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Occurrence of a soft rot of calla (*Zantedeschia aethiopica*) caused by *Pectobacterium carotovorum* subsp. *carotovorum* in central Italy

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Summary. *Pectobacterium carotovorum* subsp. *carotovorum* causing soft rot of calla (*Zantedeschia aethiopica*) plants is reported in central Italy. Bacterial isolates were identified by biochemical, physiological and pathogenicity tests and characterized by the API 50 CHE Kit and rep-PCR.

Key words: API 20 E, API 50 CHE, calla, rep-PCR.

Introduction

Pectobacterium carotovorum subsp. *carotovorum* (Jones) Hauben *et al*., *Pectobacterium carotovorum* subsp. *atrosepticum* (van Hall) Hauben *et al*., *Pectobacterium chrysanthemi* (Brenner *et al*.) Hauben *et al*., and fluorescent pectolytic Pseudomonads are the main agents of soft rot in plants, a disorder characterized by loss of host-tissue structural integrity which is mainly due to the production of bacterial pectolytic and other macerating enzymes (Perombelon, 1982). Although generally associated with vegetables, soft-rot agents are also naturally pathogenic to a wide range of ornamental plants and field crops.

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In November 2000, leaf wilting and yellowing of calla plants was observed in association with tuber soft rot in a greenhouse at Torricchio Uzzano (Pistoia, central Italy), with an incidence ranging from 40 to 60% (Fig. 1). The rot often began outside the base of leaf petioles below the soil line, then progressed up along the stems, causing leaf blighting, and down into the tubers. The rotted portion of the tubers had a consistency like cottage cheese and was separated from the healthy portion by a brown edge (Fig. 1B).

Calla soft rot was first reported in Italy (Lazio) by Petri (1934), and subsequently in Liguria by Mezzetti (1951), who identified *Bacterium aroideae* as the causal agent. This bacterium is objective synonymous with *Erwinia carotovora* subsp. *carotovora* (Bradbury, 1986), which has in turn been recently reclassified as *P. carotovorum* subsp. *carotovorum* (Hauben *et al*., 1998). The disease has also been recorded in USA (Pirone, 1978),

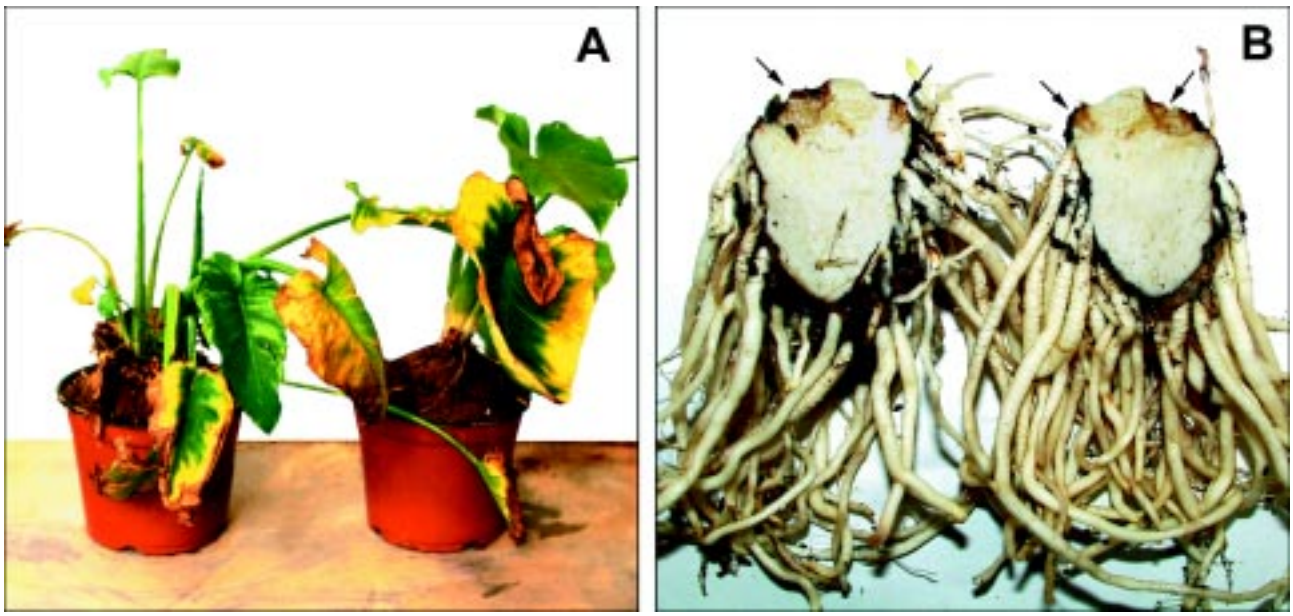

Fig. 1. Symptoms caused by *Pectobacterium carotovorum* subsp. *carotovorum* on calla (*Zantedeschia aethiopica*) plants. (A) Infected calla plants showing leaf wilting and yellowing. (B) Section of an infected calla tuber showing soft rot symptom (see arrows).

