potato, particularly during storage and transport. The most commercially important of the soft rot *Erwinias* are *Erwinia chrysanthemi*, *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica*, which cause diseases of potato and other commercially important crops (Toth et al., 2001; Czajkowski et al., 2009;

Laurila et al., 2008; De Haan et al., 2008; Diallo et al., 2009; Johnson et al., 2011). These three species in the *Pectobacterium-Dickeya* disease complex are responsible for soft rot, aerial stem rot and blackleg of potatoes. They have a wide host range, and most succulent plants are host to at least one (sub) species of the bacteria (Collmer and Keen, 1986). All known soft rot bacteria, including *Pectobacterium carotovorum* subsp. *carotovorum*, *Pecto-bacterium betavasculorum*, *Pectobacterium atrosepticum*, *Pectobacterium wasabiae*, *Pectobacterium carotovorum* subsp. *oderiferum* and *Dickeya* spp., have been isolated from several different potato growing areas of Iran (Bahar and Danesh, 1986; Ferydoni, 1994; Marefat and Ghasemi, 2000; Soheyli-Moghadam et al., 2004; Zohour-Paralak et al., 2006; Firouz et al., 2006; Baghaee-Ravari et al., 2010; Tavasoli et al., 2011).

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Abbreviations: RFLP, Restriction fragment length polymorphism; AFLP, amplified fragment length polymorphism; RecA, recombinase A; NA, nutrient agar.

The variation among these bacteria has been studied with different methods (Laurila et al., 2009) such as PCR amplification and sequencing, RFLP (restriction fragment length polymorphism) of the 16S gene and the 16S-23S rDNA intergenic spacer (Toth et al., 2001; Fessehaie et al., 2002), RFLP of *recA* gene fragments (Waleron et al., 2002) and AFLP fingerprinting (Avrova et al., 2002).

PCR-RFLP analysis of a *recA* gene fragment (Waleron et al., 2002) was found to be a suitable method for identifying the species and subspecies of *Erwinia*. PCR amplification and restriction digestion of the amplified *recA* fragments by four endonucleases (*AluI*, *HinfI*, *TasI* and *Tru1I*) was able to differentiate the 177 strains studied into 57 RFLP groups. They also separated *E. carotovora* from *Erwinia chrysanthemi* successfully.

RecA (recombinase A) is a multifunctional protein involved in homologous recombination, DNA repair and the SOS response. It is thought to be universally present in prokaryotic and eukaryotic cells as it shows a high degree of sequence conservation. RecA protein and *recA* gene sequence comparisons have been used to speculate about phylogenetic relationships among genera and species. The *recA* gene has also been used in the typing of *Acinetobacter* spp., genotyping of bacteria belonging to the former *Erwinia* genus, and identification of *Mycobacterium* species and the *Burkholderia cepacia* complex (Waleron et al., 2002). In that study, the major diversity was found with RFLP patterns produced by *TasI* digestion and not by *HinfI*.

The present study describes the genetic diversity of Iranian potato soft rot bacteria based on *recA* gene polymorphisms and sequencing analysis.

MATERIALS AND METHODS

Bacterial strains and media

Bacterial strains used in this study are listed in Table 1. Nineteen bacterial cultures isolated from different potato-producing provinces were obtained from J. Razmi, Islamic Azad University, Science and Research Branch. A single isolate isolated from corn crop (corn-Abyek) was received from Iranian Plant Protection Research Institute.

The type strains were provided by Patricia van der Zouwen (International Plant Research of Netherlands) for *Dickeya dianthicola* 980, *Dickeya dianthicola* 2114(T), *E. carotovora* subsp. *atroseptica* 161 and 1007, *P. carotovorum* subsp. *carotovorum* 1955 and 1949, and *Dickeya solani* 2222.

Furthermore, *Dickeya chrysanthemi* DSM4610 and *Pectobacterium atrosepticum* SCRI1043 were provided by Dr. Minna Pirhonen (Helsinki University, Finland). All strains were maintained in deep frozen cultures in a medium containing 10% skimmed milk supplemented with 15% glycerol at -80°C (Seo et al., 2003).

Isolation and PCR amplification of genomic DNA

The genomic DNA of strains was extracted using the alkaline lysis method (Rademaker et al., 1997). The bacterial strains were cultured on NA (nutrient agar) medium at 27°C for 24 h. 100 µl of

0.05 M NaOH was added to 10 µl of cell suspension (10^3 to 10^7 bacteria) and incubated at 95°C for 15 min. The bacterial suspensions were centrifuged for 2 min at 14,000 rpm. 1 µl from each culture supernatant was used per PCR reaction.

