JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 1825-1834

Genetic Diversity of Some Isolates of Soft rot *Pectobacterium*, from Different Host Plants in Fars Province, Iran, using RAPD Marker

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https://doi.org/10.22207/JPAM.10.3.17

(Received: 11 April 2016; accepted: 24 May 2016)

Pectobacteria are one of the most important groups of plant pathogenic bacteria which occur world-wide. The bacteria have a wide host range and infect plants with fleshy organs. Thirty five bacterial isolates were collected from potato, cabbage, sugar beet, pepper, carrot, onion, cucumber, turnip, and tomato host plants cultivated in Fars province. The bacteria were identified using biochemical and physiological tests. The genetic diversity among the isolates was evaluated using RAPD assay. The isolates were rod-shape, gram-negative, facultative anaerobes, oxidase-negative, catalase-positive, and had ability to rot the potato, while they could not induce hypersensitive reaction on tobacco leaf. All of isolates were identified as soft-rot bacteria. Based on phenotypic criteria, the isolated strains were categorized into three groups. The strains of first, second and third groups were related to *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium betavascularum* and *Dickeya chrysanthemi,* respectively. According to RAPD assay and using six primers including 19300, 91200, OPB07, OPB08, OPB11, OPB18, isolates categorized in three groups. The used primers showed significant diversity among the strains and the specific primers GTGC4, GAGC4 and GTG5 confirmed the classification. The diversity among the isolates has been confirmed based on the molecular works and clustering based on RAPD. This is the first RAPD assay to investigate the genetic diversity of soft-rot bacteria.

Keywords: *Dickeya*; genetic diversity; pectobacteria; RAPD; soft-rot.

Pectobacteria, the causal agents of soft-rot, belonging to *Enterobacteriaceae* and cause many losses in most of crops, ornamental plants and vegetables (Alpini *et al.*, 1997; Charkowski, 2006; De Haan *et al.*, 2008; Duarte *et al.*, 2004; Kim *et al.*, 2009; Pitman *et al.,* 2008). They are able to produce cell wall degrading enzymes, especially pectolytic enzymes and can cause decay of parenchyma tissues (Permobelon & Salmond, 1995). Bacterial soft-rot occurs on plants with soft and fleshy storage parts or leaves and stems. Symptoms in diseased plants are very similar in all types of hosts. The bacterium can cause various damages including reduce in germination, chlorosis, necrosis, stem rot, blackleg and eventually plant death (Avrova *et al.,* 2002; Helias *et al.*, 2000). Based on molecular and biochemical tests and host range, the genus *Pectobacterium* contains some species and subspecies including *P. carotovorum* subsp. *carotovorum, P. carotovorum* subsp. *odoriferum, P. brasiliensis, P. betavascularum, P. atrosepticum* and *P. wasabiae* (Garden *et al.,* 2003; Ma *et al.,* 2007; Van Der Merwe *et al.,* 2010). In soft-rot pectobacteria, two species, *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya chrysanthemi*, have more economic importance and wider host rang. These two species infect crops of tropical and subtropical regions while *P. atrosepticum* exclusively is found associated with stem of

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potatoes and cause blackleg disease in temperate climate (Permobelon, 1992). Bacterial soft-rot disease first reported from carrot by Jones in 1901 in America. In 1902, Van Hall from Netherland and Apple from Germany independently introduced the blackleg disease from potato (Goto & Motsumoto, 1987; Graham, 1964). In Iran, *Erwinia* was first isolated from rotted corms of cyclamen (Hedjarood, 1967). By phenotypic tests, the isolated bacterium was identified as *E. carotovora* (Amani, 1969). Some other studies were conducted to identify the isolates from different region of Iran. Most of these investigations were done based on physiological and biochemical tests, comparisons of the patterns of protein electrophoresis and the length of PCR amplified fragment using specific primers (Bahar & Danesh, 1986; Soltaninejad *et al.,* 2005; Zohoor *et al.,* 1998).

Performing molecular and biochemical tests and also host preference, is of importance to achive accurate taxonomy, diagnosis, pathogen detection and epidemiological studies (Van Der Merwe *et al.,* 2010). Many studies have been conducted for taxonomical studies and identification of the soft rot bacteria so far. Some molecular methods such as RFLP (Darrasse *et al.,* 1996; Sleds *et al.,* 2000), AFLP (Avrova *et al.,* 2002), rep-PCR (Toth *et al.,* 1999), RAPD (Maki Valkama & Karjalainen, 1994) and the analysis of 16S rRNA sequences (Hauben *et al.,* 1998) have been done to characterization of soft rot bacteria.

