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Phenotypic and genetic characterization of *Erwinia carotovora* ssp. *carotovora* (Jones) Bergey *et al.* isolates from grafted tomato in Sardinia, Italy

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Summary. A disease symptomatically similar to that caused by *Erwinia carotovora* occurred on "Cuore di Bue" and "Cencara" tomato plants grafted on "Beaufort" and "He-Man", or ungrafted, in greenhouses in Sardinia (Italy). Symptoms were: dark brown/black longitudinal stem lesions, soft stem rot, pith breakdown of the stems, hollow stems, vascular tissue discoloration, wilting and collapse of the plants. Numerous bacterial colonies from stem tissues were isolated on yeast extract salts (YS) medium. Seven isolates (DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7 e DPP Pu8) were selected on the basis of their ability to cause rot on potato pieces and a hypersensitivity reaction in "White burley" tobacco leaves. Pathogenicity tests revealed that five of these isolates infected artichoke, basil, dwarf bean, fennel, marrow, melon, pepper, eggplant, grafted and ungrafted tomato, and white cabbage. Of the remaining two isolates, one (DPP As-1) did not infect white cabbage, and the other (DPP Pu8) did not infect basil, marrow or white cabbage. Phenotypic properties and ELISA, also performed on naturally infected tissues, revealed that all the isolates were *E. c.* ssp. *carotovora* (Jones) Bergey *et al*. PCR-RFLP analysis placed two (DPP As-2 and DPP As-3) of the seven isolates in RFLP group 8. Five isolates belonged to a hitherto unknown RFLP group. Prevention and control measures for this disease are suggested.

Key words: *Lycopersicum aesculentum,* stem rot, ELISA, PCR-RFLP.

Introduction

A bacterial disease resembling that caused by *Erwinia carotovora* ssp. *carotovora* (*Ecc*) (Jones) Bergey *et al*. on tomato (Dhavantari and Dirks, 1987; Malathrakis and Goumas, 1987; Khlaif, 1993; Schuerger and Batzer, 1993) has been observed in some greenhouses of central and southern Sardinia on tomato plants "Cencara" and "Cuore di Bue" grafted on "Beaufort" and "He-Man", and on ungrafted "Camone" and "Cencara" since the winter of 2001. At first only a few plants were affected, but in the next two years the number increased. The disease was particularly harmful in one greenhouse of central Sardinia and in three others located in the south of the island. A disease incidence of about 3% was common, but it reached nearly 8% of plants in the wetter parts of the greenhouses.

Symptoms occurred in December during ripening of the first fruits. Affected plants were initially stunted, with the lower leaves showing a pale yellowing that progressed slowly upwards, and finally plants wilted and collapsed. In particular dis-

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eased plants showed 10–15-cm-long dark-brown/black longitudinal stem lesions, soft stem rot, pith breakdown, hollow stems and vascular tissue discoloration. In grafted plants the rootstock did not present any symptoms (Fig. 1).

Microscopic examination of diseased plant tissues revealed large numbers of motile bacteria. When a dense suspension of these tissues was incubated on yeast extract salts (YS) medium (Dye, 1968) at 27°C, two different colony types appeared within 96 h: a few colonies were pale yellow, and many were whitish translucent. In a subsequent biological assay these latter colonies caused soft rot on potato pieces and a hypersensitivity reaction on "White burley" tobacco leaves (Klement, 1963).

The aim of the present work was to characterize the bacteria that formed whitish translucent colonies on YS both by traditional techniques and by the more precise molecular tests such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The primers used in the PCR allowed us to identify *Erwinia carotovora* species and subspecies except *E. c.* ssp. *betavasculorum* (*Ecb*) (Darrasse *et al.,* 1994); RFLP analysis of the amplified *pel* genes (encoding pectate lyases that are involved in soft rot disease) also allowed us to discriminate *E. carotovora* subspecies (Helias *et al*., 1998).

Materials and methods

Bacterial isolation

Isolations were performed on several infected tomato plants, grafted and ungrafted, grown in greenhouses of central and southern Sardinia, Italy (Provinces of Oristano and Cagliari).