The oligonucleotide primers were designed based on the sequence of *recA* in *Erwinia* species (Waleron et al., 2002). The forward and reverse primer sequences were 5'-GGTAAAGGGTCT-ATCATGCG-3' and 5'-CCTTCACCATACATAATTTGGA-3', respectively. These sequences were adopted from scientific sources and synthesized by Eurofins MWG Operon Corporation, Germany. The concentration of each primer was adjusted to 10 pmol/µl by ddH₂O and stored at -20°C.

DNA amplification was done according to a conventional method described by Waleron et al. (2002). The PCR reaction mix was in a final reaction volume of 50 µl and contained 5 µl 10X reaction buffer (Fermentas), 1.25 µl MgCl₂ (1.5 mM), 0.5 µl each of dATP, dCTP, dGTP and dTTP (10 mM), 1 µl each primer (10 pmol), 1 to 1.5 µl DNA template and 1.5 U *Taq* DNA polymerase.

The reaction involved initial denaturation (95°C, 3 min), followed by 32 cycles of denaturation (94 °C, 1 min), annealing (47°C, 1 min) and extension (72°C, 2 min), with a final extension (72°C, 5 min). The amplified products were electrophoretically separated in a 1.5% (w/v) agarose gel at 70 V for 1 h in TBE buffer and visualized with UV light after staining in a solution of ethidium bromide (0.5 µg ml⁻¹).

Restriction fragment length analysis

The amplified DNA fragments were digested separately with four restriction endonucleases (*AluI*, *HinfI*, *TasI* and *Tru1I*). 2.5 U of each restriction enzyme was used for digestions, which were incubated overnight at the temperature recommended by the manufacturer (Fermentas). The restriction patterns were compared after electrophoresis on a 1.5% (w/v) agarose gel at 70 V for 1.5 h in TBE buffer followed by ethidium bromide staining (0.5 µg ml⁻¹) and visualized with UV light.

Data analysis

The band profiles were analyzed by NTSYSpc Ver. 2.2 software. The unweighted pair-group method of averages (UPGMA) and DICE correlation coefficient within NTSYSpc Ver. 2.2 were then used to construct dendrograms from the similarity matrices. Dice's coefficient produced a high correlation coefficient and the most adequate genetic divergence as compared to other correlations.

DNA sequencing and sequence analysis

The PCR products were purified by the British Gene Service Company, and the same primers were used for sequencing and PCR, and had the following sequences: forward primer: 5'-GGTAAAGGGTCTATCATGCG-3'; reverse primer: 5'-CCTTCACCATACATAATTTGGA-3'.

The PCR products of 11 selected isolates from different RFLP groups were sequenced by Geneservice, England, for further characterization. Multiple sequence alignments were determined using the ClustalX software. The sequences were then compared with the databases available at the National Centre for Biotechnology Information (NCBI).

RESULTS

PCR-RFLP analysis

The specific primers were designed to bind within *recA*

Table 1. Origin and *recA* PCR-RFLP groups of the bacterial strains used in this study

Number	Bacterial strain	Geographical origin	Host	RFLP group	
2	83	Hamedan	Potato	1	
3	117A	Tehran	Potato	1	
5	88-1	Hamedan	Potato	3	
6	128A	Fars	Potato	3	
7	74B	Hamedan	Potato	1	
8	31A	Kordestan	Potato	8	
9	CA	Ghazvin-abyek	Corn	14	
10	58B	Kordestan	Potato	8	
11	104B	Tehran	Potato	7	
12	116B	Tehran	Potato	5	
14	87D	Hamedan	Potato	6	
16	112A	Tehran	Potato	10	
17	45A	Kordestan	Potato	6	
18	17B	Ardebil	Potato	10	
19	108A	Tehran	Potato	6	
20	123D	Tehran	Potato	6	
21*	2114	<i>D. dianthicola</i>	Type strain	Dianthus	11
22*	1949	<i>P. carotovorum</i> subsp. <i>carotovorum</i>	Netherlands	Potato	10
23*	161	<i>E. carotovora</i> subsp. <i>atroseptica</i>	Netherlands	Potato	9
24*	2222	<i>D. solani</i>	Netherlands	Potato	13
25*	980	<i>D. Dianthicola</i>	Netherlands	Potato	11
26*	1007	<i>E. carotovora</i> subsp. <i>atroseptica</i>	Netherlands	Potato	9
27*	1955	<i>P. carotovorum</i> subsp. <i>carotovorum</i>	Netherlands	Potato	10
28*	DSM4610	<i>D. chrysanthemi</i>	Type strain	Potato	12
29*	SCRI1043	<i>P. atroscopicum</i>	Type strain	Potato	9
30*		<i>Dickeya</i> sp.	Type strain	Potato	12
33	120B	Tehran	Potato	4	
36	117B	Tehran	Potato	1	
38	84	Hamedan	Potato	1	
41	110A	Tehran	Potato	2	

*Reference strains. RFLP, Restriction fragment length polymorphism.

directed amplification of fragment of about 730 bp from all 30 bacterial strains during PCR analysis (Figure 1).

