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. *sepedonicus*), 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...
**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 $27+2^\circ$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 Eppendorf tubes and kept in refrigerator for further identifications.

**Biochemical and physiological tests**

In order to identify Pcc, the Jordanian isolates were grown at $27^\circ$C 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 $37^\circ$C, 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 $27^\circ$C, 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 $37^\circ$C.

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 -20°C for further PCR work.

**PCR amplification, purification and sequencing**

Oligonucleotide primers EXPCCR (5'-GCCGTAATGCTACCTGCTTAAG-3') and EXPCCF (5'-GAACCCGCCACCCTGACCTTTCA-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 MgCl₂, 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.
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 27°C (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±2°C 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
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*                             | 5.e-42  | 89                   | CP009678.1   |
|     |                                     | *Ecc*                             | 2.e-35  | 86                   | AF046928.1   |
| 2   | Jo-S97 (MF535187)                   | *Ecc*                             | 2.e-26  | 97                   | AF046928.1   |
| 3   | Jo-Q16 (MF535188)                   | *Pco*                             | 2.e-79  | 98                   | CP009678.1   |
|     |                                     | *Ecc*                             | 8.e-30  | 94                   | AF046928.1   |
|     |                                     | *Pcc* PC1 strain                  | 1.e-157 | 92                   |              |
| 4   | Jo-Q19 (MF535189)                   | *Ecc*                             | 8.e-10  | 94                   | AF046928.1   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 92                   |              |
| 5   | Jo-A11 (MF535190)                   | *Pco*                             | 3.e-133 | 98                   | CP009678.1   |
|     |                                     | *Ecc*                             | 4.e-122 | 94                   | AF046928.1   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 92                   |              |
| 6   | Jo-A2 (MF535191)                    | *Ecc*                             | 4.e-122 | 94                   | AF046928.1   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 92                   |              |
| 7   | Jo-Q14 (MF535192)                   | *Ecc*                             | 4.e-122 | 93                   | AF046928.1   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 91                   |              |
| 8   | Jo-Q29 (MF535193)                   | *Ecc*                             | 1.e-127 | 98                   | CP009678.1   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-116 | 94                   | AF046928.1   |
|     |                                     |                                    | 4.e-147 | 92                   | CP001657.1   |
| 9   | Jo-A5 (MF535194)                    | *Pco*                             | 7.e-125 | 98                   | CP009678.2   |
|     |                                     | *Ecc*                             | 9.e-115 | 94                   | AF046928.2   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 92                   | CP001657.1   |
| 10  | Jo-Q23 (MF535195)                   | *Pco*                             | 3.e-133 | 97                   | CP009678.2   |
|     |                                     | *Ecc*                             | 4.e-122 | 93                   | AF046928.2   |
|     |                                     | *Pcc* PC1 strain                  | 2.e-155 | 90                   | CP001657.1   |
| 11  | Jo-Q27 (MF535196)                   | *Pco*                             | 3.e-133 | 98                   | CP009678.2   |
|     |                                     | *Ecc*                             | 4.e-122 | 94                   | AF046928.2   |
|     |                                     | *Pcc* PC1 strain                  | 3.e-148 | 91                   | CP001657.1   |
| 12  | Jo-Q30 (MF535197)                   | *Pco*                             | 5.e-126 | 85                   | CP009678.2   |
|     |                                     | *Ecc*                             | 7.e-115 | 88                   | AF046928.2   |
| 13  | Reference strain NCPPB312           | *Pco* BC S7                       | 9.e-154 | 98                   | CP009678.1   |
|     |                                     | *Ecc*                             | 1.e-131 | 94                   | AF046928.2   |
|     |                                     | *Pcc*1 PC1 strain                 | 2.e-120 | 91                   | 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.

|   | Sample Code | Location     | First Test | Second Test |
|---|-------------|--------------|------------|-------------|
| 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| S97/2/1     | 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| M114/2/4    | 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    |
sequence of a \textit{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 \textit{Pcc} which will yield 380 bp bands. On the other hand, our results of nested PCR for some isolates which were confirmed as \textit{Enterobacter} \textit{spp.} and \textit{Serratia} \textit{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 \textit{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 \textit{Pc} \textit{subsp. odoriferum} (\textit{Pco}), and are in contrast with Kang et al. (2003), who reported that specific primers were able to differentiate \textit{Pcc} strains among other subspecies. \textit{P. carotovorum} \textit{subsp. odoriferum} was reported as a typical \textit{Pc} \textit{subsp. atrosepticum} strain and it is pathogenic to chicory only and produce odorous volatile (Gallois et al., 1992), which consequently differs from \textit{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 \textit{Pcc} rather than \textit{Pco}.

\textbf{CONCLUSION AND RECOMMENDATIONS}

Soft rot disease is widely common in different potato

|   |   |   |   |
|---|---|---|---|
| 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%) |
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 need 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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