Egypt. J. Phytopathol., Vol. **44**, No. 1, pp. 11-23 (2016)

Identification and Differentiation of Egyptian Isolates of *Erwinia amylovora* using 16S rRNA Gene and RAPD-PCR

Hanan A. Shaheen; Rabab M. Abd-El-Aziz and A.E. Tawfik

Plant Pathol. Res. Inst., ARC, Giza, Egypt.

Erwinia amylovora the causative of fire blight disease on pear in Egypt was identified with some classical tests and molecular methods. Random Amplified Polymorphic DNA (RAPD) technique was used to identify the genetic variability among 6 Egyptian isolates of *E. amylovora* isolated from different governorates (Behera, Gharbiya, Nubariya, Menufiya, Kaluobiya and Ismailiya) and compared with one reference isolate. Profiles were obtained by amplification of DNA from these isolates with six random primers. The RAPD band patterns resulting from these primers were analyzed using UPGMA method to construct a similarity matrix and generate a dendogram indicating the relationships between the examined isolates. The results obtained indicated that primers P4d, P5e and P6f were chosen because they reliably and reproducibly detected polymorphism among the selected isolates. Also, no variation in bacterial genome was observed among the examined isolates. In addition, 16SrRNA gene sequence was used to identify *E. amylovora* isolates using universal primers (8F and U1492R). The results showed that sequence of the isolates collected from different locations was matched with the sequence found in the Genbank database. The sequence of the fragment (16S rRNA gene) showed percentage homology with strain DSM30165 of Genbank strains ranged from 89–97%. The selected isolates were found to have the closest homology to *E. amylovora*.

Keywords: *Erwinia amylovora,* 16S rRNA and RAPD-PCR.

Fire blight caused by the bacterium *Erwinia amylovora* (Burrill) Winslow *et al*., 1920 is the most destructive disease of pear in Egypt and other pome fruit trees worldwide. The pathogen may infect all parts of tree, including leaves, blossoms, fruitlets, shoots, limbs and trunks (Van der Zwet and Beer, 1995).

In Egypt fire blight was first detected by El-Helaly *et al*. (1964) and the severe outbreak in Nile Delta by Abo El-Dahab *et al*. (1983). The disease spread rapidly in most governorates and was responsible for serious economic losses in pear growing regions. The diagnostic and identification tests were performed on all isolates from different provinces by Tawfik *et al*. (2000). The development of molecular techniques has allowed the variation in the genetic material of pathogenic bacteria for plant to be revealed, both in their chromosome and DNA plasmid. There has been intensive work on *E. amylovora* reviewed by Vanneste (1995); Momol and Aldwinckle (2000) and Gehring *et al*. (2011).

Momol *et al*. (1997) reported that several molecular techniques have revealed genetic variability among *E. amylovora* strains, random amplified polymorphic DNA (RAPD) analyses allowed the differentiation of strains isolated from pear (*Pyrus communis*), apple (*Malus domestica*), quince (*Cydonia oblonga*) and strains obtained from raspberry (*Rubus* spp.).

Manulis *et al*. (1998) studied genomic diversity by RAPD analysis using 10 primers. All the strains examined (45 Israeli and 11 from Egypt, Cyprus and Greece) produced the same RAPD patterns with each one of the primer used. Amplification patterns were indistinguishable from those produced by strains isolated from the neighbouring countries. The results suggested that the population of *E. amylovora* in Israel is homogenous.

For many years, sequencing of the 16S ribosomal RNA (16S rRNA) gene has served as an important tool for determine phylogenetic relationships between different species of bacteria. 16S rRNA gene is present in all bacteria (Weisburg *et al*., 1991). In this respect, Woese (1987) pioneered the use of this gene in phylogenetic studies, in addition to the amplification of mitochondria and chloroplast rRNA.

Recently, a bacterial pathogen *Erwinia pyrifoliae* was characterized as a novel *Erwinia* species on Asian pear (Kim *et al*., 1999). This pathogen resembled *E. amylovora* on European pear. The nucleotide sequence of 16S rRNA from *E. pyrifoliae* was identical with the corresponding sequence of *E. amylovora* and also *E. pyrifoliae* produced ooze on immature European and Asian pears.

The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been the most common housekeeping genetic marker used for a number of reasons. These reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rRNA gene (1,500 bp) is large enough for informatics purposes (Janda and Abbott, 2007).

