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First Record of *Pseudomonas syringae* pv. *syringae* in Iraq using Conventional and Specific PCR Protocol

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Abstract: *Bacterial canker and gummosis of stone fruits caused by Pseudomonas syringae pv. syringae Van Hall (Pss) were mostly found on dormant buds, blossoms, twigs and trunks rather than leaves and fruits in Duhok, Northern of Iraq. In this work, 262 symptomatic diseased samples collected from apricot, plum and peach trees (dormant buds, flowers, twigs and trunks) during early spring and late summer 2012. The conventional and Polymerase Chain Reaction (PCR) techniques managed to detect and characterize Pss . PCR protocol described uses specific primers C-Duhok B1 and B2 to identification main virulence factor Syringomycin (syrB) gene for the detection of Pss . Diseased samples were initially screened on 5% Nutrient Agar Sucrose (NAS) and 5% Nutrient Broth Sucrose (NBS), selective media of Pseudomonas Agar Base (PAB). Therefore, 139 samples were considered P. syringae . Subsequently, confirmations of 62 isolates from 139 were considered to be of P. syringae on Cetrimide Agar (CA). These isolates found to be gram negative bacilli, short rods and non spore-form and characterized by negative oxidase and leaven positive on 5% NAS. PCR was carried out for 62 P. syringae isolates for detection of Pss , using syrB primer with size 752 bp. Results showed that 13 of 62 (21%) isolates carried syrB gene and similarly in the biochemical characters using Analytical Profile Index (API 20NE). Thus, characterization of Pss strains to hydrolysis Esculin, Gelatin and fruit contents of Glucose, Arabinose, Mannose, Mannitol, Capric and Malic acid. Other biochemical analysis was also documented. PCR identification of Pss considers the first certified record for these suspected and serious phytopathogenic bacteria in Iraq.*

Key Word: *Canker, Bacterial Disease, Stone Fruit Trees, Pseudomonas syringae Pv. syringae .*

I. Introduction

Bacterial canker is the most devastating disease in apricot, peach, plum and cultivated cherry as well as wild cherry. It become a serious problem in many parts of the world that results from limited tree orchard life duration and direct reduction in yield between 10-20% in young orchards and even up to 80% under favorable climatic conditions (**Cameron, 1962; Ogawa and English, 1991; Young, 1991; Mohammadi et al., 2001**). Bacterial canker of stone fruits is caused by two closely related pathovar, *Pseudomonas syringae* pv. *syringae* (Van Hall) and pv. *morsprunorum* (Wormald), they belong to genomospecies 1 and 2, respectively and are an important disease of apricot and other stone fruits (**Young, 1991; Gardan et al., 1999; Roberts and Smith, 2002; Kennelly et al., 2007**). The Identification of *P. syringae* and pathovars immediately after 1980, the detection of plant pathogenic species could still be based on simple phenotypic tests (**Young, 2010)**. However, the use of determinative tests with primary reliance was shown to be of limited value for classification and differentiation of *P. syringae* pathovars **(Young and Triggs, 1994; Palleroni, 2005).** Increasingly orientated towards poly-phasic and molecular methods for identify, differentiate and classify closed bacteria at the sub species level **(Najafi and Taghavi, 2014).** As mentioned by **Palacio-Bielsa et al. (2009)** PCR primers can offer a reliable method for the confirmation of identity pathovars.

This work aimed to identification of the isolated pathogenic bacterial pathovars at first time in Iraq using traditional then confirmed by PCR techniques.

II. Material and Methods

2.1 Samples Collection:

Diseased samples of apricot, plum and peach trees (dormant buds, flowers, twigs and trunks) with typical bacterial canker and shot-hole symptoms were collected from six localities of Duhok province (Green Valley, Shendokha, Bagera, Akre, Dlebe and Fedeye) (Table 1), during early spring and late summer of 2012. A total number of 262 symptomatic diseased samples were assembled, including 104 samples originated from peach, 104 from apricot and 54 from plum tissues.

