

FIRST REPORT ON BACTERIAL LEAF BLIGHT OF STRAWBERRY CAUSED BY *XANTHOMONAS ARBORICOLA* PV. *FRAGARIAE* JANSE *et al.* IN TURKEY

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SUMMARY

In the summer of 2004 two bacterial strains were isolated from strawberry (*Fragaria x ananassa*) cv Camarosa propagation plants produced in Turkey. Symptoms including necrotic leaf spots and lesions along leaf margins were incited on primarily symptomless strawberry plants by keeping them in a growing chamber at 20°C and 100% relative humidity. On the basis of biochemical, physiological, serological and PCR tests the bacteria isolated were identified as *Xanthomonas arboricola* pv. *fragariae*. This is the first report on the bacterial leaf blight pathogen *X. arboricola* pv. *fragariae* of strawberry in Turkey.

Key words: bacterial angular leaf spot, fatty acid analysis, biochemical tests, serology, PCR.

INTRODUCTION

Angular leaf spot, caused by *Xanthomonas fragariae* Kennedy and King, was the only bacterial disease known to be present on strawberry (*Fragaria x ananassa*) until 1993-95 when another bacterial disease, called bacterial leaf blight, was observed in Northern Italy by Scortichini, 1996. Bacterial leaf blight symptoms are dry, brown necrotic leaf spots and large brown V-shaped lesions along the leaf margin, midrib and major veins. The causal agent of the new disease was identified as *Xanthomonas arboricola* pv. *fragariae* Janse *et al.* clearly different from *X. fragariae* (Janse *et al.*, 2001). The disease has been reported from Italy (Scortichini, 1996) and it may also be present in France because some strains previously attributed to *X. fragariae* were finally identified as *X. arboricola* pv. *fragariae* (Janse *et al.*, 2001). The economic significance of the disease is still not clear.

In the summer of 2004 symptomless strawberry propagation plant material of cultivar Camarosa, pro-

duced in the Mediterranean region of Turkey, was analyzed for the presence of *X. fragariae* before planting in Manisa province (Aegean Region). This test is done because *X. fragariae* is present in the quarantine list of Turkey (Anonymous, 2003) and in the A2 quarantine list of EPPO (Anonymous, 2001).

Propagation material was placed in a growth chamber under warm, humid conditions (20°C and > 90% RH) to see whether symptoms of angular leaf spot developed. After one week symptoms appeared that were more typical for bacterial leaf blight than of angular leaf spot, i.e. brown necrotic spots on the leaf surface, never water soaked or translucent, and large necrotic lesions along the leaf margin (Fig. 1). From the diseased leaf parts bacteria were isolated that produced yellow colonies on culture media.

The present study shows, by using biochemical, physiological, serological, pathological and molecular methods that the bacteria isolated are *Xanthomonas arboricola* pv. *fragariae*.

MATERIALS AND METHODS

Bacterial strains. In the PCR test the following isolates of *X. a. fragariae* were used: PD 2780 (pathovar reference strain); for *X. fragariae* PD 885 (type strain). For comparison in fatty acid analysis (FAA) the database of the PD containing 15 strains of *X. a. fragariae* and *X. fragariae* each, including the PVR5 and type strain) was used in the statistical analysis of the strains obtained from strawberry propagation material in Turkey. For the pathogenicity tests two reference strains from the PD



Fig. 1. Symptoms on naturally infected strawberry leaves after incubation in growth chamber.

Table 1. Results of biochemical and physiological tests.

