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Full Length Research Paper

Detection and identification of bacterial soft rot of potato *Pectobacterium carotovorum* subsp. *carotovorum* using specific PCR primers in Jordan

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Potato soft rot is one of the major destructive diseases affecting potato plants throughout the world. In a survey of different potato growing seasons in different regions of Jordan, samples of rotten potato tubers were collected and 131 isolates identified biochemically as *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*). The PCR primer pair (EXPCCR/EXPCCF) was used to detect these Jordanian isolates. The primer set amplified a single fragment of 550 bp in size from the total genomic DNA, which was extracted independently from 67 *Pcc* strains. In a nested PCR, the primer set (INPCCR/INPCCF) amplified the expected single fragment of 400 bp from the PCR product of first PCR amplification. The use of these primers was not reliable in detecting all isolates identified biochemically as *Pcc*. Different rots causal agents were detected by PCR amplification and further sequenced. The sequencing data revealed similarities to different genera; *Pseudomonas*, Enterobacteriaceae genera such as *Enterobacter* spp., *Serratia* spp. and *Klebsiella* spp., in addition to *P. carotovorum* subsp. *carotovorum*. So far this is the first study where *Pcc* has been identified by using PCR and sequencing approaches in Jordan.

Key words: *Pectobacterium carotovorum* subsp. *carotovorum*, specific primers, nested PCR, sequencing.

INTRODUCTION

Different bacterial diseases have been reported to attack potatoes around the world leading to high economic losses in yield and quality under favorable environmental conditions; of these are brown rot (*Ralstonia solanacearum*), common scab (*Streptomyces scabies*), ring rot (*Clavibacter michiganensis* subsp. *sepodonicus*), black leg (*Pectobacterium carotovorum* subsp. *atrosepticum*) and soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*).

However, potato soft rot is one of the most important diseases of potatoes; it causes a great reduction in yield resulting in economic losses in field and during transit. Hence, it is reportedly caused by various species like *Bacillus*, *Pseudomonas*, *Enterobacter cloacae* and

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Erwinia (Liao and Wells, 1987; Bishop and Davis, 1990; Agrios, 2005; Schroeder et al., 2009). However, *Erwinia carotovora* subsp. *carotovora* is reported as the most common causal agent of bacterial soft rot of potato and other commercially important crops (Perombelon, 2002; Agrios, 2005; Monilola and Abiola, 2011).

Bacterial soft rot is one of the destructive diseases prevalent in subtropical regions. It occurs worldwide wherever fleshy and stored vegetables are present (Bhat et al., 2010b). Soft rot disease causes greater total losses of produce than any other bacterial disease, hence it is considered among the factors contributing to yield losses, and among the most prevalent and destructive bacterial diseases that affect vegetables which is difficult to control (Perombelon and Kelman, 1980; Bhat et al., 2010b).

Soft rot disease caused by *P. carotovorum* subsp. *carotovorum* (*Pcc*) (Dye, 1969) is listed among the top ten plant pathogenic bacteria (Mansfield et al., 2012) with a wide host range in tropical and subtropical regions infecting vegetable species belonging to different major vegetable families including cabbage, cauliflower, lettuce, onion, pepper, carrot and potato (Rajeh and Khlaif, 2000; Bhat et al., 2010a).

In the last 30 years, Polymerase Chain Reaction (PCR) has been used for specific and rapid method for detection and identification of pathogen (Czajkowski et al., 2009). PCR technique greatly enhances detection sensitivity compared with other methods of detection (Toth et al., 1999; Kang et al., 2003; Czajkowski et al., 2009).

In Jordan, *Pcc* was identified as the causal agent of soft rot disease of vegetables, whose detection and identification was carried out through traditional techniques such as isolation on selective media and biochemical characterization. The pathogen infects and causes disease on a wide variety of hosts belonging to different families of vegetables either in field or in storage in different areas mainly Jordan Valley and Uplands. Soft rot of potatoes is a tuber borne disease where the contaminated mother tubers were reported to be the main source of inoculum. However, this bacterium was found to survive in the soil with population trends that vary with the fluctuation in soil temperature (Rajeh and Khlaif, 2000).