This study was aimed to use phenotypic characteristics as well as RAPD marker to evaluate the genetic diversity among the soft-rot bacteria isolated from potato, cabbage, carrot, pepper, tomato, cucumber, turnip and date palm tree in Fars province.

MATERIALS AND METHODS

Sampling

During the summer months of 2013, the samples were collected from potato, cabbage, carrot, pepper, tomato, cucumber, turnip and date palm trees with the symptoms of pectobacterial infection, in different regions of Fars province. Two reference isolates (EccSCR and EchSCR) which were provided by Scottish Crop Research Institute were used to compare with the isolated bacteria. The information on the isolates is given in Table 1.

Isolation and purification

In order to isolate the bacteria, the infected plant parts were washed with tap water. Bacteria were isolated from the interface between healthy and infected portions of the rotted tissue. The tissues were dipped in 10% sodium hypochlorite for 30 s and then washed with sterile distilled water to disperse the bacterial cells into water. Finally, the pieces were transferred into sterilized Petri dishes containing a few ml of distilled water and was shaken for 15-30 min. The content of Petri dishes were transferred into sterilized mortar and then homogenized. This suspension is then streaked on nutrient agar (NA) medium and the Petri dishes were incubated at 27-30°C. After 24-48 h, to purify the isolated bacteria, the relatively slimy and milky small colonies were sub-cultured on fresh NA medium. The small and relatively embossed colonies with bright green color were transferred to Eosin Methylene-Blue (EMB) medium and the purified colonies were obtained.

Investigation of phenotypic characteristics

To identify the soft-rot pectobacteria, the tests of gram, oxidase, urease, catalase, potato rot, hypersensitive reaction and growth at 37 and 40°C, reductive materials production from sucrose and indole and phosphate production were conducted according to Schaad *et al.* (2001), and aerobic/anaerobic growth assay was carried out as described by Hugh & Leifson (1953). Erythromycin sensitivity assay was carried out based on the method described by Kelment *et al.* (1964).

DNA extraction

DNA was extracted by method described by Kaung *et al.* (2003). Bacteria were grown on LB medium (0.5% yeast extract, 1% Tripton, 1% Sodium Chloride), for 20-24 h. Three ml of bacterial culture was centrifuged at 14000 rpm for three min. The pellet was dissolved in 500 µl of CTAB buffer (2 g of CTAB, 0.6 g of Tris, 2.1 g of NACL and 0.4 g of EDTA) and heated at 65°C for 30 min. Chloroform-isoamylalchol solution (1:24) was added into the tubes and centrifuged at 13000 rpm for eight min, then the supernatants were transferred into new tubes. Equal volumes of cold isopropanol were added into the tubes and centrifuged at 13000 rpm for eight min for DNA precipitation. Finally, the tubes were washed using 70% ethanol and then were dried. 50 µl of TE buffer (Tris-EDTA) or

sterilized stilled water were added to the tubes and stored at -20°C. Two µl of the extracted DNA was subjected to PCR.

Pathogen identification by using specific primers

Identifying the bacteria was carried out using Y1/Y2 specific primers (Y1: 5' TTACCGGACGC CGAGCTGTGGCGT 3'/ Y2: 5' CAGGAAGATGTCGTTATCGCGAGT 3') (DARRASSE *et al.* 1996), with annealing temperature of 65°C and expected product size of 434 bp. *P. carotovorum* subsp. *carotovorum* was also identified using EXPCCR/EXPCCF specific primers (EXPCCR: 5' GCCGTAATTGCC TACCTGCTTAAG 3'/ EXPCCF: 5' GAACTT CGCACCGCCGACCTTCTA 3') (Kaung *et al.,* 2003), with annealing temperature of 60°C and expected product size of 550 bp. Finally, ADE1/ ADE2 specific primers (ADE1:5' GATCAGA AAGCCCGCAGCCAGAT/ 3'/ADE2:5' CTGTGGC CGATCAGGATGG 3') with annealing temperature of 65°C and expected product size of 434 bp were used to identify *Dickeya* spp. (Nasser *et al.,* 1994). The primers were generated in Pishgam Biotech Company, Iran. PCR was performed in a reaction mixture of 25 µl, containing 2 µl of DNA, 2.5 µl of 10X PCR Buffer, 1.5 µl of $MgCl_2$, 0.7 µl of dNTPs (25 mM), 0.5 µl of *Taq* DNA Polymerase and 1 µl of each primer (10 pmol). PCR products were stored at -20°C until gel electrophoresis.