The objective of this study was to establish the genetic relationship between different isolates, which do not fit any recognized biochemical profiles, of *E. amylovora* isolated from some provinces using RAPD- PCR and 16S rRNA gene sequencing.

Materials and Methods

Bacterial isolates and pathogenicity tests:

Infected blossoms and leaf samples showing fire blight typical symptoms of pears (*Pyrus communis*) collected from different provinces of Behera (1Be), Gharbiya (2Gh), Nubariya (4Nu), Menufiya (5Mo), Kaluobiya (6Ka) and Ismailiya (7Es) were used for isolation on Miller and Schroth (MS) medium as highly selective medium for *Erwinia amylovora* according to Miller and Schroth (1972). Single colonies observed after 48 h incubation at 28^{o}C were isolated and transferred on King's B medium (King *et al*., 1954). One reference isolate (3Ea) obtained from

Bacterial Disease Research Dept. Plant Pathol. Res. Inst., Agric. Res. Centre was used in this study. All isolates were kept at – 80°C in 20% glycerol for later use. The pathogenic ability of the resulted isolates was tested on green immature fruits according to Schaad (1980).

Random amplified polymorphic DNA- using polymerase chain reaction (RAPD-PCR) technique for E. amylovora:

This test was done according to the protocol of Biotechnology. Central Lab. Plant Pathol. Res. Inst., Agric. Res. Centre. Six pathogenic isolates of *E. amylovora* isolated from the previously mentioned six different sites in Egypt and the reference isolate were used in this technique. The sequences of the six used primers are shown in Table (1).

Table. 1. PCR primers used for differentiation of *E. amylovora*

Primer	5'-Sequence-3'	G + C (%)
1	GGTCCCTGAC	70
2	TGCCGAGCTG	70
3	GGGTAACGCC	70
4	AATCGGGCTG	60
5	AGGGGTCTTG	60
6	CAGGCCTTCA	60

DNA extraction:

The six pathogenic bacterial isolates were cultured on King's B medium for 48 hrs at 28°C and the resulted growth was centrifuged at 4000 rpm for 30 min.to obtain bacterial pellet. Fresh bacterial pelts were homogenized in 400 µL sterile salt homogenizing buffer (200 mMTris-HCl, pH 8.5, 250 mMNaCl, 25 mM EDTA,0.5% SDS). Six µL 20 mg/mL RNase A was added and mixed well. The samples were incubated at 65°C for 10 min, after which 130 µL 3 M sodium acetate, pH 5.2, were added to each sample. Samples were vortexed for 30 sec at maximum speed, and incubated at -20°C for 10 min. The resulted lysate was centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was transferred to new tubes. An equal volume of isopropanol was added to each sample, mixing well, and incubated at -20°C for 10 min. Samples were then centrifuged for 20 min at 4°C, at 6000 rpm. The DNA pellets were washed twice using 700 µL washing solution (100 and 70% ethanol, respectively). The DNA pellets were subsequently air dried in an oven at 40°C for at least 10 min. The resultant DNA pellet was then re-suspended in 100 µL 1X TE (10 mMTris-HCl, 1 mM EDTA) buffer, pH 8.0 (Abd-Elsalam *et al*., 2007).

DNA quantification and gel documentation:

Seven microliters of the previously isolated DNA and 3 µL of 10X loading dye were loaded in a lane of 1.5% (w/v) agarose gel containing 0.05 µg/ml ethidium bromide, to check the quality of the DNA. For quantitative measurements, a charge-coupled device camera imaging system and UVI soft analysis (Gel Documentation

and Analysis Systems, Uvitec, Cambridge, UK) were used to capture the image and to calculate the band intensities.

RAPD-PCR analysis:

RAPD analysis was performed in 25-µl reaction volumes containing PCR buffer (Promega, Mannheim, Germany), 0.2 Mmol/l dNTPs, 0.5 Mmol/l primer, 4.0 Mmol/l $MgCl_2$, 1.25 units of Taq Polymerase (Promega, Mannheim, Germany) and 10–20 ng genomic DNA. PCR reactions were carried out in a T-Gradient thermal cycler (Biometra, Germany) using the following profile: 94 °C for 1 min, 36 °C for 1 min and 72 °C for 1 min for 30 cycles, and a final extension at 72 °C for 5 min. Following amplification, the samples were separated by electrophoresis in 1.4 % agarose gel, stained with 0.5 µg/ml of ethidium bromide and viewed under ultraviolet light. A 300- to 1500-bp ladder (Promega, Mannheim, Germany) was used as a molecular mass marker. The similarity coefficients were used to construct a dendrogram by UPGMA (Un-weighted pair-Group Method with Arithmetical Averages) using statistical program.