RFLP of *recA* gene fragments digested by *TasI* resulted in 13 distinct banding patterns; digestion with *HinI* resulted in 4 different banding patterns. Thus, these enzymes yielded the most and least diversity in their RFLP patterns, respectively. *AluI* and *TruI* produced six and nine RFLP patterns, respectively (Figure 2). In total, all strains were divided into 14 groups (Figure 3). The *P. carotovorum* subsp. *carotovorum* strains were distributed in six different RFLP groups (groups 1, 3, 5, 7, 6 and 10) whereas *P. carotovorum* subsp. *wasabiae* strains were scattered in just three RFLP groups (groups 2, 4 and 8).

The two strains of *P. carotovorum* subsp. *carotovorum* 88-1 and 128A and two isolates of *P. carotovorum* subsp. *wasabiae* 31A and 58B were placed in groups 3 and 8 with the highest degree of similarity about 100%. The *Dickeya Dianthicola* 2114 and 980 were clustered in group 11. The two strains of *P. carotovorum* subsp.

carotovorum 1949 and 1955 were placed in the same group with similarity above 90%.

The DNA sequences obtained for 11 selected strains were compared with available sequences deposited in GenBank (<http://www.ncbi.nlm.nih>). Most of these strains were highly similar (>96%) with GenBank sequences. Seven isolates, numbers 6, 11, 12, 14, 17, 20 and 38, were similar to *P. carotovorum* subsp. *carotovorum*; corn isolate CA was similar to *Dickeya* sp.; and three isolates, numbers 33, 10 and 41, were similar to *Pectobacterium carotovorum* subsp. *wasabiae*. The determined sequences of *recA* of the above-mentioned strains were deposited in NCBI under the accession numbers of HQ424862.1 to HQ424871.1 and HM854829.1 (Figure 4).

DISCUSSION

A huge amount of seed potatoes has been imported from

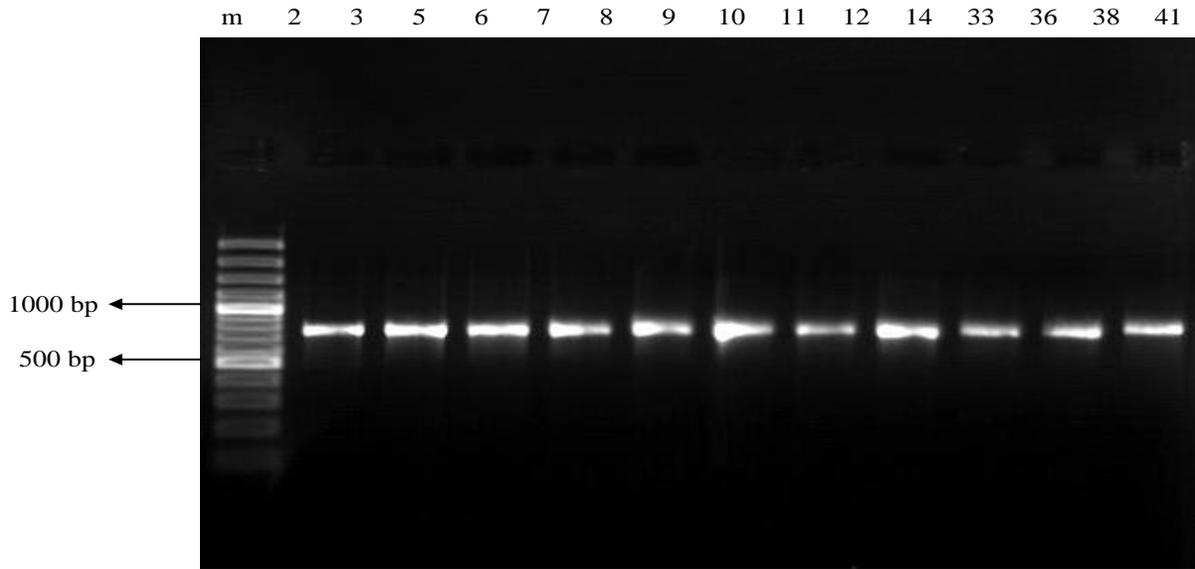


Figure 1. Agarose electrophoresis of PCR-amplified DNA from several strains. Lane/bacterial strains: lane 2, 83; lane 3, 117A; lane 5, 88-1; lane 6, 128A; lane 7, 74B; lane 8, 31A; lane 9, CA; lane 10, 58B; lane 11, 104B; lane 12, 116B; lane 14, 87D; lane 33, 120B; lane 36, 117B; lane 38, 84; lane 41, 110A. M: 1-kb DNA ladder.