Pieces of diseased stems, about 5 cm in length, were taken 10 cm above the collar, and in case of grafted plants, from the rootstock. Pieces were washed in tap water and surface-disinfected by quick immersion in 0.5% sodium hypochlorite. After washing several times with sterile distilled water (SDW), fragments of internal tissue (pith and vessels) were removed from the margin of diseased tissues and crushed in a sterile mortar with a small amount of phosphate buffer (pH 7; 0.05 M). Tenfold dilutions of the dense suspensions were plated on YS medium and incubated at 27°C for 4 days.

After this period colonies were purified twice on YS and streaked on nutrient agar (NA and CM3, Oxoid, Basingstoke, Hampshire, UK) for long-term storage at 4°C.

Pathogenicity tests

Based on their capacity to cause rot on potato pieces and to elicit a hypersensitive reaction in "White burley" tobacco leaves, seven isolates (DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6,

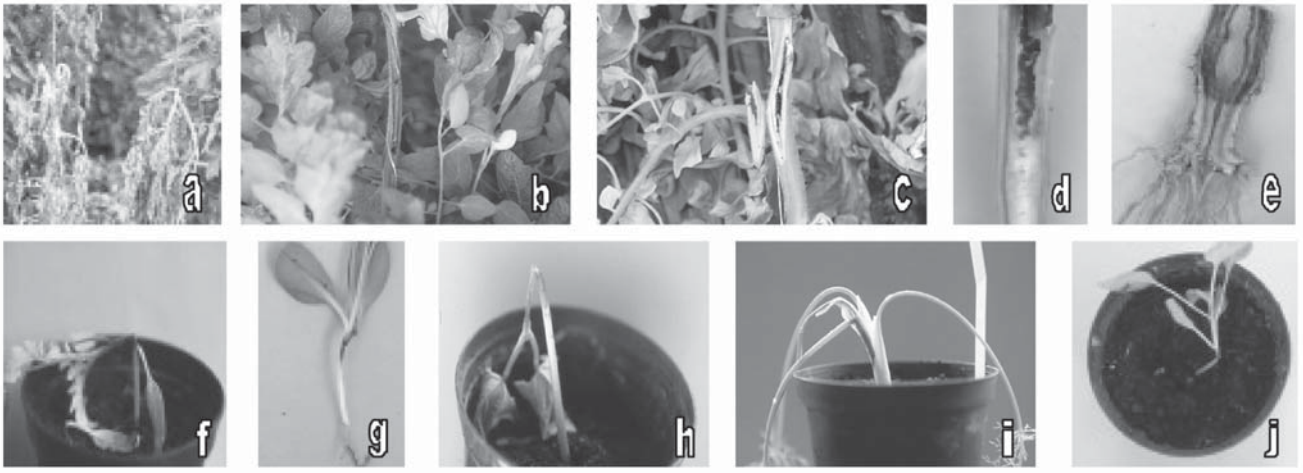

Fig. 1. Symptoms in naturally infected tomato plants: (a) wilted plants; (b) brown/black longitudinal stem lesions; (c and d) stem hollowness and vascular tissue discoloration; (e) symptoms on a grafted plant. Symptoms in artificially inoculated plants: (f) tomato, (g) artichoke, (h) bean, (i) fennel and (j) pepper plants.

DPP Pu7 and DPP Pu8) were selected for pathogenicity tests.

Five one-month-old plants of artichoke, basil, dwarf bean, white cabbage, fennel, eggplant, marrow, melon, pepper, grafted tomato and ungrafted tomato were inoculated with each isolate.

Inoculum was prepared from fresh cultures grown for 48 h on NA and suspended in SDW to give a concentration of about 10^6 cfu ml^{-1}.

Plant stems at the primary leaf node were wounded with a sterile scalpel and a drop of bacterial suspension was placed on the wound site. Artichoke, white cabbage, and fennel plants were infected through the petioles of the external leaves.

Plants were kept at 22–25°C in a wet chamber and were transferred to the greenhouse after 24 h. Symptoms were examined daily for 10 days.

For each isolate the pathogenicity tests were repeated twice. All isolates were re-isolated on YS before being tested for differential biochemical and physiological features.