This research was conducted in order to isolate and identify the causal agent of potato soft rot from different potato growing areas and storages in Jordan using biochemical and physiological tests. We also aimed to characterize the obtained isolates by PCR technique using specific set of primer.

MATERIALS AND METHODS

Samples collections

Potato rotted tubers samples were randomly collected during the years 2013 to 2015 from different potato growing areas in Jordan. The collected samples were placed in an ice box for further work in the laboratory.

Bacterial isolation

Infected potato tubers were surface sterilized with 0.5% sodium hypochlorite; thereafter, 10 g of rotted tubers were cut into small pieces placed in sterile bottle with 90 ml of sterile distilled water placed on a shaker at 200 rpm at room temperature. Series of serial dilutions were then prepared up to 10^{-3} dilution, and 0.1 ml of the 10^{-3} dilution spread onto the surface of three Logan's medium plates (Schaad et al., 2001). The inoculated plates were incubated at 27±2°C and checked periodically. Appearance of bacterial colonies with wide pink centers within the first 24 h of inoculation was suspected to be *Pcc* (Fahy and Parsley, 1983). Single colonies were re-streaked onto new nutrient agar (NA) plates. The obtained bacterial isolates were kept as SDW suspension in sterile Eppendroff tubes and kept in refrigerator for further identifications.

Biochemical and physiological tests

In order to identify *Pcc,* the Jordanian isolates were grown at 27ºC for 24 h on nutrient agar plates and were then subjected to the biochemical and physiological tests; oxidase, catalase potato soft rot, oxidative fermentative, growth at 37ºC, sodium chloride tolerance, reducing substances from sucrose, urease production and acid production from carbohydrates as described by Schaad et al. (2001). The same tests were run against a reference culture of *Pcc* isolate NCPPB312 obtained from Food and Environment Research Agency (*fera*), United Kingdom and against sterile water as a negative control.

DNA extraction

Bacterial DNA was extracted from 24 h old pure bacterial cultures grown on NA plates at 27ºC, obtained and identified by biochemical and physiological tests as *Pcc* isolates. Pure bacterial colonies were picked with a sterile loop and mixed in 4 ml of nutrient broth in a sterile and labeled culture tubes incubated overnight at 37ºC, with shaking at 150 rpm.

Genomic DNA extraction was done using DNeasy Blood and Tissue Kit (Qiagen, Valancia, CA); the protocol was performed according to the manufacturer's instructions designed for purification of total DNA from gram-negative bacteria.

The quantity and quality of the extracted genomic DNA were measured using the spectrophotometer with DNA visualized by electrophoresis in 1.0% agarose gel in Tris-Acetate-EDTA(TAE) buffer stained with ethidium bromide (0.5 µg/ml). The extracted DNA was stored at -20ºC for further PCR work.

PCR amplification, purification and sequencing

Oligonucleotide primers EXPCCR (5-GCCGTAATTGCCTACCTGCTTAAG-3) and EXPCCF (5-GAACTTCGCACCGCCGACCTTCTA-3) were used in standard PCR (Kang et al., 2003; Mahmoudi et al., 2007; Palacio-Bielsa et al., 2009). The PCR reactions were performed in 25 µl PCR mixture containing 25 mM $MgCl_2$, 5X Crimson *Taq* buffer, 10 mM dNTPs, 10 µM primer, 5U/µl*Taq* polymerase. PCR amplification was carried out as follows: one cycle of 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C and a final extension for 7 min at 72°C.

After amplification reaction, 10 µl of PCR products were separated on 1.5% agarose gel in TAE buffer and visualized by staining with ethidium bromide; also, 100 bp was used as a molecular DNA marker. Electrophoresis was performed at 110 V for 35 m in gels which were photographed under ultraviolet (UV) light.