Test	<i>X. fragariae</i> PD885 (Type strain)	<i>X. arboricola</i> pv <i>fragariae</i> PD2780 (PVRS)	Turkish strawberry isolates
Oxidase	-	-	-
HR on tobacco	-	+	+
Arginine dihydrolase	-	-	-
Levan formation on NSA	-	-	-
Oxidative metabolism of glucose	+	+	+
Fermentative metabolism of glucose	-	-	-
Nitrate reduction	-	-	-
Esculin hydrolysis	-	+	+
Starch hydrolysis	-	+	+
Gelatin hydrolysis	+	+	+
Urease activity	-	-	-
H ₂ S production	+	+	+
Cellulose hydrolysis	-	+	- (+w)
Tributyryn hydrolysis	+	+	+
Acid from:			
Sucrose	+	+	+
D-galactose	-	+	+
Maltose	-	+	+
Cellobiose	-	+	+
Arabinose	-	+	+
Trehalose	-	+	+
2% NaCl tolerance	-	+	+
Growth at 4°C	-	+	+
Growth at 35°C	-	+	+
Presence of pectolytic enzymes	-	+	+
Soft rot on potato slices	-	+	+

+ = positive reaction; +w = weak reaction; - = negative reaction.

collection, *X. arboricola* pv. *fragariae* PD2782 and *X. fragariae* PD885 and two strains obtained from strawberry propagation plants from Turkey were used.

Isolation. Strawberry propagation material cv Camarosa was placed in a growth chamber at 20 ± 1°C and > 90% humidity to observe eventual symptom development. When symptoms appeared (after 10 days) isolations were made by grinding leaf pieces near to necrotic areas in sterile water, using a disinfected sterile pestle and mortar. Aliquots of 0.1 ml of suspension were plated onto nutrient sucrose agar (NSA = Oxoid nutrient agar supplemented with 5% w/v sucrose) and incubated for 4 days at 25 ± 1°C. Yellow colonies, consistently isolated from diseased tissue, were transferred to nutrient agar (NA, Oxoid) to obtain pure cultures that were later used to characterise the isolated bacteria.

Biochemical and physiological tests. Gram stain, oxidase reaction, assimilation of carbon sources (sucrose, d-galactose, maltose, cellobiose, arabinose and trehalose), hydrolysis of esculin, starch and gelatin, arginine dihydrolase, 2% NaCl tolerance, growth at 4 and 35°C, hypersensitive reaction (HR) on tobacco and tomato were performed as described by Lelliott and Stead (1987). Macerating activity on potato slices was determined according to Janse *et al.*, (2001).

Fatty acid analysis. Whole-cell fatty acid methyl ester (FAME) profiles were analysed using the Microbial Identification System (MIS, Microbial ID, Inc (MIDI), Newark, DE, USA) according to Janse (1991 and 2001). For statistical analysis (principal component analysis) the MIDI software package was used, analyzing a larger number of strains of both *X. fragariae* and *X. a.* pv. *fragariae*.

Serological tests. Immunofluorescence tests were performed following Janse (1988) using anti- *X. fragariae* polyclonal serum IPO9534 BCD1 (titre 1600 with reference strain of *X. fragariae* PD2664 = NCPPB1822 from the USA) produced by Plant Research International, Wageningen, NL.

PCR tests. PCR tests were performed as described in Janse *et al.* (2001) using *X. fragariae*-specific primers *Xf9* and *Xf11* and *Xf9* and *Xf12* developed by Roberts *et al.* (1996) and 241A and 241B developed by Pooler *et al.* (1996).

Pathogenicity tests. Pathogenicity tests were performed using pot grown strawberry plants cv Camarosa in a growth room at 25 ±1°C and 80-100% RH. Leaves were inoculated by puncturing major veins with a bacterial suspension of 10⁸ CFU ml⁻¹. Leaves inoculated with sterile phosphate buffered saline (PBS, 0.01M) served as a control. Inoculated plants were covered with plastic bags for 3 days and incubated at 25 ± 1°C and 80-100% relative humidity for 3 weeks afterwards. Reisolations were performed from any leaves showing symptoms.

RESULTS

Isolation. Yellow, circular, mucoid and slimy colonies 1-2 mm in diameter were observed on NSA 72 h after isolation from diseased parts of strawberry plants.

Biochemical and physiological tests. Results of biochemical and physiological tests are shown in Table 1.

Fatty acid analysis. When the strawberry isolates were matched with the fatty acid profile library at the Plant protection Service (PD), Wageningen, NL a close relationship of strawberry strains from Turkey with *X. arboricola* pv. *fragariae* was found (0.840 similarity). The similarity with *X. fragariae* was found to be low (0.328).