Table 1: Location, host, plant parts and designation of bacterial isolates during 2012 from different situations in Duhok governorate

Location	Host	Plant part	*Designation of isolates	Location	Host	Plant part	*Designation of isolates
Akre	Peach	Twigs	AkPeTw	Green Valley	Peach	Flowers	GvPeFl
		Trunks	AkPeTr			Buds	GvPeBu
	Apricot	Twigs	AkApTw			Twigs	GvPeTw
		Trunks	AkApTr			Trunks	GvPeTr
	Plum	Twigs	AkPlTw		Apricot	Flowers	GvApFL
		Trunks	AkPlTr			Buds	GvApBu
Shendokha	Peach	Flowers	ShPeFl			Twigs	GvApTw
		Buds	ShPeBu			Trunks	GvApTr
		Twigs	ShPeTw		Plum	Flowers	GvPlFl
		Trunks	ShPeTr			Buds	GvPlBu
	Apricot	Flowers	ShApFl			Twigs	GvPlTr
		Buds	ShApBu			Trunks	GvPlTw
		Twigs	ShApTw	Dlebe	Peach	Flowers	DlPeFl
		Trunks	ShApTr			Buds	DlPeBu
	Plum	Flowers	ShPlFl			Twigs	DlPeTw
		Buds	ShPlBu			Trunks	DlPeTr
		Twigs	ShPlTw		Apricot	Flowers	DlApFl
		Trunks	ShPlTr			Buds	DlApBu
Bagera	Peach	Flowers	BaPeFl			Twigs	DlApTw
		Buds	BaPeBu			Trunks	DlApTr
		Twigs	BaPeTw	Fedeye	Peach	Flowers	FePeFl
		Trunks	BaPeTr			Buds	FePeBu
	Apricot	Flowers	BaApFl			Twigs	FePeTw
		Buds	BaApBu			Trunks	FePeTr
		Twigs	BaApTw		Apricot	Flowers	FeApFl
		Trunks	BaApTr			Buds	FeApBu
	Plum	Flowers	BaPlFl			Twigs	FeApTw
		Buds	BaPlBu			Trunks	FeApTr
		Twigs	BaPlTw				
		Trunks	BaPlTr				

*Isolates design constitute the abbreviation of the first two letters for each location, host and plant part, respectively.

2.2 Isolation of *Pseudomonas syringae* pv. *syringae* :

Pseudomonas species in general have simple nutritional requirements and were readily isolated from disease tissues. *Pss* isolation was carried out by modified method of **Fahy and Persley (1983)** in the Research Laboratory in Plant Protection Department, Faculty of Agriculture and Forestry using 5% Nutrient Agar Sucrose (NAS), 5% Nutrient Broth Sucrose (NBS) and *Pseudomonas* Agar Base (PAB).

2.3.1 Identification of *Pseudomonas syringae* pv. *syringae* using traditional methods:

After the incubation period, light cream to yellow and mucoid sheen colonies of suspected *Pss* appeared on NAS and PAB plates, respectively with yellowish white supernatant fluid on NBS. The suspected colonies on both media agar plates were confirmed by streaking on Cetrimide Agar (CA) and identified morphologically as *Pss* .

Microscopic Study: The morphological study was performed by Gram's staining method. It was used to determine the size, shape, arrangement and gram reaction of isolates.

Oxidase Test: Looking for cytochrome enzymes a filter paper was saturated with 1% Kovac's oxidase reagent (tetra-methyl-p-phenylenediamine dihydrochloride) and placed in a clean Petri dish. A suspected colony of *Pss* from CA was transferred with wooden stick to the filter paper and rubbed onto the reagent for 5-10 second (**York et al., 2004**).

Biochemical Tests (API 20NE): API 20NE is a standardized, easy and quick system for the identification of non-fastidious, non-enteric gram-negative rods (e.g. *Pseudomonas* , Acinetobacter, Flavobacterium, Moraxella, Vibrio, Aeromonas, etc.), combining 8 conventional and 12 assimilation tests. Further, the identification of bacterial strains using API 20NE gave a basic confirmation for the Family Pseudomonadaceae aimed to approve the identification of the genus and species level of the epiphytic strains.