Biochemical and physiological tests

To characterise the bacterial isolates under study, and their respective re-isolates, the following biochemical and physiological tests were done: observation of bacterial cells by transmission electron microscopy (Zeiss TEM 109, Oberkochen, Germany); Gram stain; fluorescence (King *et al*., 1954); arginine dihydrolase (Thornley, 1960); oxidase (Kovacs, 1956); pectolytic activity on crystal-violet pectate (CVP) medium (Schaad, 1988); levan, nitrate reduction, indole, acetoin, hydrogen sulphide, action on litmus milk, aesculin and starch hydrolysis (Dye, 1968); hydrolysis of tyrosine (Lelliott *et al*., 1966); lecithinase (Billing and Luckhurst, 1957); reducing substances from sucrose and sensitivity (15 mg/disk) to erythromycin (De Boer and Kelman, 2001); fermentation of glucose (Hugh and Leifson, 1953); acid production on Ayers *et al*. (1919) medium from cellobiose, α-methyl glucoside, inulin, D-lactose, maltose, melibiose, palatinose, D-raffinose, trehalose, L-arabitol, sorbitol, citrate, malonate, L-tartrate and DL-tartrate; growth at 36 and 37°C on YS broth, and tolerance of sodium chloride at 5% on YS broth (Dye, 1968).

The type strain of *Ecc* from the Collection Française de Bactéries Phytopathogènes (CFBP) 2046 was used as a control.

Serology

Isolates and re-isolates were tested by slide agglutination and ELISA using an antiserum obtained according to De Boer *et al*. (1979). ELISA was also used to check for the bacterium in plant samples. The ELISA test was performed using the method described by Clark and Adams (1977) and Clark (1981). Preliminary titration tests indicated the following optimal dilutions: coating 1:200; enzyme/serum ratio 1:1; conjugate dilution 1:100. Absorbance values were read with a photometer (Mod. DV990BV4, GDV, Rome, Italy) and were considered positive when they were 2.5 times higher than the average of absorbance of blank. The *Ecc* type strain CFBP 2046 was used for comparison.

PCR-RFLP

Isolates DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7, DPP Pu8 and six control strains (*Ecc* CFBP 2046 and EII 354-86; *E. c.* ssp. *atroseptica* [*Eca*] CFBP 1526; *E. c.* ssp. *odorifera* [*Eco*] CFBP 1878; *Ecb* CFBP 1539 and 2122) were evaluated for PCR and RFLP analysis (Darrasse *et al.,* 1994; Hélias *et al*., 1998).

For PCR, total DNA was extracted as indicated by Wilson (1987) and was measured by ethidium bromide fluorescence quantification.

The procedure described by Fiori and Schiaffino (2004) was followed.

The primers *pel* Y (Y_1 and Y_2) specific for *E. carotovora*, were used (Darrasse *et al*., 1994). Amplification was carried out in a 100 ml reaction mixture and PCR was performed in a PCR-Express apparatus (Hybaid, Ashford, Middlesex, UK) as follows: 1 pre-denaturation step at 94°C for 5 min; 35 cycles of 30 s at 94°C, of 30 s at 65°C and of 45 s at 72°C; a final extension at 72°C for 5 min.

Amplification products were analysed by electrophoresis on agarose gel and the images were acquired with a Gel Doc 2000 System (Bio-Rad Laboratories, Hercules, CA, USA). The size of the products was estimated using a 1 kb DNA ladder (Invitrogen, Carlsbad, CA, USA).

An amplification product of 434 bp was precipitated and aliquots of 5 ml of the precipitated products were digested at 37°C for 4 h with the restriction endonucleases *Alu* I, *Hae* II, *Hpa* II and *Sau*3A I. Restriction patterns were analysed by electrophoresis in agarose gel.

The molecular weight of the digestion fragments

was estimated by means of the software Quantity One (Bio-Rad) and 1 kb DNA ladder (Invitrogen).

RFLP groups were determined on the basis of the results obtained after digestion with the four enzymes mentioned above (Darrasse *et al*., 1994).