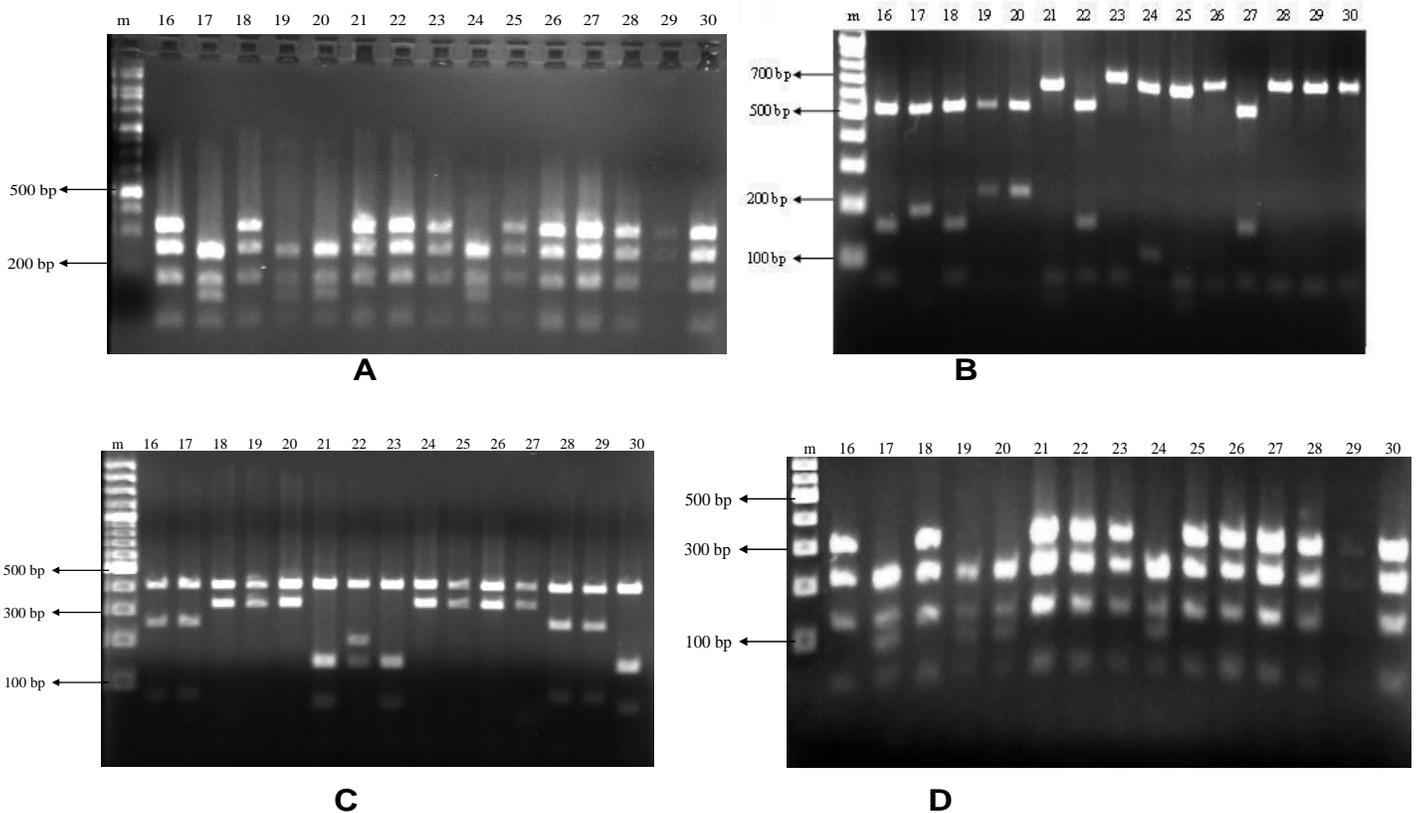


Figure 2. Restriction analysis of *recA* gene fragments amplified by PCR. RFLP patterns were obtained after digestion of PCR products with *AluI* (A), *Tru1I* (B), *TasI* (C) and *HinfI* (D). Lane/bacterial strains: lane 16, 112A; lane 17, 45A; lane 18, 17B; lane 19, 108A; lane 20, 123D; lane 21, 2114; lane 22, 1949; lane 23, 161; lane 24, 2222; lane 25, 980; lane 26, 1007; lane 27, 1955; lane 28, DSM4610; lane 29, SCRI1043; lane 30. M: 1-kb DNA ladder.