Study on genetic diversity using RAPD

To compare the genetic diversity in the populations, RAPD analysis carried out by using six short random primers (Table 2). The assay was performed in a reaction mixture of 25 µl, containing 2 µl of DNA, 2.5 µl of 10X PCR Buffer, 2.5 µl of $MgCl_2$, 0.7 µl of dNTPs (25 mM), 0.5 µl of *Taq* DNA Polymerase and 1 µl of each primer (10 pmol). PCR was performed in a thermocycler for all primer. One cycle of initial denaturation at 94°C for one min, 40 cycles including denaturation at 94°C for one min, annealing at 35°C for one min and extension at 72°C for two min, and a final extension at 72°C for 10 min. PCR products were stored at -20°C until gel electrophoresis.

Gel electrophoresis

To carry out PCR reaction, 6-7 µl of each PCR product were mixed with 2 µl of loading buffer and were electrophoresed in 1% agarose gel at 80-90 volt for 1 h. Ruler 100 bp DNA ladder was used for determination of the fragments size. The gel was dyed using ethidium bromide and then distained in stilled water. The stained gels were observed with the UV 254 transilluminator and photographed.

Analysis of molecular data

The genetic similarities among strains were analyzed by NTSYS-PC ver. 2.02 software (Numerical Taxonomy and Multivariate Analysis System), on the basis of electrophoresis profiles. Genetic distance or similarity among the strains based on molecular markers, was scored as the presence/absence of band in the gel. The matrix of similarity of coefficients was subjected to un-weighted pair-group method analysis (UPGMA) to generate dendrogram, using the average linkage procedure. (Rohlf, 2000).

RESULTS

Phenotypic characteristics

All isolates were rod-shape, gram-negative, facultative anaerobe, oxidase-negative, catalase-positive and were able to cause potato rot while they could not induce hypersensitive reaction. They did not produce yellow colonies on YDC medium, but they showed bright green colonies on EMB medium (Gallios *et al.,* 1992; Schaad *et al.,* 2001). According to differential physiological characteristics (Table 3), the isolates were categorized into three groups. The first group which grown at 37°C but not at 40°C and were negative for indole production, sucrose reduction, phosphatase activity and insensitive to erythromycin, were similar to *P. carotovorum* subsp. *carotovorum (Pcc)* (Fahy & Persly, 1983). The second group were grown at 37°C, unable to grow at 40°C and were negative for indole production, phosphatase activity and insensitive to erythromycin, but positive to sucrose reduction, were similar to *Pectobacterium betavascularum (Pb)* (Thomson & Schroth, 1981). Third group were grown at 40°C, were positive for indole production, phosphatase activity and sensitive to erythromycin, were found to be similar to *Dickeya chrysanthemi (Dch)* (Nasser *et al.,* 1994).

PCR reaction and characterization of the bacterial isolates

Except for *P. betavascularum,* the primers Y_1/Y_2 were amplified the expected product size of 434 bp, and characterized the *Pectobacterium*

Table 1. *Pectobacterium* isolates from different hosts in Fars Province

Isolate	Origin	Host	Isolate	Origin	Host
O_3	Marvdasht	Onion	S_2	Zargan	Sugar beet
Tu_1	Kazerun	Turnip	S_3	Zargan	Sugar beet
Tu_2	Shiraz	Turnip	S_4	Zargan	Sugar beet
Tu_3	Kazerun	Turnip	S_5	Zargan	Sugar beet
P_5	Marvdasht	Potato	S_6	Zargan	Sugar beet
P_6	Marvdasht	Potato	P_1	Kazerun	Potato
Cu_2	Kazerun	Cucumber	P_2	Kazerun	Potato
C_3	Shiraz	Carrot	Cu_1	Marvdasht	Cucumber
C_4	Fasa	Carrot	Pa_1	Farashband	Palm
Pe_1	Fasa	Pepper	O_1	Fasa	Onion
Pe_2	Fasa	Pepper	Pa_2	Farashband	Palm
Ca_1	Marvdasht	Cabbage	O_2	Farashband	Onion
Ca_2	Marvdasht	Cabbage	P_7	Shiraz	Potato
To_1	Shiraz	Tomato	P_8	Shiraz	Potato
To_2	Marvdasht	Tomato	C_1	Mianrud	Carrot
Pa_4	Asaluyeh	Palm	C_2	Mianrud	Carrot
EccSCR	Scotland	Potato	P_3	Kaftarak	Potato
EchSCR	Scotland	Potato	P_4	Kaftarak	Potato
S_1	Shiraz	Sugar beet			