Results

Bacterial isolation

Based on their morphological characteristics on YS, forty isolates were selected. The colonies of these isolates after 72 h were circular, slightly raised to lenticular with smooth margins, generally whitish translucent in colour, 1–1.5 mm in diameter and easily suspended in water. Of these isolates, only seven (DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8) caused rot on potato pieces and a hypersensitivity reaction in "White burley" tobacco leaves.

Pathogenicity test

As shown in Table 1, five of the isolates, DPP As-2, DPP As-3, DPP As-14, DPP Pu6 and DPP Pu7 infected all the species inoculated. Isolate DPP As-1 did not infect white cabbage, and DPP Pu8 did not infect basil, white cabbage or marrow.

Through the stems and petioles of infected plants three types of reactions were observed. 1. Rot occurred at the inoculation point within 24 h. After 48 h the entire stem was rotted and bent down. This was followed by the collapse and death of the plants (Fig. 1). 2. Rot spread more slowly: in the first 48 h it remained restricted to the inoculation site; but in the following three days the stems began to rot, and the collapse and death of the plants followed as with type 1. 3. Inoculated plants did not show clear symptoms of infection. In this case the only symptom observed was a browning of the area surrounding the inoculation point.

Bacterial characterization

The bacterial cells were rod-shaped with rounded ends and were either single or in pairs; their average size was $0.7 \times 2.3\ \mu$m; they were motile due to peritrichous flagella. The isolates and re-isolates were Gram negative, optimum growth temperature was between 25 and 27°C.

The biochemical and physiological characteristics of the seven isolates are shown in Table 2, together with those of the *E. carotovora* subspecies.

In the slide agglutination test, tomato isolates reacted positively with anti *Ecc* CFBP 2046 serum.

The ELISA gave the following average optical density values: blank 0.04; homologous *Ecc* strain CFBP 2046 maximum value 1.8; values for tomato isolates (DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8) and re-isolates ranged from 1.2 to 0.6. Moreover about 42% of the naturally infected plant material tested was positive.

Table 1. Results of experimental infection on different host plants tested with *Erwinia carotovora* ssp. *carotovora* tomato isolates.

Host	Isolate						
	DPP As–1	DPP As–2	DPP As–3	DPP As–14	DPP Pu6	DPP Pu7	DPP Pu8
Artichoke	++	++	++	++	++	+	+
Basil	++	++	++	++	++	++	–
White cabbage	–	++	++	++	++	++	–
Dwarf bean	++	++	++	++	++	++	++
Eggplant	+	++	+	+	++	++	+
Fennel	+	+	+	+	++	++	++
Marrow	+	++	+	+	+	++	–
Melon	++	++	++	++	++	++	++
Pepper	++	++	++	++	++	+	+
Tomato grafted	++	++	++	++	++	++	++
Tomato ungrafted	++	++	++	++	++	++	++

++, Death of plants within 48 hours after infection; +, death of plants within a week from infection; –, negative result.

The results of the pathogenicity, biochemical, physiological (Tables 1 and 2) and serological tests indicated that the tomato isolates DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8 and their re-isolates were *E. c.* ssp. *carotovora*.

PCR-RFLP

As expected, the PCR gave an amplification product of 434 bp for isolates DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7, DPP Pu8, *Ecc* strains CFBP 2046 and EII 354-86, *Eca* strain CFBP 1526 and *Eco* strain CFBP 1878. No

Table 2. Biochemical and physiological characteristics of tomato isolates compared with *E. carotovora* subspecies.