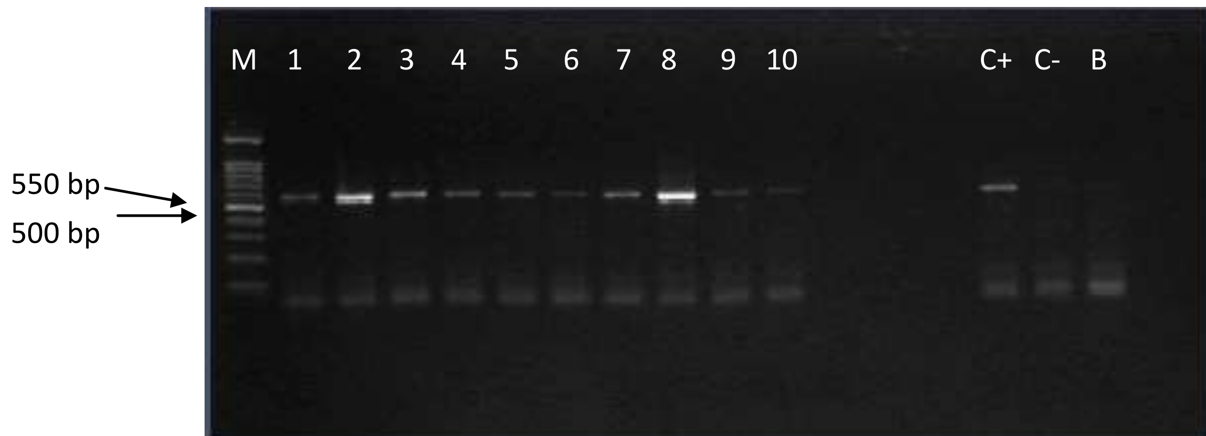

Figure 1. Agarose gel electrophoresis for PCR-amplified DNA of *Pectobacterium carotovorum* subsp. *carotovorum* isolates using EXPCC primers set with the expected amplified product of 550 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1- 10: isolates Jo-Q 16, Q19, A11, A2, Q14, Q21, Q29, A5, Q23 and Q27, respectively. Lanes C+: Positive control (reference isolate NCPPB312). C: Negative control isolate *Escherichia coli.* B: Buffer.

For nested PCR, primers INPCCF (5-TTCGATCACGCAACCTGCATTATC-3) and INPCCR (5-GGCCAAGCAGTGCCTGTATATCC-3) were used for amplification of an expected 0.4 Kb (Kang et al., 2003). Two microliters of the amplified template from the first PCR were used in nested PCR reaction under the same conditions as stated previously.

The PCR products were purified using the Wizard Purification System (Promega, Madison, Wisconsin) with the protocol performed according to the manufacturer's instructions. After purification, the DNA fragments were sequenced in both directions by Macrogen Korea (Seoul, Rep. of Korea) or Quintara Biosciences (South San Francisco, CA).

The DNA sequence data were analyzed for homology using Basic local alignment searching tool (BLAST) at NCBI server. Blast search was performed only for nucleotide sequence using BLASTn (Stephen et al., 1990).

RESULTS AND DISCUSSION

The bacterial isolates isolated from rotted potato from different regions were all identical in their cultural and biochemical properties and similar to that of reference culture of *Pcc* isolate NCPPB312. Logan's medium small circular bacterial colonies with pink to red purple centers were developed 24 h after incubation at 27ºC (Fahy and Parsley 1983).

However, bacteria from purified colonies were found to be oxidase negative, catalase positive, fermentation of glucose positive, rotting induced on inoculated potato slices, urease enzyme was produced, growth developed on nutrient agar plates incubated at 37±2ºC and on 5% NaCl. Also, all isolates were able to oxidize the alcoholic sugar and discharge it in the media to acidic reaction and were not able to reduce substances from sucrose. The above mentioned physiological reactions support the identification of these isolates as *Pcc*.

Pectobacterium species and subspecies have been increased over the recent years, and as a result, their identification and differentiation by classical methodology became more challenging (De Boer et al., 2012).

PCR assays

A total of 67 (51%) out of 131 isolates which were biochemically identified as *Pcc* showed a 550-bp bands with the specific EXPCC set of primers (Figure 1); also, bands of 380 bp were observed when PCR products amplified with EXPCC primers set were used as templates (nested PCR) using INPCC set of primers (Figure 2) (Kang et al., 2003).