Identification using 16Sr RNA sequencing:

One of the most attractive potential uses of 16S rRNA gene sequence informatics is to provide genus and species identification for isolates that do not fit any recognized biochemical profiles, so we didn't do the biochemical testes for the isolates. These tests were done according to Sigma Scientific Services Co. (23 EL Esraa st ., Elmoalmean City, Lebanon Square , El Giza, Egypt).

Amplification of 16Sr RNA gene sequencing system:

It was especially important to identify the isolates of *E. amylovora* in Egypt through determining the sequence encoding 16S rRNA gene which consider the most common method adopted by Clarridge (2004). Firstly DNA was extracted, 24 hrs after incubation of bacteria on King's B medium, by use protocol of Gene Jet genomic DNA purification Kit (Fermentas). Then PCR was done by using Maxima Hot Start PCR Master (Fermentas). Fragment of 16S rRNA gene was amplified for all 6 bacterial isolates using universal primers namely 8F and U1492R as follows:

R:- AGA GTT TGA TCC TGG CTC AG

F:- GGT TAC CTT GTT ACG ACT T

PCR amplification was performed using the recommended thermal cycling conditions outlined in the following Table:

Table. 2. Thermocycling conditions

Step	Temp.	Time	Number of cycles
Initial denaturation / enzyme activation	95	10 min	1
Denaturation	95	30 s	35
Annealing	65	1min	
Extension	72	1 min30s	
Final Extension	72	10 min	1

The PCR products were separated on a 1.5% agarose gel in TBE buffer and stained with ethidium bromide and photographed under UV light. The amplified products of 16S rRNA gene (1500 bp) were purified using Gene JET™ PCR

Purification Kit (Fermentas). The products were sequenced on GATC Company by use ABI 3730xl DNA sequencer.

Results and Discussion

Isolation, pathogenicity test and identification:

The isolates obtained from infected blossoms and leaves showed colonies typically orange coloured with deep orange centre on MS medium. These isolates were used in inoculation pear fruitlets. All tested isolates were able to infect pear fruitlets after 4 days with no substantial difference in degree of oozing between them. No variation between all isolates obtained was noted and no fluoresce was observed under ultraviolet light when grown on King's B medium. The isolated organisms confirmed to the characteristics of *Erwinia amylovora* were designated according to the abbreviation of governorate from which were isolated as 1(Be), 2(Gh), ,4(Nu), 5(Me), 6(Ka), 7(Is) and 3(Ea) reference isolate.

Identification of E. amylovora using Random Amplified Polymorphic DNA (RAPD-PCR):

Six primers were used for RAPD-PCR screening to detect polymorphism and characterize the genetic diversity or variability analysis between *E. amylovora* isolates and one isolate as a reference.RAPD-PCR analysis of the individual DNA samples of the bacterial isolates was done using the six primers that detected a band specific to the individual as it distinguished from other isolates. Genomic DNA of seven isolates was successfully amplified with oligonucleotide primers.The present results showed no clear differences among the seven isolates on the basis of amplified product band pattern observed with each primer [Fig.(1) P1a, P2b, P3c, P4d, P5e and P6f)]. Figure (1) shows a typical profile and polymorphic bands generated with the primers. All of the profiles generated were examined visually and polymorphic bands were scored (as present or absent). The fingerprints of these isolates consisted of 5 to 12 bands per isolate, ranging from 100 to 2000 bp. High similarity (100%) was observed between isolates (3 and 4) with P1a and isolates (2,3) with P4d and (2, 3, 5, 7) with P6f. Results also revealed that the similarity between all isolates being 60% with (P3c, P1a) and 75% with (P4d) and 74% with primer (P5e). On the other hand, the primer (P6f) revealed the similarity between all isolates reached 65% except the isolate number one from Behera province which being 42% together with all isolates from different provinces. The RAPD band patterns resulting from the use of the six primers were analyzed using UPGMA method indicating the close relationship between the seven examined isolates. The tree obtained indicated that genetic similarity between these isolates named (1 to 7). Results showed that no variation was observed among the examined isolates from different governorates with six primers. So, *E. amylovora* isolates in Egypt are homogenous. These results agree with those reported by Manulis *et al.* (1998). They mentioned that the population of *E. amylovora* isolates in Israel are homogenous. Results obtained from this study indicated that primers P4d (75%), P5e (74%) and P6f (60%) were chosen because they reliably and reproducibly detected polymorphism among the selected isolates. Figure 1 showed a typical profile and polymorphic bands generated with these primers.