Test	Tomato isolate							*E. carotovora* subspecies[a]			
	DPP As–1	DPP As–2	DPP As–3	DPP As–14	DPP Pu6	DPP Pu7	DPP Pu8	*Ecc*	*Ecb*	*Eco*	*Eca*
Fluorescent pigment	–	–	–	–	–	–	–	–	–	n.d.	–
Arginine	–	–	–	–	–	–	–	–	–	–	–
Oxidase	–	–	–	–	–	–	–	–	–	–	–
Pectate liquefaction	+	+	+	+	+	+	+	+	+	+	+
Levan	–	–	–	–	–	–	–	–	n.d.	n.d.	–
Nitrate reduction: to NO_2	+		+	+	+	+	+	+	+	+	+
beyond NO_2		+									
Indole	–	–	–	–	–	–	–	–	–	–	–
Acetoin production	+/–	+	+	+/–	+/–	+/–	+	+	+	n.d.	+
H_2S production	+	+	+	+	+	+	–	+	n.d.	n.d.	+
Action in litmus milk	+*	+*	+*	+*	+*	+*	+*	+*	n.d.	n.d.	+*
Hydrolysis of: aesculin	+	+	+	+	+	+	+	+	n.d.	n.d.	+
starch	–	–	–	–	–	–	–	–	+	–	–
tyrosine	+	+/–	–	–	–	–	–	–	–	–	n.d.
Lecithinase	–	–	–	–	–	–	–	–	–	n.d.	–
Reducing substance from sucrose	–	–	+	+	–	–	–	–	+	+	+
Sensivity to erythromycin	+	+	+	+	+	+	+	–	–	n.d.	–
Oxidation/fermentation of glucose	+	+	+	+	+	+	+	+	n.d.	n.d.	+
Gas from glucose	–	–	–	–	–	–	–	–	n.d.	n.d.	–
Acid production from: cellobiose	+	+	+	+	+	+	+	+	+	+	+
α-methyl glucoside	–	+	+	–	–	–	–	–	+	+	+
inulin	–	–	–	–	–	–	–	–	+	–	–
D-lactose	+	+	+	+	+	+	–	+	+	+	+
maltose	–	–	–	–	–	–	–	–	+	n.d.	+
melibiose	+	+	+	+	+	+	–	+	–	+	+
palatinose	–	+	+	–	–	–	–	–	+	+	+
D-raffinose	+	+	+	+	+	+	–	+	–	n.d.	+
threalose	+	+	–	+	+	+	–	+	+	+	+
arabitol	–	+	+	–	+	+	+	–	–	+	–
sorbitol	–	+	+	–	+	+	+	+	–	+	–
citrate	–	–	–	–	–	–	–	–	–	+	+
malonate	–	–	–	–	–	–	–	–	–	–	–
L-tartrate	–	+	–	–	+	+	+	–	–	–	–
DL-tartrate	–	–	–	–	–	–	–	–	n.d.	–	n.d.
Growth at 37°C	–	+	+	–	–	+	–	+	+	+	–
Growth at 36°C	+	+	+	+	+	+	+	+	+	+	–
Tolerance NaCl 5%	+	+	+	+	+	+	+	+	+	+	+.

+, Positive reaction; –, negative reaction; +/–, variable reaction; +*, utilisation of glucose and coagulation of casein; n.d., not determined.

[a] *Ecc*, *E. c.* subsp. *carotovora* CFBP 2046; *Ecb, E. c.* subsp. *betavasculorum* CFBP 1539; *Eco*, *E. c.* subsp. *odorifera* CFBP 1878; *Eca*, *E. c.* subsp. *atroseptica* CFBP 1526.

amplification was obtained with *Ecb* strains CFBP 1539 and 2122.

RFLP analysis by digestion of the amplification products with the restriction enzymes *Alu* I, *Hae* II, *Hpa* II and *Sau*3A I showed that the tomato isolates were *E. c.* ssp. *carotovora*. Two isolates (DPP As-2 and DPP As-3) belonged to RFLP group 8 (Darrasse *et al.*, 1994) (Fig. 2), whereas five isolates (DPP As-1, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8) could not be assigned to a previously described RFLP group (Fig. 2). The RFLP pattern of these five isolates was like that of RFLP 11 and RFLP 15 but differed in the size of the fragments obtained with *Alu* I in the case of RFLP 11, and with *Sau*3A I in the case of RFLP 15.