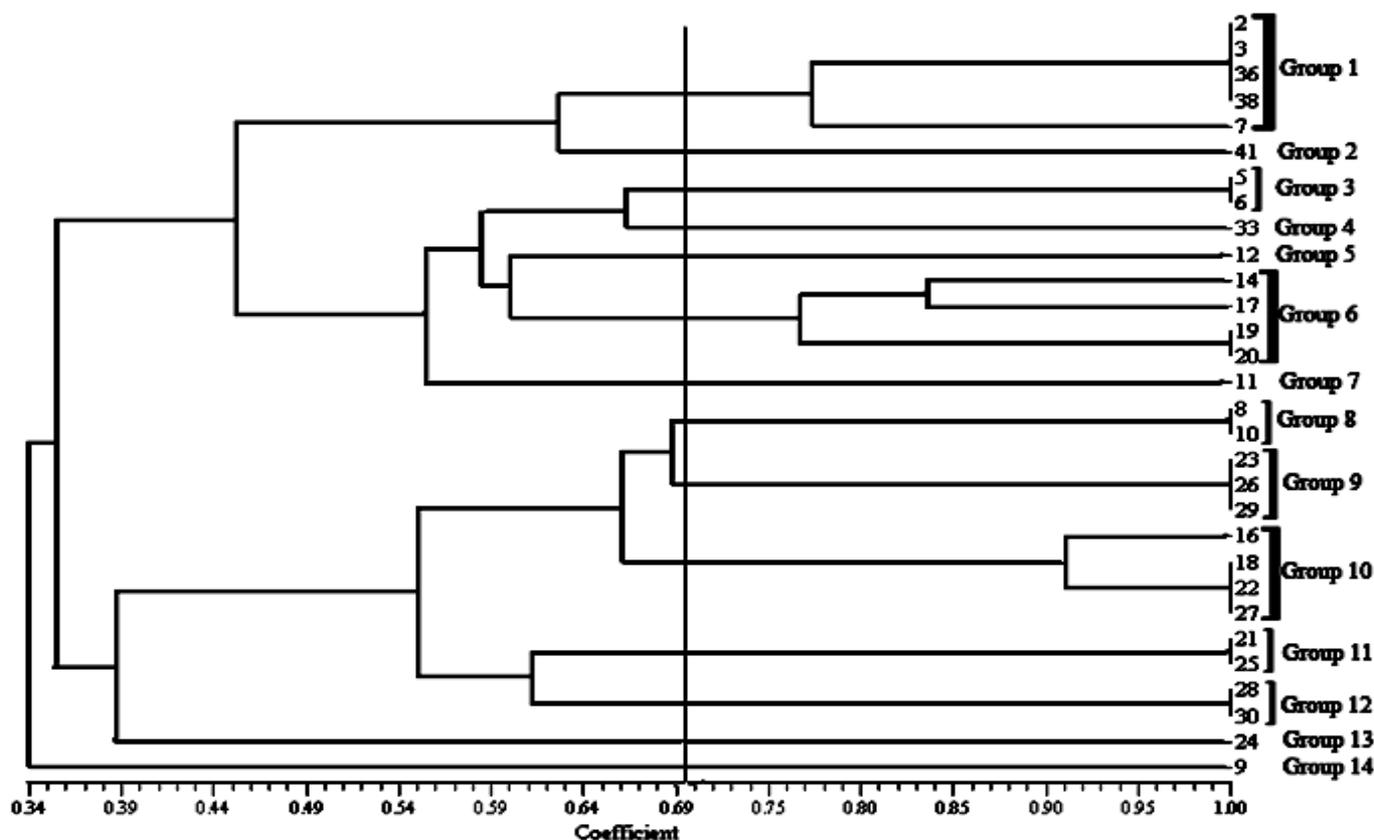


Figure 3. The Dendrogram showing similarities among restriction fragment length polymorphisms of tested isolates derived by cluster analysis using NTSYSpc Ver. 2.2 software. The *P. carotovorum* subsp. *carotovorum* strains are in groups 1, 3, 5, 6 and 7. The *P. wasabiae* strains are in groups 2, 4 and 8. The corn isolate, identified as *Dickeya* sp., was in group 14. *P. carotovorum* subsp. *atroseptica* reference strains are in group 9. *P. carotovorum*, reference strains are in group 10 and *Dickeya* sp. reference strains are in groups 11, 12 and 13.

neighboring and overseas countries with reports of increasing incidence and dispersal of important bacterial potato diseases in the main potato growing areas. However, only a few studies are available concerning the genetic diversity of these pathogens in Iran (Marefat, 2009; Baghaee-Ravari et al., 2010; Tavasoli et al., 2011). In most cases, the phenotypic characteristics of soft rot bacteria do not match with the traits published in diagnostics key tables. This indicates that a considerable diversity exists among Iranian isolates.

