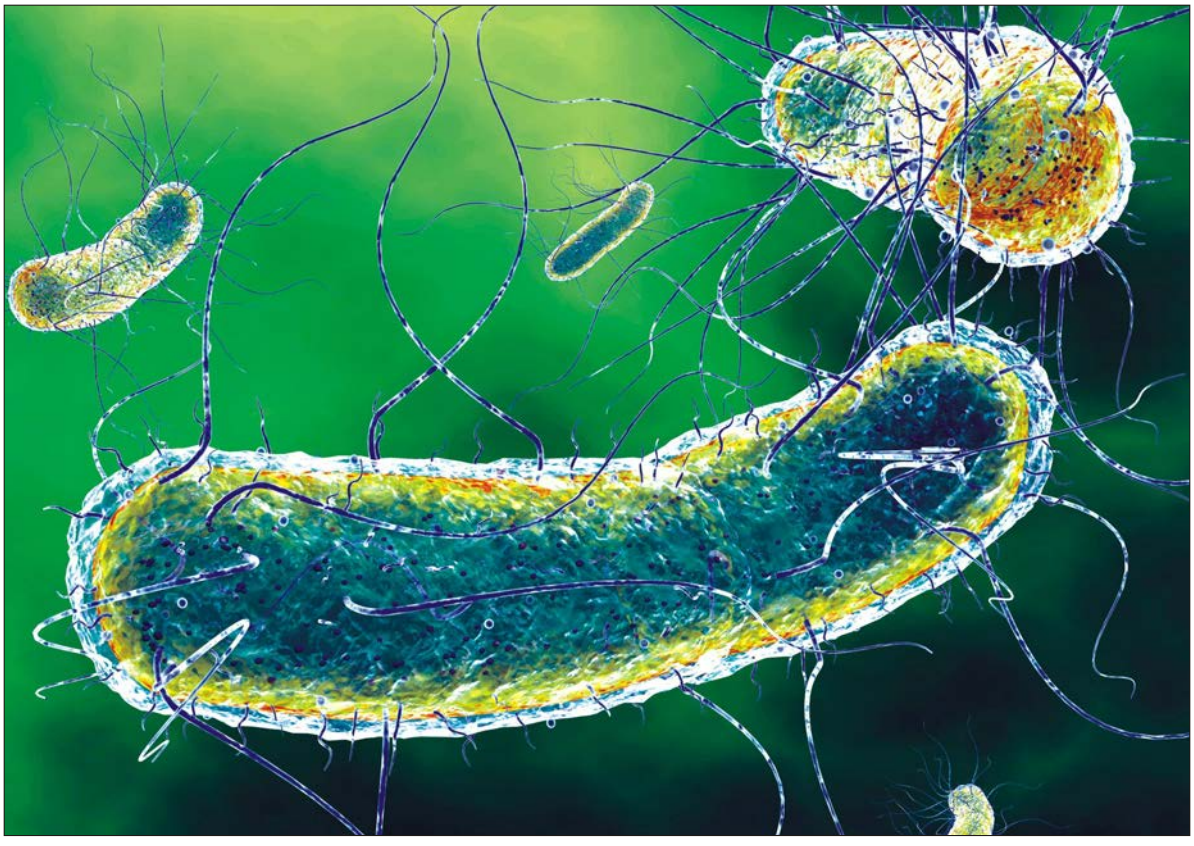

Current Research in Bacteriology

ISSN 1994-5426

science alert

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Current Research in Bacteriology 6 (1): 1-4, 2013
ISSN 1994-5426 / DOI: 10.3923/crb.2013.1.4

Identification Bacterial Agent of the Bark Canker of Peach in the Province of Ilam