isolates (Fig. 1). Moreover, by amplifying the expected 550 bp products, the oligonucleotid primers EXPCCR/EXPCCF found to be suitable for separating *Pcc* that isolated from various hosts (Fig. 2).

RAPD assay

RAPD assay was performed using the primers 19300, 91200, OPB07, OPB08, OPB11, OPB18. All primers successfully amplified DNA fragments and clearly showed genetic diversity among soft-rot isolates (Figs. 3-5). Complementary analysis using genetic fingerprinting from three RAPD primers showed that the isolates fall into three groups (Figs. 6 and 7). The first group includes all *Pb* isolates which produced exclusive electrophoresis patterns. The second and the third groups include *Dch* and all of *Pcc* isolates, respectively. In this way, thirty five isolates from various hosts which were identified as *Pcc*, *Pb* and *Dch* using phenotypic characteristics, were easily identified by RAPD assay.

Table 2. Primers used in RAPD test for characterization of *Pectobacterium* isolates from different hosts in Fars Province

Primer name	Primer sequence
19300	CGATTCGGCG
91200	GATAACGCAC
OPB-07	GGTGACGCAG
OPB-08	GTGACGTAGG
OPB-11	GTAGACCCGT
OPB-18	CCACAGCAGT

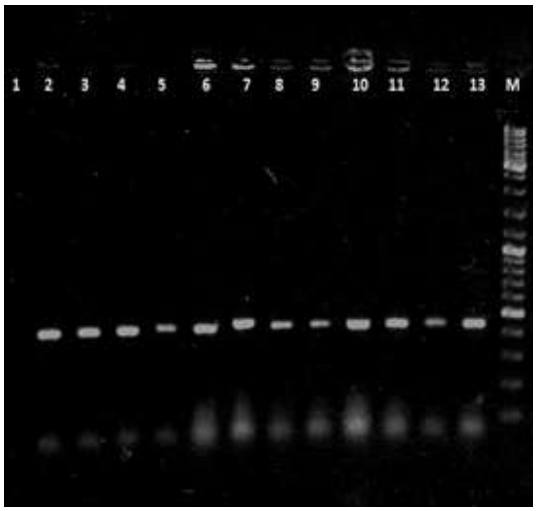

Fig. 1. DNA fingerprinting pattern of *Pectobacterium* sp. isolates generated by Y_1 and Y_2 primers 1: Water control, 2: Standard isolate, 3: P_1, 4: Pa_1, 5: C_1, 6: P_3, 7: O_3, 8: Tu_1, 9: P_5, 10: Pe_1, 11: To_1, 12: C_3, 13: Ca_1, M: Marker (100bp)

Table 3. Differential physiological properties of *Pectobacterium* isolates from different hosts in Fars Province

Test	Bacterial isolates																																					
	S_1	S_2	S_3	S_4	S_5	S_6	EccSCR	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	Cu_1	Cu_2	C_1	C_2	C_3	C_4	Tu_1	Tu_2	Tu_3	Pe_1	Pe_2	Ca_1	Ca_2	To_1	To_2	O_1	O_3	Pa_1	Pa_4	EchSCR	Pa_2
O_2																																						
Gram test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aerobe growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobe growth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Potato soft rot	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HR on Tobacco	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 40°C	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-
Yellow pigment on YDC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phosphatase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Erythromycin sensitivity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Reducing substrate from sucrose+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pectobacterium strain	*Pb*	*Pb*	*Pb*	*Pb*	*Pb*	*Pb*	*Dch*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Pcc*	*Dch*	*Dch*	*Dch*

Pb: Pectobacterium betavascularum Dch: Dickeya chrysanthemi *Pcc: Pectobacterium carotovorum* subsp. *carotovorum*