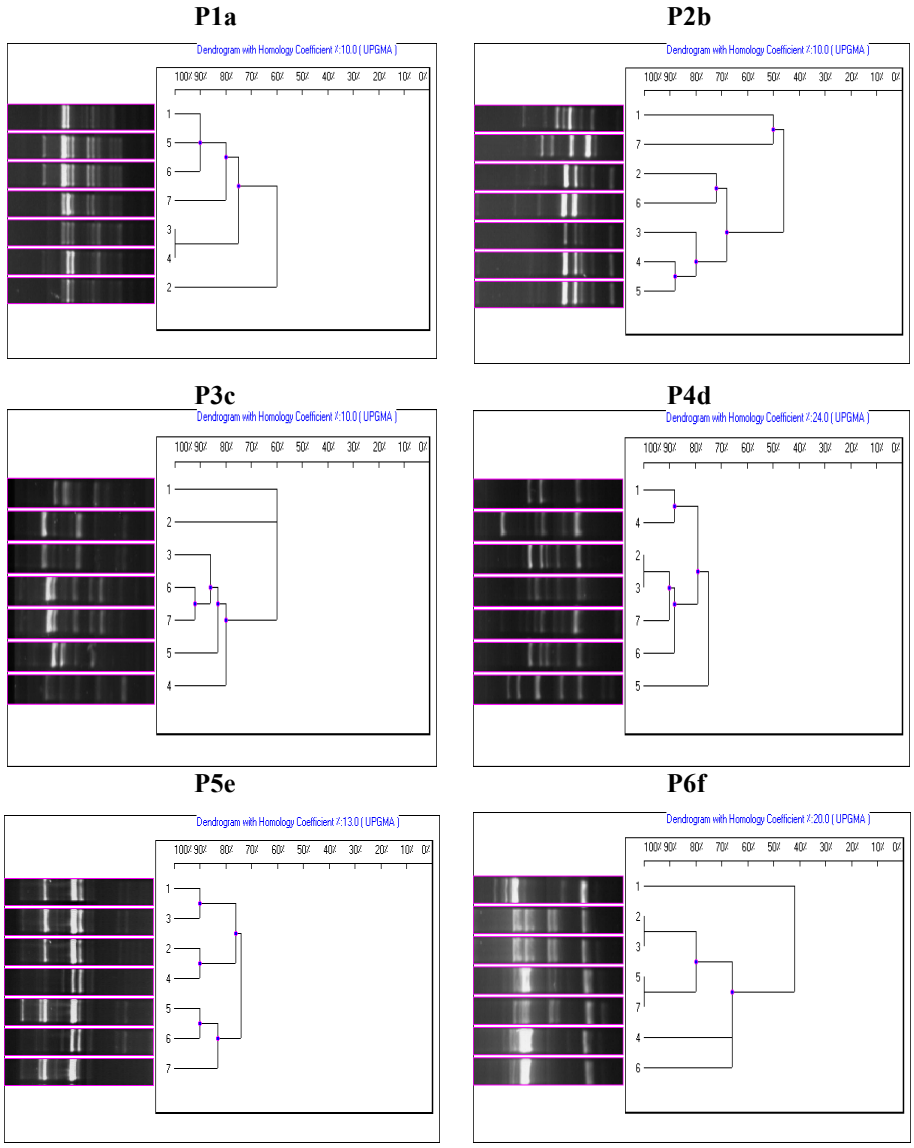

Fig. 1. Cluster analysis (unweighted pair-group method using arithmetic averages, UPGMA) of *Erwina amylovora* isolates based on DNA fingerprinting using RAPD profile (Primers 1 to 6). Lane 1: 1(Be) from Behera, Lane2:2(Gh) from Gharbiya, Lane3: reference isolate,Lane4: 4(Nu) from Nubariya, Lane5:5(Me) from Monifiya, Lane6:6(Ka) from Kaluobiya and Lane7:7(Is) from Ismailiya. A 300- to 1500-bp ladder (Promega, Mannheim, Germany) was used as a molecular mass marker.