Sequence analysis

Maximum nucleotide similarity results of BLASTn for selected *Pcc* isolates amplified with EXPCC species-specific set of primers are presented in Table 1, showing maximum similarity percentage with closely related reference strains, E-value and accession numbers in the GenBank.

BLASTn results showed that most of *Pcc* Jo-isolates showed high similarity with the strain *E. carotovora* subsp. *carotovora* from Korea (Acc. no. AF046928.1) and with *Pcc* strain PC1 from USA (Acc. no.CP001657.1). Maximum similarity percentage ranged from 86 to 98%; some *Pcc* Jo-isolates showed high similarity with the reference strain *P. carotovorum* subsp. *odoriferum* (Acc. no. CP009678.1) from China, whereas their maximum similarity percentage ranged from 85 to 98%.

In order to determine the specificity of EXPCC primers (Kang et al., 2003), eleven bacterial isolates sequenced on the bases of EXPCC primers and their sequences

Figure 2. Agarose gel electrophoresis for PCR-amplified DNA of *P. carotovorum* subsp. *carotovorum* isolates using EXPCC primers set followed by nested PCR using INPCC primers set with the expected amplified product of 380 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1-10: Isolates Jo-Q16, Q19, A11, A2, Q14, Q21, Q29, A5, Q23 and Q27, respectively. Lanes C+: Positive control (reference isolate NCPPB312); C: Negative control isolate *E. coli.* B: Buffer.

Figure 3. Agarose gel electrophoresis for PCR-amplified DNA of *P. carotovorum* subsp. *Carotovorum* isolates using EXPCC primers set followed by nested PCR using INPCC primers set with the expected amplified product of 380 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1-11: Isolates Jo-S97/2/1, S97/2/2, Q111, G60/2/1, G60/2/2, G42/1/1, G42/1/2, M2/3, M2/4, G40/2/1 and G40/2/2, respectively. Lanes C+: Positive control (reference isolate NCPPB312). B: Buffer.

showed maximum similarity percentage with other closely related species. Interestingly, nested PCR gave the expected product size of 380 bp (Figure 3), although they were identified as *Enterobacter* spp., *Serratia* spp. and *Klebsiella* spp., based on their sequencing, rather than *P. carotovorum* subsp. *carotovorum*.

Specificity of PCR with the species-specific set of primers (EXPCCF/EXPCCR), which was designed to test specificity of *Pcc* isolates was more limited because they also amplified the expected 550 bp product from some isolates identified as species other than *Pcc* as shown in Figure 2 and did not amplify DNA from other isolates that were identified as *Pcc* on the basis of biochemical test (Table 2). Similar results were obtained by De Boer et al. (2012) where the specific primer set for *Pcc* amplify the expected size of strains identified as *P. wasabiae* and did not amplify other strains biochemically identified as *Pcc*.

Our results are also in agreement with previous finding of Azadmanesh et al. (2013) where none of the 12 Iranian *Pcc* tested isolates produced the 550 bp products in PCR in contrast to two standard *Pcc* isolates that produced the desired bands and they found that these

Table 1. Maximum nucleotide similarity (BLASTn) between *Pectobacterium carotovorum* subsp. *carotovorum* Jo-isolates amplified with EXPCC set of primers and the most closely related species/subspecies.