Japan (Horita, 1994), Brazil (1997), New Zealand (Wright, 1998) and Lithuania (Snieskiene, 1995). Other soft-rot bacteria including *Pectobacterium carotovorum* subsp. *atrosepticum* have also been associated with soft rot of calla (Wright and Burge, 2000).

We show here that *P. carotovorum* subsp. *carotovorum* is the causal agent of soft rot observed on calla plants in central Italy. Biochemical and molecular characterization of some isolates of the bacterium is also reported.

Isolation and characterization of the pathogen

To isolate the causal agent, small portions of infected calla tubers close to the margin of the rot were macerated in a few drops of sterile water and the suspension streaked onto nutrient agar (NA). After incubation at 27°C for 24 h, white, circular (less than 1 mm in diameter), slightly raised bacterial colonies with wavy margins were consistently obtained. These colonies formed pits on crystal violet-pectate medium (Cupples and Kelman, 1974) and were not fluorescent on King's medium B (King *et al*., 1954). Single bacterial colonies were selected, streaked in purity on NA plus 5% sucrose, and stored in a 15% glycerol solution at –80°C. Three bacterial isolates were used for identification tests.

The pathogenicity test was carried out on approx. 50-cm-high calla plants. To prepare the inoculum for this test, bacterial isolates were grown on NA at 27°C for 24 h, suspended in sterile deionised water and spectrophotometrically adjusted to 10^6 cfu ml^{-1}. The upper part of the calla tuber, at the base of the leaves, was infiltrated with the bacterial suspensions using a syringe. Plants infiltrated with water served as controls. Plants were kept in a greenhouse at 18–24°C, RH 60–80%, under natural light conditions. All bacterial isolates caused soft rot in the tubers at the inoculation sites about one week after inoculation. Subsequently, poor shoot growth and yellowing of the leaves were observed. The shoots were easily detached from the tubers and new shoots often started to grow from the bottom part of the tuber. No symptoms were observed in the control plants. The pathogen was reisolated from tubers of the inoculated plants and their cultural characteristics were identical to the bacterial isolates used for inoculation.

To identify the causal agent, bacterial isolates, one re-isolate, and reference strain LMG 2408 of *Pectobacterium carotovorum* subsp. *carotovorum* isolated from soft-rotted calla in Denmark were

tested for Gram reactions, presence of catalase and oxidase, growth at 37°C, oxidation/fermentation, production of reducing sugars from sucrose (Schaad *et al.*, 2001) and for the API 20 E system (bioMérieux, Marcy l'Etoile, France), which was performed according to the manufacturer's instructions. To characterise one calla isolate for comparison with the reference strain, the assimilation of 50 substrates by API 50 CHE Kit (bioMérieux) was carried out according to manufacturer's instructions. Further characterisation was performed by rep-PCR on the isolates, the re-isolate and strains LMG 2408 and NCPPB 2577 of *Pectobacterium carotovorum* subsp. *carotovorum* according to the procedures described by Rademaker and De Bruijn (1997), using BOX, ERIC and REP as primers. For template preparation, bacteria were cultured for 24 h at 27°C on NA and bacterial cells were treated with sterile NaOH 0.5 M at 95°C for 15 min.

The isolates, the re-isolate and the reference strain were Gram negative, oxidase negative and catalase positive, grew at 37°C, had both oxidative and fermentative metabolism and did not produce reducing sugars from sucrose. In the API 20 E system all bacteria tested were positive for: nitrate reduction, β-galactosidase, citrate utilization, acetoin production, gelatinase, assimilation of glucose, mannitol, rhamnose, sucrose, melibiose, amygdalin and arabinose and negative for: oxidase, arginine dihydrolase, lysine and ornithine decarboxylase, H_2S production, urease, tryptophane deaminase, indole production, and assimilation of sorbitol. The only difference between the isolates or the reisolate from callas and the reference strain was that the reference strain did not assimilate inositol. When the API 20 E results were expressed as a 7-digit code, the code no. 1207373 was obtained for bacteria (isolates and reisolate) from callas, and 1207173 for the reference strain. This last code is identical to that reported by Mergaert *et al*. (1984) for the same strain. In the API 50 CHE system the calla isolate and the LMG 2408 strain of *P. carotovorum* subsp. *carotovorum* assimilated the following 25 carbon sources: glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, rhamnose, inositol, mannitol, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, lactose, melibiose, sucrose, trehalose, D-raffinose, β-gentiobiose, gluconate and 5-keto-gluconate. Unlike the reference strain the calla isolate assimilated maltose, starch and glycogen.

Rep-PCR analysis, whose electrophoretic gel is shown in Fig. 2, showed that: i) bacteria from callas gave identical fingerprinting profiles, independent of the primer used; ii) the reference-strain profiles were different from each other and from those of the isolates or re-isolate from calla. A neighbour-joining dendrogram built using the fingerprinting obtained with the three primers and the Dice index values confirmed that the isolates and the re-isolate from callas were identical, and that there were distances of about 10% and 30% between them and the reference *Pectobacterium carotovorum* subsp. *carotovorum* strains LMG 2408 and NCPPB 2577 respectively.