Genotypic characterization of E. amylovora using 16S rRNA gene sequence:

16SrRNA was used to study the classification and identification of *E. amylovora* using universal primers (8F and U1492R).PCR amplification of ribosomal RNA was carried out with universal forward and reverse primers of 16S rRNA and produced a fragment of approximately 1500 bp (Fig. 2). This size corresponded to the expected size as compared to other bacteria.

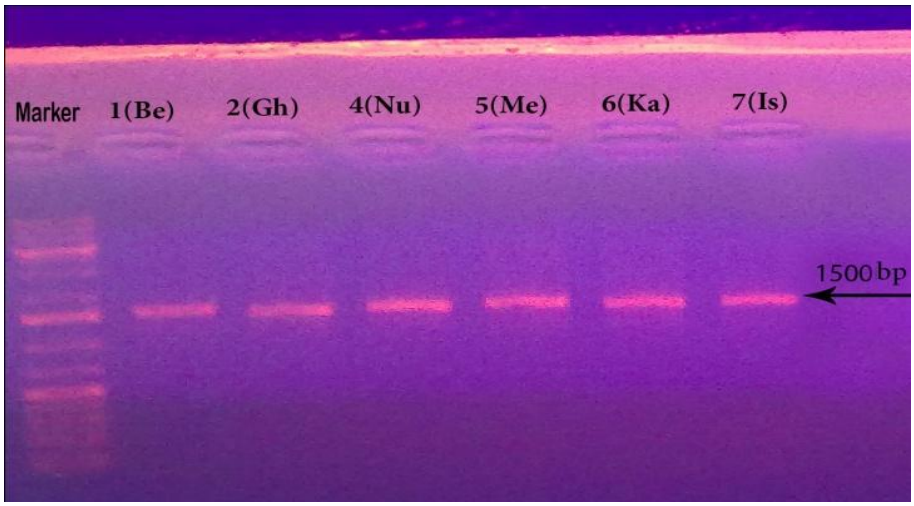

Fig. 2. 16S rRNA-PCR amplification of *E. amylovora* isolates.

Systematic study coupled with molecular study is well established to reveal the true identity of the organisms. Since the organisms have been potentially identified by the 16S rRNA method, the partial 16S rRNA sequences of six *E. amylovora* isolates were compared with other strains of bacterium from the similarity matrix, calculated by number of base differences, depending upon the highest level of similarity.1Be (1197bp), 2Gh (1073bp), 4Nu (1203bp), 5Me (999bp),6Ka (1166),6Is (1253bp) revealed maximum score hit compared with other strains of bacterium on the basis of nucleotide similarity matrix which are used to align nucleic acid sequences.

Results in the present data showed that the sequence of isolates collected from pear of different locations in Egypt was matched with the sequence found in the international database. The sequence of the fragment showed percentage ranged from 89 – 97 % homology with strain DSM30165 ((*Erwinia amylovora* 16S rRNA gene (strain DSM 30165) -1497 bp DNA linear BCT 17-Nov.1999, from genbank, Locus: AJ233410.1)).

The selected isolates were found to have the closest homology to other *E. amylovora*. These results were shown in Fig. (3) which revealed six isolates of *E. amylovora* collected from different provinces. Obtained data are in harmony with those of Sproeer *et al*. (1999).

The 16SrRNA gene is used as the standard for identification of microbes because it is present in most microbes and shows proper changes. It is interested to notice that the type strains of 16SrRNA gene sequence for most bacteria are available on public databases. However, the quality of the sequences found on these databases is often not validated. Therefore, the databases which collect only 16SrRNA sequences are widely used.

In addition 16SrRNA gene sequences contain hyper variable regions useful for bacterial identification. As a result it can be concluded that 16SrRNA gene sequencing has become prevalent as a rapid and cheap alternative to phenotypic methods of bacterial identification. As well as, although it was originally used to identify bacteria, 16S sequencing was subsequently found to be capable of reclassifying bacteria into new species or even genera. Generally, 16S rRNA sequencing has played an important tool for identification of bacterial isolates and determining phylogenetic relationships between bacteria. Also it useful to notice that 16S rRNA sequences has a powerful mechanism for identifying new pathogens with suspected bacterial disease.

