Study on some bacterial diseases of stone and pome fruit trees in Algeria

1,4Said Sadallah, 2Messaoud Benchahane, 3Murat Yildiz, 3Zisan Turan and 3Fikrettin Sahin

1Department of Botany, National High School of Agronomy (ENSA)-El Harrach 16200, Algiers, Algeria.
2Department of Agronomy, Faculty of Agro-Veterinary and Biological Sciences, Saad Dahlab University, Baida, Algeria.
3Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Yeditepe University Istanbul Turkey.

Address For Correspondence:
Said Sadallah, Department Department of Agronomy, Faculty of Sciences,20 August 1955 University, Skikda, Algeria.
E-mail: Sadallah2@hotmail.com; Tel: (+213773312895)

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ABSTRACT
This study was carried out to identify bacterial diseases on the main stone and pome fruit trees; sweet cherry (Prunus avium L.), plum (P.domestica), peach (P.persica), apricot (Prunus armeniaca L.), apple (Malus pumila) and pear (Pyrus communis), cultivated in eastern Algeria. Samples of diseased plant material exhibiting bacterial disease symptoms (cankers and gummosis on branches, leaf spots, blossom blights and tip dieback), were collected from cherry, plum, peach, apricot, apple and pear trees in Constantine, Khencela, Skikda, Setif and Souk Ahras localities (Eastern Algeria ) during 2008-2010. Out of 120 bacterial strains were isolated from margins of diseased tissue. All the isolates were identified as genus and species levels using morphological and biochemical tests. Fatty Acid Methyl Esters analysis (FAME) was used for confirmation. Pathogenicity tests were performed on green immature sweet cherry and pear fruitlets and sweet cherry shoots. All investigated strains were Gram negative and Hypersensitive response on tobacco leaves (HR), positive. Based on positive pathogenicity tests and differential LOPAT (Levan production, oxidase reaction, potato soft rotting, arginine dihydrolase and tobacco hypersensitivity) and GATTa tests (Gelatin liquefaction, Aesculin, Tyrosinase activity and Ttratrate utilization), investigated isolates were divided into three distinct groups: the first group of strains were classified as Pseudomonas syringae pv. syringae. The second group of strains identified as Pseudomonas syringae pv. morsprorum and The third group of isolates were identified as Pseudomonas viridiflava. Fatty Acid Methyl Esters analysis (FAME) confirmed the identification of bacterial strains from stone fruits as P. syringae with similarity indices of 0.65 to 0.89.

KEYWORDS: Pome and stone fruit trees, symptoms, Pseudomonas syringae, LOPAT and GATTa, Fatty acid.

INTRODUCTION
Stone and pome fruit trees of the rosaceous family are attacked by many bacterial diseases around the world [1]. Among these diseases; those caused by bacteria of the genus Pseudomonas mainly, on stone fruits; bacterial canker of stone fruit trees due to Pseudomonas syringae pv. syringae Van Hall and P. syringae pv. morsprorum (Wormald) [4,8,14,18], bacterial decline of peach tree due to P.s.pv.persicae [22,25], hyperplastic canker of almond caused by P.amygdali [8], and bacterial canker of wild cherries ( Pseudomonas syringae pv. Avii) [24], on pome fruit trees; blossom blast of pear and apple caused by P.s.pv.syringae [7,11,21], and blister spot of apple caused by P.s.pv.papulans [5,30]. Under favorable conditions, damages of these diseases may be of economic importance [1]. Other diseases of fruit trees are bacterial spot of stone fruit trees caused by Xanthomonas arboricola pv. pruni [3,17] and fire blight of pome trees due to Erwinia amylovora [1], considered with bacterial peach decline as regulated diseases [1,25].

In previous studies, certain of these diseases were identified in many fruit production areas in Algeria [2, 10,27]. The aims of this study were the identification of bacterial diseases of stone and pome fruit trees in some...
fruit production areas in Algeria using biochemical, physiological tests LOPAT and GATTa tests and by gas chromatographic analysis of Fatty Acid Methyl Esters (GC-FAME).

MATERIALS AND METHODS

Source, Isolation and purification of bacteria:
Bacteria were isolated from cankers on branches and stems of cherry trees during 2008 and 2010 from Constantine and Khemchela localities (Eastern Algeria). Individual samples were selected from different trees. Symptomatic tissues were surface sterilized with 0.25% aqueous sodium hypochlorite for 30 sec and rinsed in sterile distilled water. Small pieces of tissue were excised from canker and lesion margins, macerated in 3 ml of sterile distilled water for 30 min and loopfuls of the resulting suspensions were streaked onto nutrient agar (NA). Plates were incubated at 27°C for 3-4 days [8]. After incubation, characteristic single colonies were subcultured on NA or King’s medium B (KB) [16]. This procedure was repeated at least twice to ensure purity. Cultures were stored on Nutrient Agar slants at 4°C.

Biochemical and physiological tests:
Bacterial isolates were identified on the basis of the following tests: Gram reaction using potassium hydroxide solubility test (KOH test) [29], fluorescence on king’s medium B, Nitrate reduction, Glucose metabolism, LOPAT tests (Levan production, Cytochrome oxidase, Potato soft rot, Arginine hydrolysis and Tobacco hypersensitivity) [20], GATTa tests (Gelatin liquefaction, Aesculine hydrolysis, Tyrosine and L(+)-tartrate utilization and L-Lactate utilization test [18].

Identification of bacterial species by MIS:
All the strains were compared by fatty acid methyl esters (FAME) analysis. Preparation and analysis of FAME from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer’s manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA) [26,28]. FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm) with cross-linked 5% phenyl methyl silicone. FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package. The identity of bacterial strains was revealed by computer comparison of FAME profiles of the unknown test strains with those in the library (Laboratory of Genetics and Bioengineering, Yeditepe University).