PCR tests. The strawberry isolates from Turkey and the *X. a.* pv. *fragariae* control strain PD 2780 were negative by PCR using *X. fragariae*-specific primers *Xf9* and *Xf11*, as well as *Xf9* and *Xf12* (Roberts *et al.*, 1996) and

241A and 241B (Pooler *et al.*, 1996), whereas *X. fragariae* strain PD885 used as a control yielded the expected product of 537, 458 or 550 bp respectively.

Serological tests. In the immunofluorescence (IF) test one of the Turkish strawberry isolates (S-Tr-1) reacted with polyclonal serum against *X. fragariae* at a high titre of 1600, but although clearly defined cells were observed, the cell wall was thinner than that of reference strain *X. fragariae* PD 2664. The other isolate (S-Tr-2) isolate gave a negative reaction with the same antiserum.

Pathogenicity tests. On leaves of inoculated pot plants small, brown spots 1-2 mm in diameter were observed 5-7 days after inoculation. Only leaf spots induced by isolate S-Tr-1 from Turkey were surrounded by a chlorotic halo. In time spots enlarged and became slightly angular (Fig. 2). Infection also spread on midribs, major and secondary veins, petioles, peduncles and flower sepals.

DISCUSSION

In this study bacteria isolated from symptoms on initially symptomless strawberry propagation plants cv Camarosa produced in Turkey (incited by keeping the plants in a growth chamber at 20°C and 80-100% humidity), were identified on the basis of biochemical, physiological, serological, pathological and molecular methods. Symptoms were brown necrotic spots on the leaf surface, never water soaked or translucent, and large necrotic lesions along the leaf margin. The biochemical and physiological characters of the bacteria isolated were in accordance with those of *X. arboricola* pv. *fragariae* as described by Janse *et al.* (2001). In serological studies one of the isolates reacted with polyclonal antiserum against *X. fragariae* but the other did not react. Weak reactions of some *X. arboricola* pv. *fragariae* strains with anti-*X. fragariae* antisera were reported previously by Janse *et al.* (2001). PCR tests performed with *X. fragariae*-specific primers were negative. A pathogenicity test on strawberry cv Camarosa confirmed the pathogenicity of the Turkish isolates. On the basis of results of all our tests the Turkish strawberry isolates were identified as *X. arboricola* pv. *fragariae*.

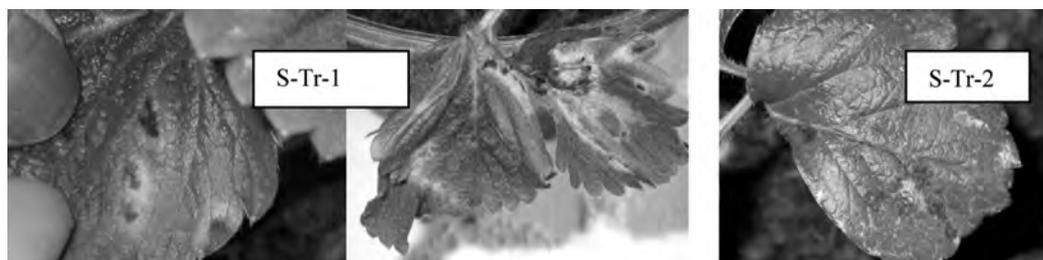


Fig. 2. Symptoms induced following artificial inoculation with two bacterial strains. Chlorotic haloes are present only on leaves infected by isolate S-Tr-1.

This is the first report on the bacterial leaf blight pathogen *X. arboricola* pv. *fragariae* on strawberry in Turkey. This also appears to be the first report on presence of the bacterium on asymptomatic symptomless strawberry propagation plants.

ACKNOWLEDGEMENTS

The technical assistance of Ms. P. Smits-Gorkink and K. Gaisch is highly appreciated.

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Received July 4, 2006

Accepted August 28, 2006