S/N	Isolate and accession no. in GenBank	Closely related species/subspecies	E-value	Maximum % similarity	Accession no.
1	Jo-G59 (MF535186)	*Pco* *Ecc*	5 e^{-42} 2 e^{-35}	89 86	CP009678.1 AF046928.1
2	Jo-S97 (MF535187)	*Ecc*	2 e^{-26}	97	AF046928.1
3	Jo-Q16 (MF535188)	*Pco* *Ecc* *Pcc* PC1 strain	2 e^{-79} 8 e^{-30} 1 e^{-157}	98 94 92	CP009678.1 AF046928.1 CP001657.1
4	Jo-Q19 (MF535189)	*Pco* *Ecc* *Pcc* PC1 strain	4 e^{-82} 8 e^{-10} 2 e^{-155}	98 94 92	CP009678.1 AF046928.1 CP001657.1
5	Jo-A11 (MF535190)	*Pco* *Ecc* *Pcc* PC1 strain	3 e^{-133} 4 e^{-122} 2 e^{-155}	98 94 92	CP009678.1 AF046928.1 CP001657.1
6	Jo-A2 (MF535191)	*Pco* *Ecc* *Pcc* PC1 strain	3 e^{-133} 4 e^{-122} 2 e^{-155}	98 94 92	CP009678.1 AF046928.1 CP001657.1
7	Jo-Q14 (MF535192)	*Pco* *Ecc* *Pcc* PC1 strain	3 e^{-133} 4 e^{-122} 2 e^{-155}	97 93 91	CP009678.1 AF046928.1 CP001657.1
8	Jo-Q29 (MF535193)	*Pco* *E cc* *Pcc* PC1 strain	1 e^{-127} 2 e^{-116} 4 e^{-147}	98 94 92	CP009678.1 AF046928.1 CP001657.1
9	Jo-A5 (MF535194)	*Pco* *Ecc* *Pcc* PC1 strain	7 e^{-125} 9 e^{-115} 2 e^{-155}	98 94 92	CP009678.2 AF046928.2 CP001657.1
10	Jo-Q23 (MF535195)	*Pco* *E cc* *Pcc* PC1 strain	3 e^{-133} 4 e^{-122} 2 e^{-155}	97 93 90	CP009678.2 AF046928.2 CP001657.1
11	Jo-Q27 (MF535196)	*Pco* *Ecc* *Pcc* PC1 strain	3 e^{-133} 4 e^{-122} 3 e^{-148}	98 94 91	CP009678.2 AF046928.2 CP001657.1
12	Jo-Q30 (MF535197)	*Pco* *E cc*	5 e^{-126} 7 e^{-115}	85 88	CP009678.2 AF046928.2
13	Reference strain NCPPB312	*Pco* BC S7 *Ecc* *Pcc*1 PC1 strain	9 e^{-154} 1 e^{-131} 2 e^{-120}	98 94 91	CP009678.1 AF046928.2 CP001657.1

two isolates could not be identified by PCR using *Pectobacterium* subsp. specific primers. Also, Kang et al. (2003) found that only genomic DNA of 29 strains of *Pcc* out of 54 bacterial strains which is equal to about 54% yielded the expected 550 bp amplified product following PCR with EXPCC specific primers. These results could be due to sequence variation among strains of *Pectobacterium* isolated from different regions of the world (Azadmanesh et al., 2013). Whereas the species-specific EXPCC was generated from the nucleotide

Table 2. Polymerase chain reaction (PCR) of set of primers for all *P. carotovorum* subsp. *carotovorum* Jo-isolates collected from different regions of Jordan.

No.	Reference no.	Region	Result of biochemical tests	Primer set (Species-specific EXPCC)
1	A1	Amman	Positive	Positive
2	A2	Amman	Positive	Positive
3	A3	Amman	Positive	Positive
4	A4	Amman	Positive	Negative
5	A5	Amman	Positive	Positive
6	A6	Amman	Positive	Negative
7	A7	Amman	Positive	Negative
8	A8	Amman	Positive	Positive
9	A9	Amman	Positive	Negative
10	A10	Amman	Positive	Positive
11	A11	Amman	Positive	Positive
12	A12	Amman	Positive	Negative
13	A13	Amman	Positive	Positive
14	R123/1	AR Ramtha	Positive	Negative
15	R104/3	AR Ramtha	Positive	Negative
16	R105/2	AR Ramtha	Positive	Negative
17	R85/4	AR Ramtha	Positive	Positive
18	R105/4	AR Ramtha	Positive	Negative
19	R104/4	AR Ramtha	Positive	Negative
20	R105/3	AR Ramtha	Positive	Negative
21	R89/4	AR Ramtha	Positive	Negative
22	R89/3	AR Ramtha	Positive	Negative
23	R106/4	AR Ramtha	Positive	Negative
24	R83/3	AR Ramtha	Positive	Negative
25	R105/4	AR Ramtha	Positive	Positive
26	G29/1	Jordan Valley	Positive	Negative
27	G27/4	Jordan Valley	Positive	Positive
28	G72/2/4	Jordan Valley	Positive	Negative
29	G70/2	Jordan Valley	Positive	Negative
30	G6/4	Jordan Valley	Positive	Negative
31	G32/2/1	Jordan Valley	Positive	Negative
32	G4/4	Jordan Valley	Positive	Negative
33	G71/1	Jordan Valley	Positive	Negative
34	G68/1	Jordan Valley	Positive	Positive
35	G44/4	Jordan Valley	Positive	Negative
36	G43/1	Jordan Valley	Positive	Positive
37	G40/1	Jordan Valley	Positive	Positive
38	G59/3	Jordan Valley	Positive	Positive
39	G59/2	Jordan Valley	Positive	Positive
40	G71/2	Jordan Valley	Positive	Positive
41	G4/2	Jordan Valley	Positive	Negative
42	G68/4	Jordan Valley	Positive	Positive
43	G68/3	Jordan Valley	Positive	Positive
44	G68/2	Jordan Valley	Positive	Negative
45	G43\2	Jordan Valley	Positive	Positive
46	G60/1	Jordan Valley	Positive	Positive
47	G59/4	Jordan Valley	Positive	Positive
48	G60/2	Jordan Valley	Positive	Positive
49	G60/3	Jordan Valley	Positive	Positive
50	G60/4	Jordan Valley	Positive	Positive