A. Preparation of the API 20NE Strip: The 5 ml of distilled water was distributed into the tray bottom to create a humid atmosphere with recording the specimen number on the elongated flap of the tray, the strip was kept in the incubation box.
B. Preparation of the *Pss* Inoculum: Suspected isolates were grown on 5% NAS medium and followed by picked up 4 colonies from young cultures (18-24hrs old) with identical morphology from the agar plate and suspended in 2ml of normal saline.
C. Inoculation of the Strip: The bacterial saline suspension was distributed into the cupules of the following tests: NO_3, TRP, GLU, ADH, URE, ESC, GEL and PNPG using pipette and then approximately 200 µl was added of the remaining saline suspension to the ampule of API AUX Medium. Fill the cupules of the following tests: GLU, ARA, MNE, MAN, NAG, MAL, GNT, CAP, ADI, MLTM, CIT and PAC with the suspension to defect a flat or slightly convex. Then, mineral oil was added to the cupules of the tests GLU, ADH and URE until a convex meniscus was formed. Finally, the incubation boxes were closed and incubated at 28 ± 2°C for 24 and 48hrs before reading the results.

2.3.2 PCR identification of *Pseudomonas syringae* pv. *syringae*

Yellow light cream colonies on CA were sub-cultured on 5% NBS and then genomic DNA was extracted by Mini Kit methods. DNA was amplified by Polymerase Chain Reaction (PCR) using specific primers C-Duhok B1 and B2 of 21-mer oligonucleotides from syrB gene for the detection of *P. syringae* pv. *syringae* . The primers B1 and B2 locate into the open reading frame of the syrB gene and yield a 752-bp product (**Sorensen et al., 1998**).
Genomic DNA Extraction: Genomic DNA preparation of *Pss* isolates strains were carried out by Genomic DNA Mini Kit which provides an efficient method for purifying DNA (**Vogelstein and Gillespie, 1979**).
DNA Concentration Measuring: DNA concentration was measured by using Nanodrop instrument, where 1 µl of isolated DNA was taken and put on Nanodrop instrument and measured electronically by computer with adsorption reading at 260/280 nanometer (**Tyc, 2011**). DNA purity was measured according to the following equation. In this research we obtained pure DNA which was about (1.46- 2.56).

$$\text{DNA Purity} = \frac{\text{Adsorption reading at 260}}{\text{Adsorption reading at 280}}$$

Electrophoresis: Agarose Gel Electrophoresis, Loading Buffer and Gel Running were done according to **Maniatis et al., 1982**.
Ethidium Bromide Staining: The gel was removed carefully and put in Ethidium bromide staining solution and left for 1-1.5hrs, the gel was viewed under UV light and findings were documented by photography (**Gaafar et al., 2006; Pour and Taghavi, 2011**).
Specific PCR Protocol: PCR employed with specific primers for syringomycin (syrB) gene in the amplification reaction for detection of syringomycin toxin gen (Takemoto, 1992). The syrB1 and syrB2 primers and components were mixed in the same amplification reaction (Table 2). Appropriate thermocycling program was set on thermocycler for syringomycin toxin **(syrB1, syrB2) gene as** Pre-PCR **94 C^o for 5 min;** Thermocycling **(35 cycles):** Denaturation **94 C^o for 1.5 min,** Annealing **60 C^o for 1.5 min,** Extension **72 C^o for 3 min and** Final Extension **72 C^o for 10 min.**

Table 2: The component required for specific primers syrB1 and syrB2 amplification.

Components	**Volume of one reaction**	**n x Reaction**
Deionise distil water	15.8µl	n x15.8µl
10x buffer with $Mgcl_2$	2.5µl	n x 2.5µl
dNTP mixture	2.5µl	n x 2.5µl
Reverse primer	1µl	n x 1µl
Forward primer	1µl	n x 1µl
Taq polymerase	0.2µl	n x 0.2µl
Made a master mix and then sub-divided as 23µl aliquots to n eppendorf tubes		
Sample DNA	2µl to each respective tube	
Total	25µl final reaction volume each	