Discussion

On the basis of the results obtained with conventional and molecular analysis, isolates DPP As-1, DPP As-2, DPP As-3, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8 from diseased tomato plants were identified as *E. c.* ssp. *carotovora*. However, tomato isolates showed a certain variability both in virulence and in pathogenicity on tomato and other plant species, and infected with varying aggressiveness almost all the plant species inoculated (artichoke, basil, dwarf bean, white cabbage, fennel, eggplant, marrow, melon, pepper, grafted tomato and ungrafted tomato) with the exception of DPP As-1 and DPP Pu8 (Table 1). Moreover, the results of some phenotypic properties, such as growth at 37°C, sensitivity to erythromycin, reducing substances from sucrose, and acid production from some carbon sources (Table 2) was not in accordance with the typical determination provided by Lelliott and Dickey (1984) and De Boer and Kelman (2001); and the results of RFLP analysis obtained for five isolates (DPP As-1, DPP As-14, DPP Pu6, DPP Pu7 and DPP Pu8) were also atypical. The differences in some of the responses shown by our isolates could reflect natural bacterium variability. Such variability is reported in the literature (Smith and Bartz, 1990; Helias *et al.*, 1998; Seo *et al.*, 2002; Seo *et al.*, 2003).

Erwinia carotovora ssp. *carotovora* is an ubiquitous bacterium known as the causal agent of bacterial rot in various ornamental and horticultural plants (Pérombelon and Kelman, 1980; Smith and Bartz, 1990; Schuerger and Batzer, 1993), including tomato, on which it causes a disease known as hollow stalk (Dhavantari and Dirks, 1987; Malathrakis and Goumas, 1987; Khlaif, 1993; Schuerger and Batzer, 1993; Alippi *et al.*, 1997; Cazorla *et al.*, 2000; Hseu *et al.*, 2003). In Sardinia *Ecc* has been reported on artichoke, cardoon (Marras, 1966), pepper (Fiori and Schiaffino, 2004), and now also on tomato.

At present we can only speculate on how the disease appeared in Sardinian greenhouses. It is well known that *Ecc* survives in plant residues, in contaminated soil, in irrigation water, in hydroponically grown plants (Pérombelon and Kelman, 1980; Pérombelon and Hyman, 1986; Schuerger and

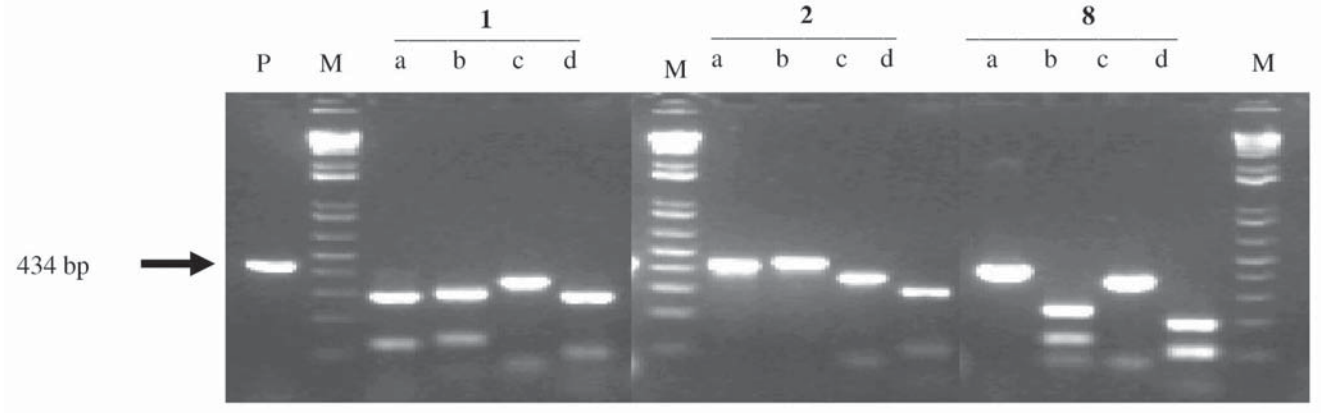

Fig. 2. RFLP patterns of (**1**) *Ecc* CFB 2046; (**2**) DPP Pu6, DPP Pu7, DPP Pu8, DPP As–1, DPP As–14; (**8**) *Ecc* EII 354–86, DPP As–2, DPP As–3 PCR amplification product, obtained with primers Y_1 and Y_2, digested with the restriction endonucleases *Alu* I, *Hae* II, *Hpa* II and *Sau*3A I. M, 1 kb marker (Invitrogen); P, amplification product of 434 bp; a, *Alu* I; b, *Hae* II; c, *Hpa* II; d, *Sau*3A I.