The results indicated that 16S rRNA and RAPD-PCR are suitable methods for rapid identification and differentiation of *E. amylovora* isolates.

1(Be)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	2021	2021	91%	0.0	97%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	2015	2015	91%	0.0	97%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	1938	1938	91%	0.0	96%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	1932	1932	91%	0.0	96%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1901	1901	85%	0.0	97%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1873	1873	91%	0.0	95%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1864	1864	91%	0.0	95%
Erwinia mallotivora strain DSM 4565 16S ribosomal RNA, partial sequence	1857	1857	91%	0.0	95%
Pectobacterium cypripedii strain DSM 3873 16S ribosomal RNA, partial sequence	1840	1840	91%	0.0	94%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1834	1834	91%	0.0	94%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1829	1829	91%	0.0	94%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1823	1823	91%	0.0	94%
Klebsiella oxytoca strain ATCC 13182 16S ribosomal RNA, partial sequence	1821	1821	91%	0.0	94%

2(Gh)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	1825	1825	85%	0.0	97%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	1808	1808	85%	0.0	96%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	1748	1748	85%	0.0	95%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	1740	1740	85%	0.0	95%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1692	1692	82%	0.0	95%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1687	1687	79%	0.0	96%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1683	1683	82%	0.0	95%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1655	1655	85%	0.0	94%
Erwinia mallotivora strain DSM 4565 16S ribosomal RNA, partial sequence	1650	1650	85%	0.0	94%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1644	1644	82%	0.0	94%
Pectobacterium cypripedii strain DSM 3873 16S ribosomal RNA, partial sequence	1644	1644	85%	0.0	94%
Klebsiella oxytoca strain ATCC 13182 16S ribosomal RNA, partial sequence	1642	1642	85%	0.0	94%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1637	1637	84%	0.0	94%

4(Nu)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	2015	2015	92%	0.0	96%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	2004	2004	92%	0.0	96%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	1932	1932	92%	0.0	95%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	1927	1927	92%	0.0	95%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1884	1884	87%	0.0	96%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1873	1873	92%	0.0	94%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1864	1864	92%	0.0	94%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1834	1834	92%	0.0	93%
Pectobacterium cypripedii strain DSM 3873 16S ribosomal RNA, partial sequence	1834	1834	92%	0.0	93%
Erwinia mallotivora strain DSM 4565 16S ribosomal RNA, partial sequence	1829	1829	87%	0.0	95%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1825	1825	86%	0.0	95%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1818	1818	92%	0.0	93%

5(Me)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	1351	1351	85%	0.0	89%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	1328	1328	85%	0.0	88%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	1279	1279	85%	0.0	88%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	1271	1271	85%	0.0	87%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1234	1234	73%	0.0	90%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1230	1230	73%	0.0	90%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1221	1221	80%	0.0	88%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1218	1218	85%	0.0	87%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1214	1214	73%	0.0	90%
Klebsiella oxytoca strain ATCC 13182 16S ribosomal RNA, partial sequence	1212	1212	73%	0.0	89%
Kluyvera cryocrescens strain 12993 16S ribosomal RNA, partial sequence	1212	1212	73%	0.0	89%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1210	1210	75%	0.0	89%
Kluyvera intermedia strain 256 16S ribosomal RNA, partial sequence	1206	1206	73%	0.0	89%

6(Ka)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	1930	1930	93%	0.0	96%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	1914	1914	93%	0.0	96%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	1847	1847	93%	0.0	95%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	1840	1840	93%	0.0	95%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1792	1792	88%	0.0	96%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1786	1786	93%	0.0	94%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1777	1777	93%	0.0	94%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1755	1755	93%	0.0	93%
Erwinia mallotivora strain DSM 4565 16S ribosomal RNA, partial sequence	1755	1755	93%	0.0	93%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1753	1753	89%	0.0	94%
Pectobacterium cypripedii strain DSM 3873 16S ribosomal RNA, partial sequence	1744	1744	93%	0.0	93%
Klebsiella oxytoca strain ATCC 13182 16S ribosomal RNA, partial sequence	1740	1740	89%	0.0	94%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1729	1729	89%	0.0	94%
Kluyvera cryocrescens strain 12993 16S ribosomal RNA, partial sequence	1724	1724	89%	0.0	94%

7(Is)