To address this issue, the PCR-RFLP method was selected due to its high precision and reliability. PCR primers designed based on published *recA* gene sequences allowed the amplification of a DNA fragment of approximately 730 bp from each of the 30 strains tested. Four restriction enzymes (*AluI*, *HinfI*, *TaqI* and *TruI*) were used, and from the resultant banding patterns, 14 different *recA* combined RFLP patterns (restriction groups) were defined (Figure 3).

The largest number of restriction fragment patterns for the *recA* gene fragments was obtained with *P. carotovorum*. On the other hand, RFLP groups 2, 4 and 8 (containing

P. carotovorum subsp. *wasabiae* strains) clustered together with *Pectobacterium* spp. and thus no unique patterns for the latter strains (58B, 110A and 120B) were observed. This issue was resolved by sequencing analysis of *P. carotovorum* subsp. *wasabiae* strains.

According to results published by Waleron et al. (2002), PCR-RFLP analysis of the *recA* gene fragment is not only a useful tool for the identification of species and subspecies belonging to the former *Erwinia* genus, but also acts as an identification tool to differentiate strains of *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*.

Using two different enzymes (*DdeI* and *HindIII*), Seo et al. (2002) were able to differentiate all tested Asian strains of *E. carotovora* subsp. *carotovora* into 10 distinct RFLP groups. Darrasse et al. (1994) developed a primer set that amplifies a fragment of the *peIY* family pectate lyases from *Erwinia carotovora* subspecies, with the exception of *E. carotovora* subsp. *betavasculorum*. Digestion of the amplified fragment with *AluI*, *HaeIII*, *Sau3AI* and *HpaII* resulted in 21 RFLP groups, a group just containing *E. carotovora* subsp. *wasabiae*, two groups possessing *E. carotovora* subsp. *atroseptica*, and