Batzer, 1993) and it can also, as reported for pepper plants (Hadas *et al*., 2001), be transmitted by infected seeds. This last could have been the origin of the primary infection reported here.

This bacterial disease represents a threat to Sardinian greenhouse crops. For the future it would be desirable to monitor other hosts besides tomato, artichoke and pepper in order to ascertain the diffusion of this disease and to verify epidemiological aspects.

Control of this tomato disease in the particular environment of Sardinian greenhouses is difficult. Rules to be followed are similar to those that help control other bacterial soft rots.

The first rule is to follow prevention and sanitation procedures. Although *Ecc* infects numerous plant species (Table 1), the best way to avoid its introduction is by using only healthy planting material starting from the tomato seeds. In the case of grafted plants, all steps involved in grafting should be performed under sanitary conditions.

When infection nevertheless occurs, early detection is essential to avoid disease spread. At the first appearance of the disease, all infected plants should be destroyed and the crop should be treated with fixed copper sprays. During cultural practices (defoliation and binding) disease incidence can be reduced by limiting the wounds made on plants.

Generally, outbreaks occur during rainy periods and maximum incidence is highest when plants remain wet long enough to allow bacterial multiplication. In those cases the spread of bacteria from diseased to healthy plants can be avoided by increasing air movement, which minimizes the time that the leaves remain wet.

Since the bacteria survive in plant residues, in the soil and in irrigation water from one cropping season to the next, the risk of infection occurring from one planting to another should be avoided by removing all plant residues left over from a previous crop, solarizing the soil during the summer, and verifying the absence of *Ecc* in the irrigation water to be used for the new crop.

Literature cited

Alippi A.M., E. Dal Bò, L.B. Ronco and P.E. Casanova, 1997. Tomato as a new host of *Erwinia carotovora* subsp. *carotovora* in Argentina. *Plant Disease* 81, 230.

Ayers S.H., P. Rupp and W.T. Johnson, 1919. A study of the alkali-forming bacteria in milk. *United States Department of Agriculture Bulletin*, 782 pp.

Billing E. and E.R. Luckhurst, 1957. A simplified method for the preparation of egg yolk media. *Journal of Applied Bacteriology* 20, 90.

Clark M.F., 1981. Immunosorbent assay in plant pathology. *Annual Review of Phytopathology* 19, 83–106.

Clark M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34, 475–483.

Cazorla F.M., A. Perez Garcia, M.E. Rivera, J.C. Codina, J.A. Torres and A. de Vicente, 2000. Bacterial diseases of tomato in southern Spain: application of a detached tissue assay to evaluate bacterial pathogenicity. *Diseases of Cucurbitaceous and Solanaceous Vegetable Crops in the Mediterranean Region*, 11–14 October 1999, Kerkyra, Greece,. *Bulletin OEPP* 30, 351–356.

Darrasse A., S. Priou, A. Kotoujansky and Y. Bertheau, 1994. PCR and restriction length polymorphism of a *pel* gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. *Applied and Environmental Microbiology* 60, 1437–1443.

De Boer S.H., R.J. Copeman and H. Vrugink, 1979. Serogroups of *Erwinia carotovora* potato strains determined with diffusible somatic antigens. *Phytopathology* 69, 316–319.

De Boer S.H. and A. Kelman, 2001. II Gram-negative bacteria. B-2 Erwinia Soft Rot Group. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (N.W. Schaad, ed.), Third ed. APS Press, Saint Paul, MN, USA, 56–72.

Dhanvantari B.N. and U.A. Dirks, 1987. Bacterial stem rot of greenhouse tomato: spatial distribution and the effect of high humidity. *Phytopathology* 77, 1457–1463.

Dye D.W., 1968. A taxonomic study of the genus *Erwinia* I. The "*Amylovora*" group. *New Zealand Journal of Science* 11, 590–607.

Fiori M. and A. Schiaffino, 2004. Bacterial stem rot in greenhouse pepper (*Capsicum annuum*, L.) in Sardinia (Italy): occurrence of *Erwinia carotovora* subsp. *carotovora*. *Journal of Phytopathology* 152, 1–6.