Description	Max score	Total score	Query cover	E value	Max ident
Erwinia amylovora strain DSM 30165 16S ribosomal RNA, partial sequence	2117	2117	98%	0.0	97%
Erwinia pyrifoliae Ep1/96 strain Ep1/96 16S ribosomal RNA, complete sequence	2100	2100	98%	0.0	96%
Erwinia tasmaniensis Et1/99 strain Et1/99 16S ribosomal RNA, complete sequence	2034	2034	98%	0.0	95%
Erwinia tasmaniensis Et1/99 strain : Et1/99 16S ribosomal RNA, partial sequence	2026	2026	98%	0.0	95%
Erwinia pyrifoliae DSM 12163 strain ICMP 14143 16S ribosomal RNA, partial sequence	1978	1978	93%	0.0	96%
Erwinia persicina strain HK 204 16S ribosomal RNA, partial sequence	1973	1973	98%	0.0	95%
Erwinia rhapontici strain DSM 4484 16S ribosomal RNA, partial sequence	1964	1964	98%	0.0	94%
Erwinia mallotivora strain DSM 4565 16S ribosomal RNA, partial sequence	1936	1936	98%	0.0	94%
Citrobacter youngae strain GTC 1314 16S ribosomal RNA, partial sequence	1925	1925	98%	0.0	94%
Pectobacterium cypripedii strain DSM 3873 16S ribosomal RNA, partial sequence	1919	1919	98%	0.0	94%
Pantoea stewartii subsp. stewartii strain ATCC 8199 16S ribosomal RNA, partial sequence	1912	1912	98%	0.0	94%
Citrobacter gillenii strain CDC 4693-86 16S ribosomal RNA, partial sequence	1903	1903	98%	0.0	94%
Buttiauxella noackiae strain NSW 11 16S ribosomal RNA, partial sequence	1895	1895	98%	0.0	93%
Buttiauxella izardii strain S3/2-161 16S ribosomal RNA, partial sequence	1895	1895	98%	0.0	93%

Fig. 3. Computer sheet (BLAST –NCBI) showing species identification of *Erwinia amylovora* isolated from different governorates and showed percentage ranged from 89–97% homology with strain DSM30165.

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(Received 24/02/2016;in revised form 31/03/2016)

التعريف و التفرقة بين العزلات المصرية لبكتيريا *Erwinia amylovora* باستخدام RAPD-PCR و 16S rRNA

حنان علي شاهين ، رباب محمد عبد العزيز ، علي السيد توفيق
معهد بحوث أمراض النباتات - مركز البحوث الزراعية - مصر.

تسبب بكتيريا الايروينيا أميلوفورا الموجودة في مصر مرض اللفحة النارية للكمثري و تم تعريفها بالتنمية علي بيئات متخصصة وأختبار القدرة المرضية و كذلك الطرق الجزيئية. وقد تم استخدام طريقة RAPD–PCR لمعرفة الاختلافات الجينية بين 6 عزلات من الإيروينيا أميلوفورا من محافظات مصرية مختلفة (البحيرة ، الغربية، النوبارية، المنوفية، القليوبية والأسماعيلية) وعزلة واحدة من قسم بحوث أمراض النباتات البكتيرية للمقارنة ، وتم الحصول علي مظهر يوضح الأرتباط بين DNA للعزلات المختبرة مع البادئات الستة العشوائية.

تم عمل تحليل للحزم الناتجة من أستخدام هذه البادئات بأستخدام طريقة UPGMA بناء علي التشابه لإيجاد صلة القرابة بين العزلات المختبرة. أوضحت النتائج المتحصل عليها أن P4d, P5e, P6f هي أفضل البادئات المستخدمة للتفرقة بين هذه العزلات. أيضا لا يوجد اختلافات في الجينوم البكتيري بين العزلات المختبرة.بالإضافةإلي ذلك،تم أستخدام التسلسل الجيني 16S rRNA لدراسة تعريف الإيروينيا أميلوفورا بأستخدام بادئات عالمية (F8وU1492R). وأظهرت النتائج أن هذا التسلسل الجيني للعزلات التي تم جمعها من مواقع مختلفة متطابقة مع التسلسل الموجود في قاعدة بيانات بنك الجينات. أظهرتسلسل جزء 16S rRNA تشابه تراوح بنسبة 89-97٪ مع سلالة DSM 30165 من سلالات بنك الجينات ، والعزلات المختارة كانت أقرب ما يكون إلى التماثل مع *E. amylovora* .