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ABSTRACT

Peach canker is a bacterial disease common on apricot, prune, plum and sweet cherry trees as well as on peach trees. Twenty-five samples were collected from peach gardens in Ilam, Iran between 2010-2011. Small tissue pieces from stem, surfaces of cankers, were removed aseptically, ground by plastic roller in bacteriological saline (0.85% w/v NaCl) and left at room temperature (20°C) for 15 min. Loopfuls of the bacterial suspension were streaked onto the surface of plates made of Nutrient Agar and incubated at 26°C. In this way, pure bacterial cultures were obtained from the infected tissue. Isolates were routinely grown on NA at 25°C and stored at 4°C for up to 2 weeks. Results showed that, based on biochemical and pathogenicity properties, the predominate pathogenic type identified was *Pseudomonas syringae* pv. *syringae*. Based our findings all 25 isolates of *Pseudomonas syringae* pv. *syringae* produced canker on the stem of peach.

Key words: *Pseudomonas syringae* pv. *syringae*, peach, canker, pathogen, isolates

INTRODUCTION

Canker is a bacterial disease on stone fruit trees. Bacterial canker of peach, caused by *Pseudomonas syringae* pv. *syringae*, affects all commercially grown *Prunus* species. Factors that weaken or injure the tree predispose it to developing cankers. These factors include wounds, frost damage and poor nutrition. Infection by other pathogens including *Pseudomonas* spp., *Verticillium* and *Nectria* can lead to more bacterial canker. Bradbury (1986) reported that *Pseudomonas syringae* pv. *syringae* is unique among most *Pseudomonas syringae* pathovars in its ability to cause disease in over 180 species of plants in several unrelated genera. Gross and DeVay (1977) and Saad and Hagedorn (1972) *Pseudomonas syringae* pv. *syringae* strains infecting beans. The objective of the present study was the identification of the causal agent of bacterial canker on peaches in the Ilam province.

MATERIALS AND METHODS

Twenty-five samples were collected from peaches gardens in Ilam province, Iran during 2010-2011. Small tissue pieces from stem, surfaces of cankers, were removed aseptically, ground by plastic roller in bacteriological saline (0.85% w/v NaCl) and left at room temperature (20°C) for 15 min. Loopfuls of the bacterial suspension were streaked onto the surface of plates made of Nutrient Agar (NA) and King's medium B (KB) and incubated at 26°C. In this way, pure bacterial cultures were obtained from the infected tissue. Isolates were routinely grown on KB at 26°C and stored at 4°C for up to 2 weeks. For longer-term storage, bacterial strains were stored in a freezing medium (Luria peptone+glycerol) at -80°C. Pathogenicity tests were conducted on young potted

12-month-old peach using 25 peach bacterial isolates. Stems were wounded with a 25G hypodermic needle. The wound was covered with cotton wool dipped in a suspension of 1×10 CFU mL^{-1} of each isolate and the inoculation site was wrapped with grafting tape. Control plants were treated with sterile distilled water. The trees were kept in high humidity (95%) for 24 h before and after inoculation. Following this, the grafting tape and cotton wool were removed and the peach seedlings were moved outdoors where they were watered overhead twice daily. Strains were characterized by the Gram test in 3% KOH, the oxidative/fermentative test 8, production of fluorescent pigment on KB, Hypersensitive Reaction (HR) in tobacco, an oxidase test, levan formation, catalase, urease, gelatin liquefaction, litmus milk, salt tolerance (5%) and gas formation from glucose. In addition, tests for arginine dehydrolase, hydrogen sulphide production from peptone, reducing substances from sucrose, tyrosinase casein hydrolase, nitrate reduction, indole production, 2-keto gluconate oxidation lecithinase, starch hydrolysis, phenylalanine deaminase, esculin and Tween 80 hydrolysis and optimal growth temperature were performed. This reference isolate was considered as a typical isolate of *P. syringae* pv. *syringae* (Hugh and Leifson, 1953; Suslow *et al*., 1982; Lelliott and Stead, 1987; Hildebrand, 1998; APS, 2001). Analysis of variance was performed on the data collected using the General Linear Model (GLM) procedure of the SPSS software) Version 16, IBM Inc.). The mean separation was conducted by Tukey analysis in the same software ($p = 0.05$).