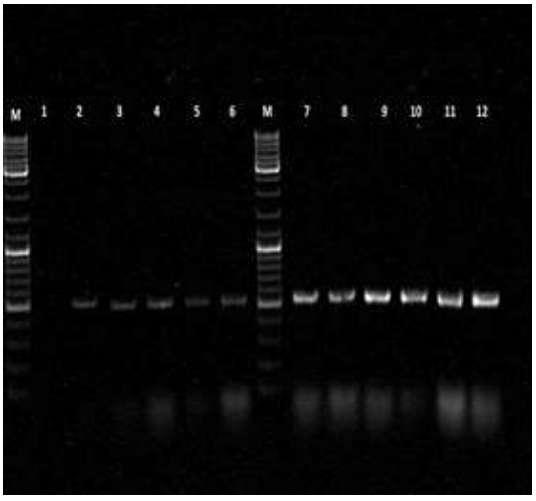

Fig. 2. DNA fingerprinting pattern of *Pectobacterium carotovorum* subsp. *carotovorum* isolates generated by EXPCCR and EXPCCF primers M: Marker (100bp), 1: Water Control, 2: P_1, 3: P_2, 4: Pa_1, 5: O_1, 6:P_3, 7: O_3, 8: C_1, 9: C_5, 10: P_4, 11: To_1, 12: Standard isolate

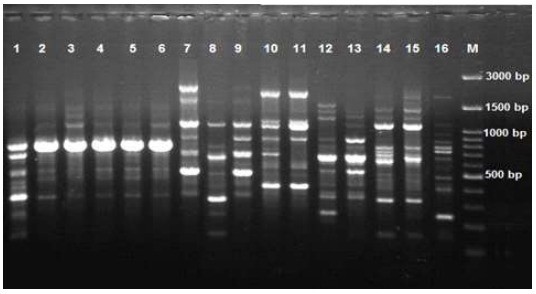

Fig. 3. DNA fingerprinting pattern of isolates generated by RAPD and primer 193001: S_1, 2: S_2, 3: S_3, 4: S_4, 5: S_5, 6: S_6, 7: EchSCR, 8: O_2, 9: Pa_2, 10: P_7, 11:P_8, 12: P_1, 13: P_2, 14: Cu_2, 15: O_1, 16: Pa_1, M: Marker (100bp).

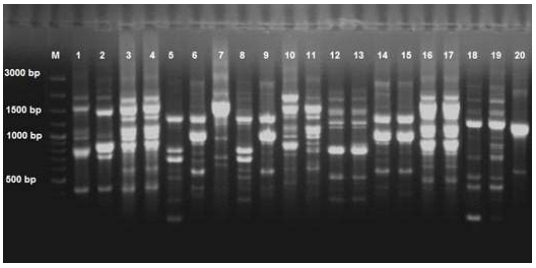

Fig. 4: DNA fingerprinting pattern of isolates generated by RAPD and primer OPB07 M: Marker (100 bp), 1: C_1, 2:C_2, 3: P_3, 4: P_4, 5: O_3, 6: Tu_1, 7: Tu_2, 8: Tu_3, 9: Cu_2, 10: P_5, 11: P_6, 12: C_3, 13: C_4, 14: Pe_1, 15: Pe_2, 16: Ca_1, 17: Ca_2, 18: To_1, 19: To_2, 20: Pa_4.

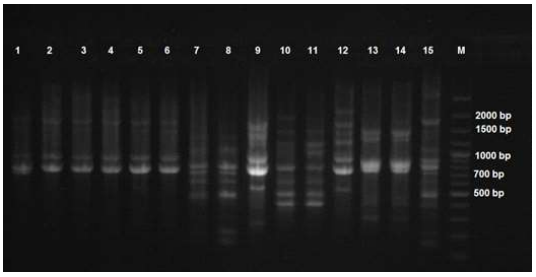

Fig. 5. DNA fingerprinting pattern of isolates generated by RAPD and primer OPB08
1: S_1, 2:S_2, 3: S_3, 4: S_4, 5: S_5, 6: S_6, 7: EchSCR, 8: O_2, 9: Pa_2, 10: P_7, 11: P_8, 12: Cu_1, 13: P_1, 14: P_2, 15: O_1, M: Marker (100bp)