Discussion

It is concluded on the basis of cultural, biochemical, physiological and nutritional characteristics and the pathogenicity tests that all bacterial isolates obtained from calla plants belonged to *Pectobacterium carotovorum* subsp. *carotovorum* and that this bacterium caused the soft rot of calla in Tuscany. *Pectobacterium carotovorum* subsp. *atrosepticum* did not cause the calla soft-rot in the present study because the isolates grew at 37°C and did not produce reducing sugars from sucrose (Schaad *et al.*, 2001). Despite the low number of strains considered, it seems that rep-PCR is suitable for bacterial characterisation but not for *P. carotovorum* subsp. *carotovorum* identification due to the variability observed. Molecular characterisation of a great number of bacterial strains using rep-PCR detected high variability within *Xanthomonas campestris* pv. *campestris* (Santangeli, 2001) and very low variability within *Xanthomonas axonopodis* pv. *vesicatoria* (unpublished data) and *Xanthomonas axonopodis* pv. *phaseoli* (Vizzarri, 2001). These rep-PCR findings suggest that the high fingerprint profile variability is at least in part due to the poliphagy of the bacterial species, as is the case with *P. carotovorum* subsp. *carotovorum* and *Xanthomonas campestris* pv. *campestris*, both of which attack several hosts.

P. carotovorum subsp. *carotovorum* infects calla at all stages of its growth cycle, from tuber planting to postharvest storage. Although any temperature higher than 10°C is sufficient for infection to occur, temperatures >27°C are optimal for soft-rot

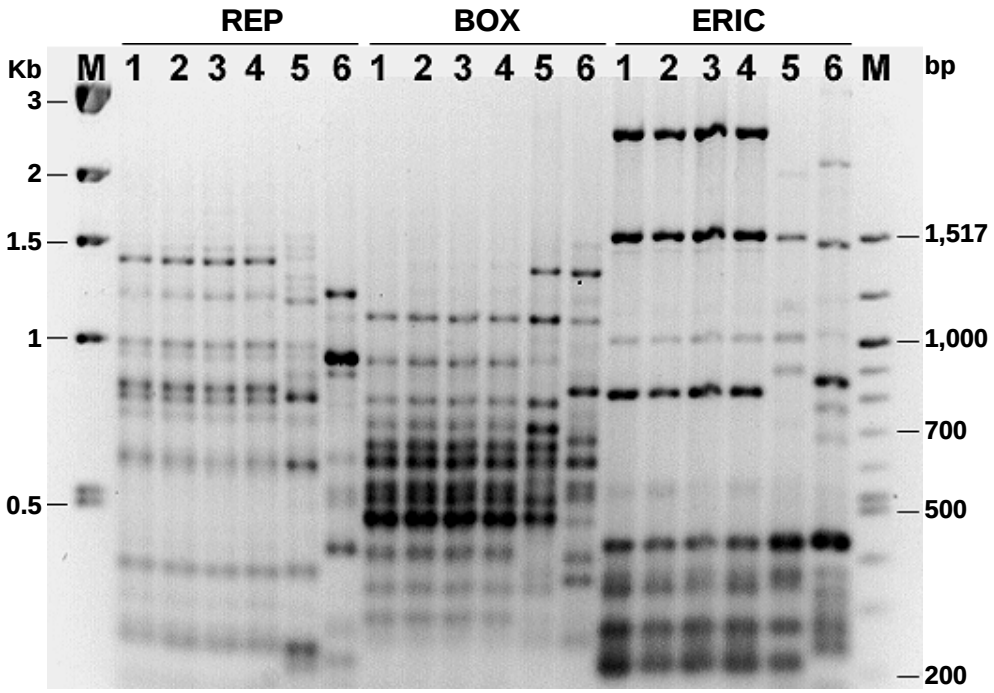

Fig. 2. Agarose gel electrophoresis of polymerase chain reaction fingerprint patterns obtained from 3 bacterial isolates (lanes 1, 2 and 3) and 1 re-isolate (lane 4) from callas and from the strains LMG 2408 (lane 5) and NCPPB 2577 (lane 6) of *Pectobacterium carotovorum* subsp. *carotovorum*. The 35-40 bp repetitive extragenic palindromic (REP) sequence, the 154 bp BOX element and the 124-127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence were used as primers in rep-PCR. M_1, 1 Kb ladder; M_2, 100 bp ladder.

development (Wright and Burge, 2000). Control of bacterial soft rot in calla is generally preventive, including measures such as the discarding of diseased tubers before planting, growing callas in well-drained soil at moderate temperatures, careful management of irrigation, crop rotation, avoiding heavy nitrogen fertilisation and stocking tubers in well-aired rooms (Wright and Burge, 2000). Investigations are in progress to determine the effectiveness of acibenzolar-S-methyl in protecting callas from the soft rot observed in a preliminary greenhouse trial.

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