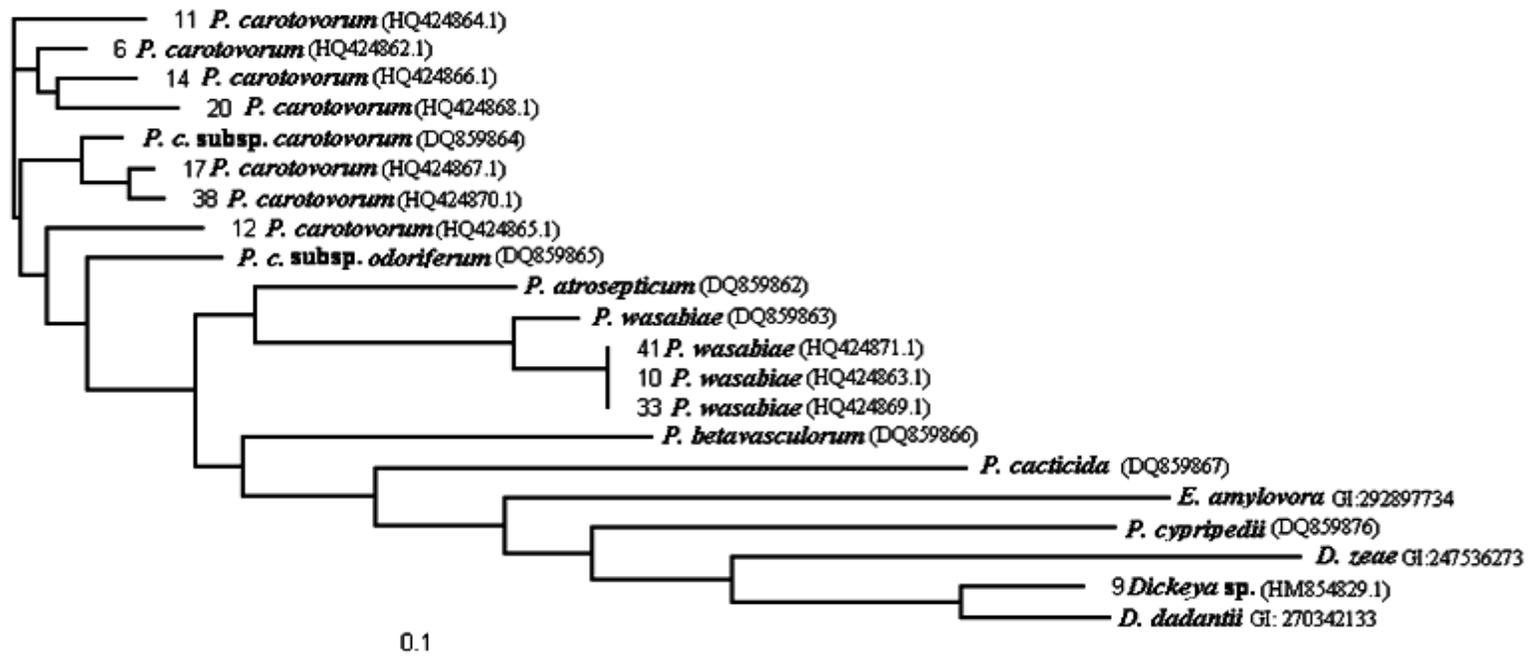


Figure 4. Phylogenetic tree showing the relationship of the plant pathogenic soft rot bacterial strains in *Enterobacteriaceae*. On the basis of *recA* sequence alignment, a phylogenetic tree was constructed using the neighbour-joining method. Stability of the tree was assessed by 1,000 bootstrap replications.

two just with *E. carotovora* subsp. *odorifera*. Three of the RFLP groups contained both *E. carotovora* subsp. *odorifera* and *E. carotovora* subsp. *carotovora* and the remaining 13 RFLP groups contained *E. carotovora* subsp. *carotovora* strains.

Nassar et al. (1996) designed a primer set which amplified a 420-bp pectate lyase gene fragment from all *E. chrysanthemi* strains tested, but not from any other soft rot *Erwinia*. Digestion of this fragment with *AluI*, *HpaII* and *Sau3AI* revealed 16 RFLP patterns among the 78 strains tested.

El Tassa et al. (2006) with application of the molecular marker of PCR-RFLP, digested the *recA* gene fragment in *Erwinia carotovora* subsp. *brasiliensis* strains with *TasI* and *HhaI* restriction enzymes. The restriction fragment length polymorphism (PCR-RFLP) analysis with *TasI* and *HhaI* enzymes generated seven and 12 patterns, respectively. Analysis of the combined results allowed the separation of 13 distinct groups and differentiation of *P. carotovora* subsp. *brasiliensis*.

Here, the largest number of fragments was

obtained following *TasI* rather than *HinfI* digestion. Our results confirm the efficacy of the *recA* PCR-RFLP method as a reliable and reproducible method for distinguishing subspecies of *Pectobacterium* and *Dickeya*. Among the tested isolates, *P. carotovora* subsp. *carotovora* was found to be the most dominant soft rot bacterium in Iranian potato field samples. The next most common was *P. carotovora* subsp. *wasabiae* with three representatives (58B, 110A and 120B). The corn strain (CA) was identified as *Dickeya* sp. All these were confirmed by phenotypic, PCR-

RFLP and sequences analyses. Unlike the recent report from Iran (Baghaee-Ravari et al., 2010), no *P. atrosepticum* was detected within the screened potato strains from five main potato-growing regions. This may indicate that this species is rare and therefore has a negligible role in bacterial soft rot disease in Iranian potato fields.