III. Results and Discussion

3.1 Isolation of *Pseudomonas syringae* pv. *syringae*

The successful isolation of *P. syringae* pv. *syringae* (*Pss*) was performed immediately after the appearance of the initial symptoms in early spring. 5% Nutrient Agar Sucrose (NAS), 5% Nutrient Broth Sucrose (NBS), *Pseudomonas* Agar Base (PAB) and Cetrimide agar (CA) were used as plating media. Symptomatic of 262 diseased samples were initially screened on NAS and NBS. Growth of bacterial colonies after 72hrs under aerobic conditions at 28 ± 2°C resulted in 262 round 2-3 mm in diameter, light cream with entire margins, dome shaped, shiny, smooth and mucoid on 5% NAS. A yellow supernatant fluid was showed when grown on 5% NBS media. The same descriptions were observed by **Mohammadi et al., (2001) and Karimi-Kurdistani and Harighi, (2008)**. PAB was used as selective media for *Pss* and resulted in 139 outcomes from 262 of the suspected bacterial isolates on NAS and NBS identified as *Pseudomonas syringae* , characterized with round colonies 1-3 mm in diameter, yellow with complete margins, conk shaped, sparkly, smooth, and fluorescent after 2-4 days at 28 ± 2°C. Selective media CA was used for confirmation and avoiding of full inhibition of the contamination as recommended by **Moore et al., (2006)** (**Figure 1**). Finally, 62 isolates were identified and considered to be *Pseudomonas syringae* (**Table 3**).

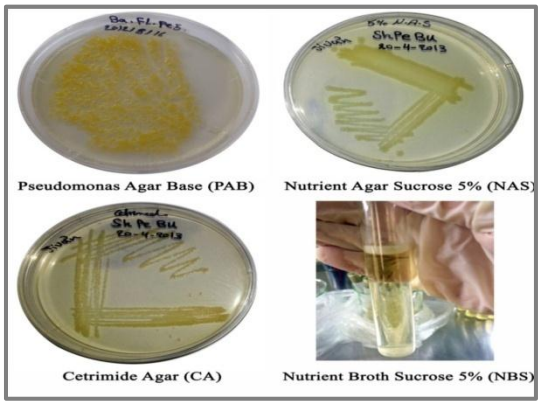

Figure 1: Growth of characteristic, mucoid and shiny colonies of *Pseudomonas syringae* pv. *syringae* , 2 days after inoculation on different media.

Table 3: Initial identification of *Pseudomonas syringae* isolates on 5% Nutrient Agar Sucrose (NAS), *Pseudomonas* Agar Base (PAB) and Cetrimide Agar (CA) media.

Host / Media	Initial Identification			% Isolation			% Contamination		
	NAS	PAB	CA	NAS	PAB	CA	NAS	PAB	CA
Apricot	104	48	26	100	46.15	54.17	0	53.85	45.83
Peach	104	57	18		54.81	31.58		45.19	68.42
Plum	54	34	18		62.96	52.94		37.04	47.06
Total	262	139	62		53.05	44.60		46.95	55.40

3.2 Identification of Bacterial Strains using traditional methods

According to standard operating procedures, all 62 of tested isolates were gram negative, short rods, no spore-form, aerobic, oxidase negative and formation of distinctive mucoid that demonstrates as levan production on NAS (leaven positive). These morphological characteristics were also revealed by **Mohammadi et al., (2001); Kaluzna et al., (2012)**.

3.3 PCR identification of Bacterial Strains

Genomic DNA Extraction: Suitable yields of genomic DNA were obtained from repeated experiments with an average yield of 50 µg DNA per µl with purity about 1.46 to 2.56 determined by Nanodrop instrument and electronically by computer with adsorption reading at 260/280 nanometer.

Amplification by the Specific Primer: However, *P. syringae* has a broad host range, isolated from different plant hosts may comprise genetically distinct groups. So, the pathovars of these phytopathogenics that are associated with stone fruits produce several effective of such phytotoxin compounds as coronatine, phaseolotoxin, syringomycin, and tabtoxin which can be used for pathovar differentiation (**Hwang et al., 2005**). Thus, detection by PCR using specific primers could offer a reliable method for the confirmation of pathovars identity (**Young, 2010; Gasic et al., 2012; Ivanovic et al., 2012**). For differentiating the pathovars of