Pathogenicity tests:
The pathogenicity of isolates was tested on cherry shoots, green immature pear and sweet cherry fruitlets cv.Burlat as described [6,13]; fruitlets, were disinfected by dipping in 50% ethanol and inoculated (5-10 fruitlets per isolate) with a sterile needle immersed in a 10⁵ cfu/ml aqueous suspension of each isolate. After inoculation, fruitlets were placed on moist filter paper in sterile Petri dishes and incubated at 22°C for 4 days.

All tests were repeated twice, and reference strains ; of P.syringae pv. syringae, and P.s pv. morsprunorum from Kifissia university -Greece were used for comparison of results.

RESULTS AND DISCUSSION

Field survey, bacterial isolation and Phenotypic characterization:
Field surveys of pome and stone fruit tree orchards during 2008-2010, showed the occurrence of symptoms of bacterial diseases such as cankers and gummosis on cherry, plum and apricot (Fig.1) and pear and apple trees in many localities of the studied area in Algeria, however, Severe damages were observed on cherry trees.

Out of 12 fluorescent Pseudomonas strains were isolated from infected tissues of pome and stone fruit trees and 55 strains were pathogenic.

Bacterial colonies on NA medium were round, white and have 2-3 mm in diameter. All studied pathogenic isolates were gram-negative, aerobic and showed fluorescence on KB medium. The isolates were identified according to LOPAT tests results (Levan production, oxidase reaction, potato soft rotting, arginine dihydrolase, and tobacco hypersensitivity), phenotypic characterization using GATTa (Gelatin liquefaction, Esculin, Tyrosinase activity and Tartrate utilization) and L-Lactate tests and pathogenicity tests. Fatty Acid Methyl Esters analysis (FAME) confirmed the identification of all tested bacterial strains from pome and stone fruits as P. syringae with similarity indices of 0.65 to 0.89.

All strains tested for pathogenicity produced clear lesions and necrosis on cherry immature fruitlets after 2-4 days incubation. Identified bacteria were classified into three distinct groups (Table 1):
- Group 1: consisted of strains 30 with Gelatin and Aesculin positive, and Tyrosinase and Tartrate negative results were classified as *Pseudomonas syringae* pv. *syringae*. strains comparable to *P.syringae* pv.*syringae* isolated from cherry (14 strains),plum (6 strains),apricot (5 strains) and Pear (5 strains).

-Group 2: 15 strains with, Gelatin and Aesculin negative and Tyrosinase and Tartrate positive results were identified as *Pseudomonas syringae* pv. *morsprunorum* isolated from cherry (10 strains) and plum (5 strains).

-Group 3: 10 strains with levan test negative and potato soft rotting activity positive identified as *Pseudomonas viridiflava* (Group II of Lelliot *et al.* [20], obtained from cherry, apricot and apple trees.

The remaining fluorescent Pseudomonas strains showing negative HR Tobacco reaction and positive potato rot (some strains),were ranged in LOPAT Groups II and III of the determinative scheme of Lelliot *et al.* [20].

Field surveys and bacteriological analysis showed that that *Pseudomonas syringae* is associated with symptoms; such as cankers, gummosis and leaf lesions, blossom blasts, tip diebacks on pome and stone fruit trees in some surveyed orchards in Algeria.

Many Pseudomonas isolates were obtained from symptomatic tissue collected from cherry, plum, apricot, pear and apple orchards. Based on LOPAT and Gatta tests, Phenotypic characterization showed that the strains belong to *Pseudomonas syringae* pv. *syringae*. *P.s. pv. morsprunorum* and *P.viridiflava*.

*Pseudomonas syringae* pv. *syringae* and *P.s. pv. morsprunorum* strains from cherry were isolated from orchards of Constantine locality, from orchards of Khenchela and Souk Ahras areas. Pear and apple strains of *P.s.pv.syringae* and *P.viridiflava* were obtained from Khenchela and Skikda orchards.

These results confirm those obtained in previous works [2, 10,27].They also agree with results of similar works over the world reporting that *Pseudomonas syringae* pv. *syringae* and *P.s. pv. morsprunorum* are the causal agents of canker disease on stone fruit trees [4, 9,31] and apple and pear diseases [21,23].These bacteria survive also as epiphytes on leaf surfaces of cherry [12,19].

![Fig. 1: Symptoms of cankers and gummosis on cherry (A) and plum (B) branches.](image)

<table>
<thead>
<tr>
<th>Groups Test</th>
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<th>2</th>
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<th>P.s.s Rs183 (Ia11)</th>
<th>P.s.m Rs31</th>
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*Positive,-, Negative,/+, variable.*Ps.s.: *Pseudomonas syringae* pv. *syringae*;Ps.m.: *P.s.pv.morsprunorum*. Rs183 (Ia11):Reference strain of *Pseudomonas syringae* pv. *syringae*, Rs 31: Reference strain of *P.s.pv.morsprunorum*. 
Conclusion:
Field surveys showed the occurrence of many symptoms of bacterial diseases in some fruit producing areas in Algeria. Biochemical, physiological LOPAT, GATTa tests and gas chromatographic analysis of Fatty Acid Methyl Esters (GC-FAME) used for identification of isolates from diseased tissues revealed two main diseases; bacterial canker of stone fruits and pear blossom blast on pear and apple. However, Cherry trees are the most infected in the studied area.

This work is continued to delimit repartition areas of these diseases in Algeria and to study their epidemiology.

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