Hadas R., G. Kritzman, T. Gefen and S. Manulis, 2001. Detection, quantification and characterization of *Erwinia carotovora* ssp. *carotovora* contaminating pepper seeds. *Plant Pathology* 50, 117–123.

Hélias V., A.C. Le Roux, Y. Bertheau, D. Andrivon, J.P. Gauthier and B. Jouan, 1998. Characterization of *Erwinia carotovora* subspecies and detection of *Erwinia carotovora* subsp. *atroseptica* in potato plants, soil and water extracts with PCR-based methods. *European Journal of Plant Pathology* 104, 685–699.

Hseu S.H., C.Y. Lin and T.C. Sung, 2003. Bacterial stem rot of tomato caused by *Erwinia carotovora* subsp. *carotovora*. *Plant Protection Bulletin* 45, 257–262.

Hugh R. and E. Leifson, 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *Journal of Bacteriology* 66, 24–26.

Khlaif H.M., 1993. Bacterial stem rot of plastic-house grown tomato in the Jordan Valley. *Phytopathologia Mediterranea* 32, 58–61.

King E.O., M.K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* 44, 301–307.

Klement Z., 1963. Rapid detection of the pathogenicity of phytopathogenic pseudomonads. *Nature* 199, 299–300.

Kovacs N., 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 178, 703.

Lelliott R.A., E. Billing and A.C. Hayward, 1966. A determinative scheme for the fluorescent plant pathogenic *Pseudomonads*. *Journal of Applied Bacteriology* 29, 470–489.

Lelliott R.A. and R.S. Dickey**,** 1984. Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209^{AL}. In: *Bergey's Manual of Systematic Bacteriology* (N.R. Krieg, J.G. Holt, ed.), Vol. 1. Williams & Wilkins Co, Baltimore, MD, USA, 469–476.

Malathrakis N.E. and D.E. Goumas, 1987. Bacterial soft rot of tomato in plastic greenhouses in Crete. *Annals of Applied Biology* 111, 115–123.

Marras F., 1966. Il "marciume radicale" del carciofo (*Cynara scolymus* L.) e del cardo (*Cynara cardunculus* L.) causato da *Pectobacterium carotovorum* (Jones) Waldee, in Sardegna. In: *Atti del Primo Congresso dell'Unione Fitopatologica Mediterranea*. 26 settembre–1 ottobre 1966, Bari-Napoli, Italy, 160–167.

Pérombelon M.C.M. and L.J. Hyman, 1986. A rapid method for identifying and quantifying soft rot erwinias directly from plant material based on their temperature tolerances and sensitivity to erythromycin. *Journal of Applied Bacteriology* 60, 61–66.

Pérombelon M.C.M. and A. Kelman, 1980. Ecology of the soft rot erwinias. *Annual Review of Phytopathology* 18, 361–387.

Schaad N.W., 1988. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 2nd ed. APS Press, Saint Paul, MN, USA, 164 pp.

Schuerger A.C. and J.C. Batzer, 1993. Identification and host range of an *Erwinia* pathogen causing stem rots on hydroponically grown plants. *Plant Disease* 77, 472–477.

Seo S.T., N. Furuya, C.K. Lim, Y. Takanami and K. Tsuchiya, 2002. Phenotypic and genetic diversity of *Erwinia carotovora* ssp. *carotovora* strains from Asia. *Journal of Phytopathology* 150, 120–127.

Seo S.T., N. Furuya, C.K. Lim, Y. Takanami and K. Tsuchiya, 2003. Phenotypic and genetic characterization of *Erwinia carotovora* from mulberry (*Morus* spp.). *Plant Pathology* 52, 140–146.

Smith C. and J.A. Bartz, 1990. Variation in the pathogenicity and aggressiveness of strains of *Erwinia carotovora* subsp. *carotovora* isolated from different hosts. *Plant Disease* 74, 505–509.

Thornley M.J., 1960. The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. *Journal of Applied Bacteriology* 23, 37–52.

Wilson K., 1987. Preparation of genomic DNA from bacteria. In: *Current Protocols in Molecular Biology*. (F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl ed.), Greene Publishing Associates & Wiley Interscience, New York, NY, USA, 2.4.1–2.4.2.

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