Pseudomonas syringae, the PCR technique is rapid, simple reproductive to identify and classify phytopathogenic *P. syringae* at pathovar level and it may be a useful diagnostic tool for these important plant pathogens **(Abu-Ashraf et al., 2000**). In the present study, specific primer of Syringomycin (syrB) gene was used for identification of 62 *P. syringae* isolates from conventional methods. The syrB gene was encoded synthesize syringomycin because these toxins are considered the main virulence factor of *Pss* (**Young, 1991; Pour and Taghavi, 2011; Abbasi et al., 2013**). Out of 62 isolates 13 (21%) were amplified with specific primer of Syringomycin (syrB) gene (752 bp) whilst, the rest isolates are not amplified with it as shown in (**Table 4 and Figure 2**). Non amplified isolates were clarified, that they did not possess the syringomycine synthesize genes (**Louws et al., 1999; Lopez et al., 2003**). Other studies reported similar results of this strain from infected stone fruits (**Sorensen et al., 1998; Scortichini et al., 2003; Gilbert et al., 2009; Kaluzna et al., 2010; Pour and Taghavi, 2011**). In this direction, the specific pathovar identification of phytopathogenic Pseudomonads is more complicated than species detection. However, identification should always be confirmed by symptoms, host range and pathogenicity test that indicate potential target pathogens.

Table 4: PCR results of 62 isolates by specific primer of Syringomycin (syrB) gene.

Bacterial Strain	syrB	Bacterial Strain	syrB	Bacterial Strain	syrB
ShPeFl1	+	AkPlTw2	-	FeApTr1	-
ShPeFl2	+	AkPlTw4	-	FeApTr2	-
ShPlFl2	+	ShPeTr1	-	AkPeTr5	-
ShPlFl3	+	ShPeTr5	-	AkApTr1	-
GvPeFl3	+	ShApTr3	-	AkApTr3	-
GvPeFl4	-	ShApTr4	-	AkApTr4	-
GvApFl2	+	ShApTr5	-	AkPlTr1	-
GvApFl4	-	GvPeTr1	-	AkPlTr2	-
GvPlFl1	-	GvPeTr2	-	AkPlTr4	-
GvPlFl3	-	GvPeTr3	-	DlPeBu4	-
BaPeFl5	+	GvApTr1	-	ShApTw5	-
ShPeBu4	+	GvApTr2	-	GvPeTw2	-
ShPeBu5	+	GvApTr4	-	GvApTw2	-
ShApBu5	+	GvPlTr1	-	GvPlTw4	-
ShPlBu2	-	GvPlTr2	+	DlApTw5	-
GvApBu4	-	GvPlTr3	-	AkApTw1	-
GvPlBu1	-	GvPlTr4	-	AkApTw2	-
GvPlBu2	+	BaApTr3	-	AkPlTw1	-
BaPeBu5	+	BaApTr4	-	DlApTr5	-
BaApBu4	-	DlPeTr2	-	FePeTr3	-
BaApBu5	-	DlPeBu3	-		

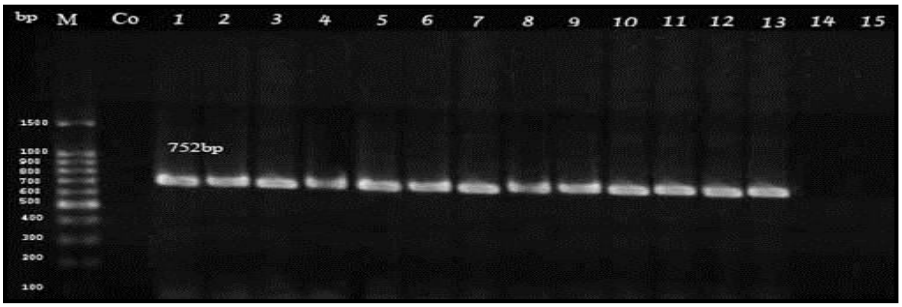

Figure 2: Agarose gel Electrophoresis of PCR with primers B1 and B2 corresponding to gene syrB, Co: Negative control, 1-13: *Pseudomonas syringae* pv. *syringae* strains 752bp, 14&15 strains of *P. syringae* , M: 100-1500bp DNA molecular marker.