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REFERENCES

- Avrova AO, Hyman LJ, Toth RL, Toth IK (2002). Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. *Appl. Environ. Microb.* 68: 1499-1508.
- Baghaee-Ravari S, Rahimian H, Shams-Bakhsh M, Lopez-Solanilla E, Antúñez-Lamas M, Rodríguez-Palenzuela P (2011). Characterization of *Pectobacterium* species from Iran using biochemical and molecular methods. *Eur. J. Plant. Pathol.* 129: 413-425.
- Bahar M, Danesh D (1986). Occurrence of potato black leg in Isfahan. *Proc. 8th Iran Plant Protec.* Isfahan, Iran, p.105.
- Collmer A, Keen NT (1986). The role of pectic enzymes in plant pathogenesis. *Annu. Rev. Phytopathol.* 24: 383-402.
- Czajkowski R, Grzegorz G, Van Der Wolf JM (2009). Distribution of *Dickeya* spp. and *Pectobacterium carotovorum* subsp. *carotovorum* in naturally infected seed potatoes. *Eur. J. Plant. Pathol.* 125: 263-275.
- Darrasse A, Priou S, Kotoujansky A, Bertheau Y (1994). PCR and restriction fragment length polymorphism of a *pel* gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. *Appl. Environ. Microb.* 60: 1437-1443.
- De Haan EG, Dekker-Nooren TCEM, Van Den Bovenkamp GW, Speksnijder AGCL, Van Der Zouwen PS, Van Der Wolf JM (2008). *Pectobacterium carotovorum* subsp. *carotovorum* can cause potato blackleg in temperate climates. *Eur. J. Plant. Pathol.* 122: 561-569.
- Diallo S, Latour X, Groboillot A, Smadja B, Copin P, Orange N, Feuilloley MGJ, Chevalier S (2009). Simultaneous and selective detection of two major soft rot pathogens of potato: *Pectobacterium atrosepticum* (*Erwinia carotovora* subsp. *atrosepticum*) and *Dickeya* spp. (*Erwinia chrysanthemi*). *Eur. J. Plant. Pathol.* 125: 349-354.
- El Tassa SOM, Duarte V (2006). Identification of *Pectobacterium carotovorum* subsp. *brasiliensis* through PCR-RFLP of the *recA* gene. *Fitopatologia Brasileira.* 31(1): 23-28.
- Ferydoni A (1994). Survey on phenotypic features of potato soft rot bacteria in some areas in Iran. M.Sc. Thesis, Tarbiat Modares University, Tehran, Iran.
- Fessehaie A, De Boer SH, Levesque CA (2002). Molecular characterization of DNA encoding 16S-23S rRNA intergenic spacer regions and 16S rRNA of pectinolytic *Erwinia* species. *Can. J. Microbiol.* 48: 387-398.
- Firouz R, Bahar M, Sarif-Nabi B (2006). Detection of the causal agents of potato soft rot and blackleg in Isfahan province. *Iran. J. Plant. Pathol.* 43: 145-162.
- Johnson DA, Dung JKS, Cummings TF, Schroeder BK (2011). Development and Suppression of aerial stem rot in commercial potato fields. *Plant Dis.* 95(3): 285-291.
- Laurila J, Ahola V, Lehtinen A, Joutsjoki T, Hannukkala A, Rahkonen A, Pirhonen, M (2008). Characterization of *Dickeya* strains isolated from potato and river water samples in Finland. *Eur. J. Plant. Pathol.* 122: 213-225.
- Marefat A (2009). Identity and genetic diversity of bacteria belonging to the family *Enterobacteriaceae* causal agents of potato soft rot in western Iran. In: *Proceedings of the 5th International Conference on Plant Pathology.* Delhi, India, p.17.
- Marefat AR, Ghasemi A (2000). Identification of *Erwinia* causing soft rot and black-leg on imported potato. *Proceeding of the 14th Iranian Plant Protection Congress.* Isfahan, Iran, p. 317.
- Nassar A, Darrasse A, Lemattre M, Kotoujansky A, Dervin C, Vedel R, Bertheau Y (1996). Characterization of *E. chrysanthemi* by pectinolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR-amplified fragments of *pel* genes. *Appl. Environ. Microb.* 62: 2228-2235.
- Rademaker JLW, De Bruijn FJ (1997). Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer assisted pattern analysis. In G. Caetano-Anollés and P.M. Gresshoff (ed.), *DNA markers: Protocols, Applications and Overviews.* J. Wiley and Sons, Inc., USA, pp. 151-171.
- Seo ST, Furuya N, Lim CK, Takanami Y, Tsuchiya K (2003). Phenotypic and genetic characterization on *Erwinia carotovora* from mulberry (*Morus* spp.). *Plant. Pathol.* 52: 140-146.
- Seo ST, Furuya N, Takeshita M, Takanami Y (2002). Genotyping of *Erwinia carotovora* subsp. *carotovora* Strains from Asia Based on *recA* Gene Restriction Fragment Length Polymorphisms. *J. Fac. Agric. Kyushu. Univ.* 47(1): 7-12.
- Soheyl-Moghadam B, Hassanzadeh N (2004). Identification of pathogenic bacteria that cause potato soft rot in Ardabil. *Proc. 16th Iran Plant Protec.* Tabriz, Iran, p. 14.
- Tavasoli E, Marefat AR, Hassanzadeh N (2011). Identity and genetic diversity of *Pectobacterium* spp., causal agents of potato soft rot in Zanjan, Iran. *Afr. J. Plant. Sci.* 5(6): 329-336.
- Toth IK, Avrova AO, Hyman LJ (2001). Rapid identification and differentiation of the soft rot *Erwinias* by 16S-23S intergenic transcribed spacer-PCR and restriction fragment length polymorphism analyses. *Appl. Environ. Microb.* 67: 4070-4076.
- Waleron M, Waleron K, Podhajska AJ, Ojkowska E (2002). Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiol.* 148: 583-595.
- Zohour-Paralak E, Rahimian H, Banihashemi Z (2006). A comparative study on pectolytic *Erwinias* isolated from potato in the Fars province. *Iran. J. Plant. Pathol.* 43: 121-144.