Table 2. Contd.

51	G40/4	Jordan Valley	Positive	Positive
52	G40/3	Jordan Valley	Positive	Positive
53	G32/2/2	Jordan Valley	Positive	Positive
54	G32/2/3	Jordan Valley	Positive	Positive
55	G32/2/4	Jordan Valley	Positive	Positive
56	G32/3	Jordan Valley	Positive	Positive
57	G37/1	Jordan Valley	Positive	Positive
58	G37/2	Jordan Valley	Positive	Positive
59	G37/3	Jordan Valley	Positive	Positive
60	G37/4	Jordan Valley	Positive	Positive
61	G29/2	Jordan Valley	Positive	Negative
62	G42/1	Jordan Valley	Positive	Positive
63	G42/3	Jordan Valley	Positive	Positive
64	G59/1	Jordan Valley	Positive	Positive
65	G40/2	Jordan Valley	Positive	Positive
66	G29/3	Jordan Valley	Positive	Negative
67	G29/4	Jordan Valley	Positive	Negative
68	G18/1	Jordan Valley	Positive	Negative
69	G18/2	Jordan Valley	Positive	Negative
70	G18/3	Jordan Valley	Positive	Negative
71	G20/4	Jordan Valley	Positive	Positive
72	S24/3	Ma'an	Positive	Positive
73	S24/4	Ma'an	Positive	Positive
74	S98/2/2	Ma'an	Positive	Positive
75	S103/2	Ma'an	Positive	Positive
76	S97/2/4	Ma'an	Positive	Positive
77	S97/2/2	Ma'an	Positive	Positive
78	S103/3	Ma'an	Positive	Negative
79	S97/1/1	Ma'an	Positive	Negative
80	S97/2/3	Ma'an	Positive	Positive
81	S102/4	Ma'an	Positive	Negative
82	S24/2	Ma'an	Positive	Positive
83	S83/4	Ma'an	Positive	Negative
84	S99/4	Ma'an	Positive	Negative
85	S102/2	Ma'an	Positive	Positive
86	M2/2	Madaba	Positive	Negative
87	M117/2/4	Madaba	Positive	Positive
88	M113/2/3	Madaba	Positive	Positive
89	M114/2	Madaba	Positive	Positive
90	M2/3	Madaba	Positive	Negative
91	M112/2	Madaba	Positive	Negative
92	M113/2/4	Madaba	Positive	Positive
93	M113/1	Madaba	Positive	Positive
94	M114/2/1	Madaba	Positive	Positive
95	M126/4	Madaba	Positive	Negative
96	M117/2/3	Madaba	Positive	Negative
97	M114/2/4	Madaba	Positive	Positive
98	M114/2/2	Madaba	Positive	Positive
99	M113/4	Madaba	Positive	Positive
100	M126/2	Madaba	Positive	Positive
101	M2/4	Madaba	Positive	Negative
102	Q111/2	Mafraq	Positive	Negative

Table 2. Contd.