3.4 Comparative PCR Results with Biochemical Tests

Actually, the polymerase chain reaction (PCR) technique was firstly used to confirm the identification of the causal agent of bacterial canker which resulted in thirteen pure strains of *Pseudomonas syringae* pv. *syringae* (*Pss*) to thrift the cost and time before application of the Analytical Profile Index (API 20NE) to recognize the biochemical characteristic features of 13 *Pss*, since the initial detection of plant pathogenic bacteria relied mainly on biochemical and physiological methods (**Schaad et al., 2001**), and according to **Jones et al.,** (**1993**), the test strips of API 20NE utilize to determine the bacterial enzymatic activity on various substrates of strip and identify the genus level only of the most bacteria.

The results revealed that all 13 bacterial strains of *Pss* have the same biochemical characters (**Figure 2 C**). Five of eight conventional tests of API 20NE were negative in reduction of nitrates (NO3), indole production

TRyptoPhane (TRP), p-galactosidase Para-NitroPhenyl-ßD-Galactopyranosidase (PNPG), Arginine DiHydrolase (ADH) and GLUcose fermentation (GLU), whereas these strains were capable for hydrolysis β-glucosidase ESCulin (ESC), hydrolysis GELatin (GEL) and UREa (UREase) (**Table 5**). These results were also reported by **Kazempour et al. (2007) and Bultreys and Kaluzna (2010)** and considered as diagnostic biochemical characters for *Pss*.

Figure 2: Biochemical reaction of API 20NE **(A)** Positive test, **(B)** Negative test, **(C)** *Pseudomonas syringae* pv. *syringae* and **(D)** *Pseudomonas syringae* .

Assimilation tests of N-Acetyl-Glucosamine (NAG), MALtose (MAL), ADIpic acid (ADI) and PhenylACetic acid (PAC) were negative, though the bacterial strains were able to utilize glucose (GLU), ARAbinose (ARA), ManNosE (MNE), MANnitol (MAN) and trisodium CITrate (CIT). Potassium GlucoNate (GNT), CAPric acid (CAP), MaLaTe (MLT) were also positive. Similar results were also reported by **Kairu, (1997); Kazempour et al., (2007) and Bultreys and Kaluzna, (2010)** to certify the identification of *Pss*.

Twelve unknown samples of *P. syringae* pathovar resulted from PCR were selected for testing their biochemical and physiological characters using strips of API 20NE. These samples were kept in a refrigerator to identify in the future when the primers of PCR become available. We found disparity in some reactions, such as GLUcose fermentation (GLU), Arginine DiHydrolase (ADH), UREase (URE), hydrolysis β-glucosidase ESCulin (ESC), GELatin hydrolysis (GEL) and p-galactosidase Para-NitroPhenyl-ßD-Galactopyranosidase (PNPG) (**Figure 2 D**). Therefore, we expect the occurrence of other pathovars of *P. syringae* , since for distinguishing pathovars within *P. syringae* that called GATTa tests are commonly used, including: Gelatine hydrolysis (G), Aesculin hydrolysis (A), Tyrosinase activity (T), Tartrate (Ta) utilization (**Lelliot and Stead, 1987**) and L-lactate utilization test (**Lattore and Jones, 1979**) in addition to more differences in the assimilation reactions.

Table 5: Biochemical characteristics of *Pseudomonas syringae* pv. *syringae* strains as assayed on different substrates by API 20NE tests.

Substrate*	Reactions / Enzymes	*Pss*
Potassium Nitrate	Reduction of Nitrates to Nitrites	-
	Reduction of Nitrates to Nitrogen	-
L-tryptophane	Indole production (tryptophane)	-
D-Glucose	Fermentation (Glucose)	-
L-Arginine	Arginine Dihydrolase	-
Urea	UREase	+
Esculin Ferric Citrate	Hydrolysis (B-Glucosidase) (Esculin)	+
Gelatin (Bovine Origin)	Hydrolysis (Protease) (Gelatin)	+
4-Nitrophenyl-Pd-Galactopyranoside	P-Galactosidase(Para-NitroPhenyl-ßD-Galactopyranosidase)	-
D-Glucose	Assimilation (Glucose)	+
L-Arabinose	Assimilation (Arabinose)	+
D-Mannose	Assimilation (Mannose)	+
D-Mannitol	Assimilation (Mannitol)	+
N-Acetyl-Glucosamine	Assimilation (N-Acetyl-Glucosamine)	-
D-Maltose	Assimilation (Maltose)	-
Potassium Gluconate	Assimilation (Potassium Gluconate)	+
Capric Acid	Assimilation (Capric Acid)	+
Adipic Acid	Assimilation (Adipic Acid)	-
Malic Acid	Assimilation (MaLaTe)	+
Trisodium Citrate	Assimilation (Trisodium Citrate)	+
Phenylacetic Acid	Assimilation (Phenylacetic Acid)	-