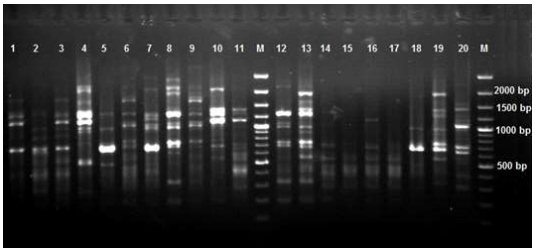

Fig 6. DNA fingerprinting pattern of isolates generated by RAPD and primer OPB18
1: C_1, 2: C_2, 3: P_3, 4: P_4, 5: O_3, 6: Tu_1, 7: Tu_2, 8: Tu_3, 9: P_5, 10: P_6, 11: Cu_2, 12: C_3, 13: C_4, 14: Pe_1, 15: Pe_2, 16: Ca_1, 17: Ca_2, 18: To_1, 19: To_2, 20: Pa_4, M: Marker (100bp)

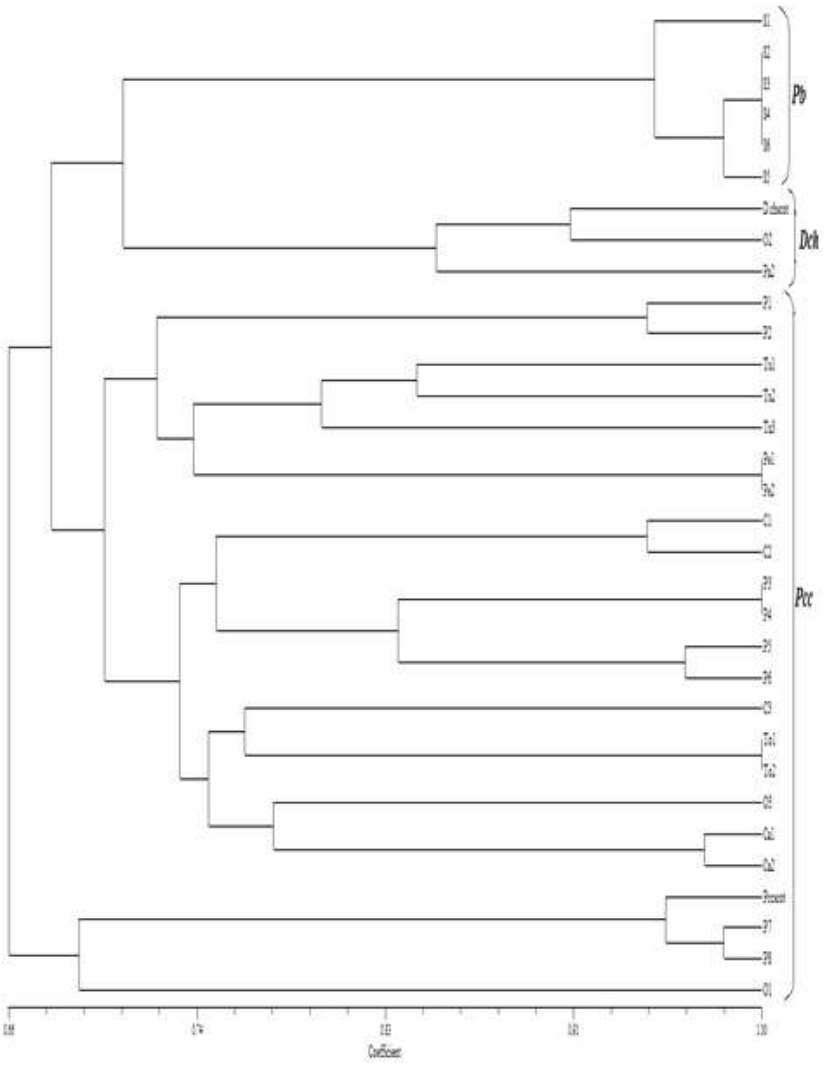

Fig. 7. Dendrogram of soft rot *Pectobacterium* strains based on 6 primers of RAPD
Pb: Pectobacterium betavascularum Dch: Dickeya chrysanthemi Pcc: Pectobacterium carotovorum subsp. *carotovorum*

DISCUSSION

In this study, significant diversity was observed among the tested isolates, in case of phenotypic characteristics. However, in some cases, an inconsistency with previously identified species was found. It has reported earlier that bacterial identification seems to require more reliable, precise and faster methods, such as DNA-based techniques. According to Toth *et al.* (2001), RAPD method can be used as a faster and more suitable method than the phenotypic methods.