103	Q8/2	Mafraq	Positive	Negative
104	Q9/2	Mafraq	Positive	Negative
105	Q14/3	Mafraq	Positive	Negative
106	Q111/4	Mafraq	Positive	Negative
107	Q12/1	Mafraq	Positive	Negative
108	Q8/3	Mafraq	Positive	Negative
109	Q9/3	Mafraq	Positive	Negative
110	Q14/4	Mafraq	Positive	Negative
111	Q12/2/2	Mafraq	Positive	Negative
112	Q12/2/3	Mafraq	Positive	Negative
113	Q9/4	Mafraq	Positive	Positive
114	Q12/2/4	Mafraq	Positive	Positive
115	Q14	Mafraq	Positive	Positive
116	Q15	Mafraq	Positive	Positive
117	Q16	Mafraq	Positive	Positive
118	Q17	Mafraq	Positive	Negative
119	Q18	Mafraq	Positive	Positive
120	Q19	Mafraq	Positive	Positive
121	Q20	Mafraq	Positive	Negative
122	Q21	Mafraq	Positive	Positive
123	Q22	Mafraq	Positive	Positive
124	Q23	Mafraq	Positive	Positive
125	Q24	Mafraq	Positive	Negative
126	Q25	Mafraq	Positive	Positive
127	Q26	Mafraq	Positive	Negative
128	Q27	Mafraq	Positive	Positive
129	Q28	Mafraq	Positive	Negative
130	Q29	Mafraq	Positive	Positive
131	Q30	Mafraq	Positive	Positive
	Total positive		131	67 (51%)

sequence of a *Pcc* universal rice primer (URPs) PCR product, although URPs were developed from repetitive sequences in the rice genome that have been used to fingerprint genomes of diverse organisms (Kang et al., 2003).

Kang et al. (2003) presented that PCR used on the bases of EXPCC set of primers should be followed by using PCR product as a template for second run of nested PCR in order to confirm the detection of *Pcc* which will yield 380 bp bands. On the other hand, our results of nested PCR for some isolates which were confirmed as *Enterobacter* spp. and *Serratia* spp. using maximum nucleotide similarity (BLASTn) gave bands of 380 bp, where these results are in conflict with Kang et al. (2003) and indicated that EXPCC was not a species specific primer and in agreement with De Boer et al. (2012). Although specific DNA markers were commonly used for detection of bacteria at subspecies level as reported in many studies, specific detection of *Pcc* isolates using molecular techniques is faced by complexity among strains associated with other subspecies. In general, molecular approach that was used to evaluate microbial population revealed a more complex soft rotting population than is usually evident from evaluations based on isolation alone.

Homology search results for all Jordanian sequenced on the bases of EXPCC set of primers showed high similarity percentage with *Pc* subsp. *odoriferum* (*Pco*), and are in contrast with Kang et al. (2003), who reported that specific primers were able to differentiate *Pcc* strains among other subspecies. *P. carotovorum* subsp. *odoriferum* was reported as a typical *Pc* subsp. *atrosepticum* strain and it is pathogenic to chicory only and produce odorous volatile (Gallois et al., 1992), which consequently differs from *Pcc* known as widely distributed pathogen and has a broad host range (Kang et al., 2003). For all of the above, this confirms that all our isolates are *Pcc* rather than *Pco.*

CONCLUSION AND RECOMMENDATIONS

Soft rot disease is widely common in different potato

growing areas in Jordan and the results of biochemical and physiological tests confirmed that the main causal agent of soft rot in Jordan is *P. carotovorum* subsp. *Carotovorum.* While using the PCR primer pair (EXPCCR/EXPCCF) was found to be not reliable in detection and identification of all soft rot Jordanian isolates of *Pcc*, DNA sequencing was found to be the most reliable way in specific detection and confirmation of the causal agent of soft rot. On the other hand, more studies needs to be implemented in order to study soft rot disease etiology and epidemiology.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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