*Provided by API 20NE diagnostic kit, (-) negative reaction, (+) positive reaction.

The investigated isolates shown in (**Table 6**) divided into three clearly distinct groups (1) positive reactions of gelatin (GEL) and esculin (ESC) hydrolysis of the isolates BaApBu4, GvApTw2, ShApTr4 and FeApTr1 may be attribute to *P. syringae* pv. *morsprunorum* race2 admitting to identification methods of (**Kaluzna et al., 2012**). (2) The isolate GvPlFl1 that didn't hydrolyze gelatin and esculin may referred to *P. syringae* pv. *morsprunorum* race1 or pv. persicae. The same result was also confirmed by **Lattore and Jones (1979) and Burkowicz and Rudolph (1994)**. (3) Isolates of GvApFl4, ShApBu5, ShPlBu2, GvApBu4, DlPeBu3, ShPeTr1 and GvApTr4 with a positive reaction of the esculin and negative in gelatin tests in their metabolic processes. Thus, according to numerical taxonomy of biochemical tests and Rep- PCR conducted by **Menard et al. (2003**) signifying that they are phenon 5 = *P.* s. pv. *papulans* or *P. syringae* or phenon 10 = *P. syringae* pv. *morsprunorum*.

Table 6: Biochemical reactions of twelve unknown bacterial isolates.

Tests	*Pseudomonas syringae* isolates											
	GvApFl4	**GvPlFl1**	**ShApBu5**	**ShPlBu2**	**GvApBu4**	**BaApBu4**	**DlPeBu3**	**GvApTw2**	**ShPeTr1**	**ShApTr4**	**GvApTr4**	**FeApTr1**
NO3	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
TRP	-	-	-	-	-	-	-	-	-	-	-	-
GLU	+	+	+	+	+	+	+	+	-	+	+	+
ADH	-	-	-	-	-	-	-	-	+	-	-	-
URE	-	-	-	-	-	-	-	-	-	-	-	-
ESC	+	-	+	+	+	+	+	+	+	+	+	+
GEL	-	-	-	-	-	+	-	+	-	+	-	+
PNPG	+	+	+	+	-	+	+	+	-	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+	+	+
ARA	+	+	+	+	+	+	+	+	+	+	+	+
MNE	+	+	+	+	+	+	+	+	+	+	+	+
MAN	+	+	+	-	+	+	+	+	+	+	+	+
NAG	+	+	+	+	+	+	+	+	+	+	+	+
MAL	+	+	+	+	+	-	+	+	-	+	-	+
GNT	+	+	+	+	+	+	+	+	+	+	+	+
CAP	+	-	+	+	+	+	+	+	+	+	+	-
ADI	-	-	-	-	-	-	-	-	-	-	+	-
MLT	+	+	+	+	+	+	+	+	+	+	+	+
CIT	+	-	+	+	+	+	+	+	+	+	+	+
PAC	-	-	-	-	-	-	-	-	-	-	-	-

IV. Conclusion

In this work we conclude that PCR identification of *P. syringae* pv. *syringae* (*Pss*) consider the first record of this suspected and serious phytopathogenic bacteria in Iraq. Detection using PCR is reliable method for the confirmation of pathovars. Syringomycine (syrB) gene the main virulence factors of *Pss*, since induces necrosis in host tissues and typical chlorosis of leaves as a result of chloroplast disruption. Therefore, thirteen isolates could synthesize this toxin that assists the identification and characterization of putative *Pss* strains.

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