Our study showed that investigation of genetic diversity using RAPD assay is a reliable method for identification and classification of *Pectobacterium* spp. In the present research, the results obtained from the primers 19300, 91200, OPB07, OPB08, OPB11 and OPB18, showed that all of the primers successfully amplified the DNA fragments. Of these primers, 19300, 91200, OPB07 and OPB11 was able to differentiate the three species of the soft rot bacteria. Analysis of genetic fingerprinting from six RAPD primers showed that the isolates which have 66% similarity, are categorized into two groups. The first group is divided to two sub-group, first includes *Pb* isolates with 95% and *Dch* isolates with 85% similarity and the second includes some isolates of *Pcc* with 70% similarity. The other isolates of *Pcc* were fallen into the second group with 69% similarity. In our study, no PCR product was amplified in case of *Pb* and *Dch* isolates. This is agree with previous findings (Helias *et al.,* 2000; Kaung *et al.,* 2003).

RAPD assay of the *Pcc* isolates showed that these are genetically diverse isolates whereas the *Pb* isolates have more genetic homogeneity. More restricted host range, smaller population size and even similar origin may are the reasons for lesser genetic diversity of the *Pb* isolates.

In *Pcc* group, the isolates P_1, P_2, C_1 and C_2 which were isolated from a same host and region share 95% similarity. However, in some cases, it is possible that two isolates from a same host and a same geographical region fall into different groups with less similarity, indicating high level of diversity in a single geographical region.

Maki-Valkama & Karjalainen (1994) successfully used RAPD to distinguish *Erwinia carotovora* subsp. *carotovora (Ecc)* from *E. carotovora* subsp. *atroseptica (Eca)*. They also showed that the similarity among the isolates of *Eca* is more than 85% while high level of genetic diversity was observed among *Ecc* samples. Rezaei & Taghavi (2008) used rep-PCR with Eric, Box and rep primers and categorized the soft-rot pectobacteria into four groups: *Pcc*, *P. carotovorum* subsp. *odoriferum (Pco)*, *Pb* and *Dch.* Furthermore, they showed that a high level of genetic diversity exists among *Pcc* isolates. Tavasoli *et al.* (2010) investigated the genetic diversity of the soft-rot pectobacterial population using BOX-PCR. Their results showed that there is a high level of diversity among the isolates. Generally, the diversity has been found in genetic fingerprint of isolates from different regions. In contrast, isolates from a single region may fall into different groups. In a study, AFLP was performed by Avrova *et al*. (2002), and the tested pectobacteria were placed into three groups. The first includes *Pco* and *Pcc,* the second contains *P. atroseptica (Pa)* and *Pb* and the third harbors *Dch*. A polymerase chain reaction (PCR) test based on amplification of 16S–23S rRNA of intergenic transcribed spacer (ITS) characterization of *P. carotovorum* was carried out by Golkhandan *et al.* (2013). They results showed that PCR by G1/L1 followed by RCR-RFLP of G1/L1 primer using *Rsa*I enzyme was a simple, rapid, and effective method for identification and differentiation of *Pcc* from *Dickeya* spp. and other species of *Pectobacterium*.

So far, RAPD assay has not been used to investigate the genetic diversity of soft-rot bacteria. However, this method has been used for some other plant pathogenic bacteria. Bysal *et al.* (2011) used ISSR method and showed that there is a high genetic diversity among the isolates of *Clavibacter michiganensis* subsp. *michiganensis* in Turkey. In another study, Samanta & Mandal (2013) were applied the ISSR test to show the genetic diversity among the isolates of *X. axonopodis.* pv. *commiphorae.* Kawaguchi (2014) used ISSR to categorize the isolates of *X. arbicola* pv. *pruni* into four groups, while the genetic diversity among the isolates was low.

The results of the present study showed that RAPD fingerprinting is a powerful tool to identify the various soft rot bacteria from different hosts and regions. The high genetic diversity was found in case of *Pcc* and *Dch* isolates. *Pb* isolates

were also found to have more genetic homogeneity.

ACKNOWLEDGMENTS

The research was supported by Research and Technology office and Post Graduate office of Yasouj University, Yasouj, Iran. The authors thank for their valuable assistance.

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