RESULTS AND DISCUSSION

All 25 isolates were gram negative, oxidase and catalase negative and were unable to utilize glucose under anaerobic conditions (Table 1). None of the isolates produced reducing compounds from sucrose, or showed lecithinase or arginine dihydrolase activity, or produced gas from glucose. All isolates were capable of hydrolysing gelatin. None of the isolates were able to, produce indole, reduce nitrate. All isolates were able to utilize citrate and produced acid from manitol, xylose, inositol, maltose, sorbitol, manose and sucrose. Serious infections occurred on young seedling. It is most likely that this growth was callus tissue caused by a reaction of the peach to wounding and the presence of *Pseudomonas syringae*. This long incubation period may also have occurred in the first year of infection of the peach trees. The pathogen has the ability to kill both young and older trees. Systemic infection and death of young trees is a perennial problem in nurseries and canker development leading to the girdling and death of scaffold limbs and entire trees is a common event that can lead to the rapid demise of older orchards. *Pseudomonas syringae* cause diseases of stone fruit trees and these pathogens utilize an impressive array of virulence factors such as effectors, toxins and phytohormones to incite disease symptoms. Lelliott *et al*. (1966) reported aspects of the systematics, ecology and genetics of *Pseudomonas syringae*. Bacterial canker is caused by *Pseudomonas syringae* pv. *syringae* and is an important disease of peach (*Prunus persica*). Canker formation is facilitated by stress events such as exposure to freezing conditions and injury from frost damage, causing a weakening and predisposition of trees to infection. Cankers formed on scaffold branches and trunks of peach are typically sunken in appearance and associated with gummosis. These cankers can become quite large, girdling branches and trunks leading to death of limbs or the entire tree. All 25 isolates of *Pseudomonas syringae* pv. *syringae* produced canker on the stem of peach. No significant differences were observed in the degree of disease symptoms. Sarhan *et al*. (2005) and Fuller *et al*. (2003) reported

Table 1: Phenotypic characteristics of *Pseudomonas syringae* pv. *syringae* strains tested

Characteristics	I	R
Gram reaction	-	-
Fluorescent pigment	+	+
Levan formation	+	+
Oxidative/fermentative	-	-
Pectinase	-	-
Arginine dihydrolase	-	-
HR on tobacco	+	+
Ice nucleation	+	+
Growth at 39°C	-	-
Acetoin	-	-
Nitrate reduction	-	-
Catalase	-	-
Oxidase	-	-
Starch hydrolysis	-	-
Gelatin hydrolysis	+	+
Esculin hydrolysis	+	+
Indole formation	-	-
H_2S from cysteine	-	-
Casein hydrolysis	-	-
Urease	+	+
Utilization of		
L-lysine	+	+
Citrate	+	+
lecithinase	-	-
Growth in 5% NaCl	-	-
Acid from		
L-Arabinose	+	+
Inositol	+	+
Manitol	+	+
Xylose	+	+
Maltose	+	+
D-Sorbitol	+	+
Sucrose	+	+
D-Manose	+	+
D-Glucose	+	+
Cellobiose	-	-
Inulin	-	+
Fructose	+	+
Lactose	-	-

I: Iranian isolates of *P. syringae* pv. *syringae*, R: Reference *P. syringae* pv. *syringae* (Hildebrand, 1998)

ice nucleation is the induction of ice formation at super cooled temperatures of -2 to-10°C in the presence of suitable ice nuclei. Hirano and Upper (2000), Morris *et al.* (2004) and Kennelly *et al.* (2007) reported role of bacterial ice nucleation in frost injury and the subsequent development of plant diseases have been extensively reviewed.

CONCLUSION

In conclusion, based on the findings all 25 isolates of *Pseudomonas syringae* pv. *syringae* produced canker on the stem of peach. Further research that elucidates the mechanisms eliciting the observed genetic diversity is needed.

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