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**Short Communication**

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# Identification of *Pseudomonas syringae* pv. *actinidiae* as Causal Agent of Bacterial Canker of Yellow Kiwifruit (*Actinidia chinensis* Planchon) in Central Italy

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## Abstract

Angular, necrotic leaf spot, longitudinal cracks along the petiole, oozing and wilting of branches were observed during summer 2008 on *Actinidia chinensis* (yellow kiwifruit) cultivar Hort16A, cultivated in different orchards located the province of Latina (central Italy). Symptoms closely resembled those incited by *Pseudomonas syringae* pv. *actinidiae* on kiwifruit *A. deliciosa*. Isolates obtained from typical lesions were assessed by means of biochemical, pathogenicity and host range tests and compared with some *Pseudomonas syringae* pathovars by enterobacterial repetitive intergenic consensus (ERIC-PCR) analysis. The isolates belong to *Pseudomonas* LOPAT group Ia, incited the death of pot-cultivated *A. chinensis* cv. Hort 16A and *A. deliciosa* cv. Hayward plants in few days, but did not cause any symptoms to the other inoculated plant species. Upon ERIC-PCR analysis, all the isolates showed similarity with *P. syringae* pv. *actinidiae* NCPPB 3739, type-strain of the pathovar. This is the first report of this pathogen on *A. chinensis* in Italy and, as far as we currently know, in the world.

## Introduction

In Italy, kiwifruit (*Actinidia deliciosa* Liang *et* Ferguson) is cultivated on approximately 22 000 hectares and the main areas of cultivation are located in Latium, Piedmont, Emilia-Romagna, Veneto and Campania. In recent years, the golden or yellow kiwifruit (*Actinidia chinensis* Planchon) has also been introduced into limited areas of Italy, especially in Latium and Emilia-Romagna regions. *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit, was reported on *A. deliciosa* in the Latium region for the first time in 1994 (Scortichini, 1994). After this record, the bacterium was isolated again in other few occasions in the same area (Scortichini, unpublished data). This phytopathogen causes important economic losses on kiwifruit also in Japan (Takikawa et al., 1989), South Korea (Koh et al., 1994) and Iran (Mazarei and Mostofipour, 1994).

During spring 2008, symptoms resembling those incited by *P. s.* pv. *actinidiae* (i.e. angular leaf spots, elongated cracks along the petiole, whitish oozing from the trunk, wilting of branches) were observed in some *A. chinensis* cultivar Hort 16A orchards cultivated in Latium. The disease caused death of branches, on up to 3–5% of the plants present in the orchard. These observations prompted us in performing isolation and identification of the pathogenic bacterium isolated from the plant lesions.

This study reports on for the first time, the identification of *P. s.* pv. *actinidiae* as the causal agent of bacterial canker of yellow kiwifruit in Italy, and as far as we currently know, in the world.

## Materials and Methods

### Isolation

Fragments of infected tissues were aseptically removed from lesion margins of leaves, petiole and trunk and ground into sterile mortars containing 5 ml of sterile saline solution, (SS) (NaCl 0.85% in sterile distilled water). Subsequently, 0.1 ml aliquots of serial 10-fold dilutions were plated on nutrient agar containing 5% of sucrose (NSA) and incubated at 25–27°C for 3 days.

### Biochemical and pathogenicity tests

With colonies grown on NSA plates, pure cultures were obtained and with representative isolates, the following biochemical tests were performed according to the procedures described by Lelliott and Stead (1987): levan production, presence of oxidase, soft rot activity on potato slices, presence of arginine dehydrolase, hypersensitivity reaction in tobacco leaves (LOPAT

tests), metabolism of glucose, hydrolysis of aesculin, gelatine hydrolysis and fluorescence on the medium B of King et al. (1954) (KB). In addition, the utilization of sorbitol, inositol, erythritol, adonitol, glycerol, mannitol, L-alanine, L-tyrosine, arabinose, D-xylose and D-fructose was assessed.

Pathogenicity and host range tests were performed using the 10 isolates obtained from yellow kiwi fruit Hort16A and belonging to LOPAT tests group Ia and *P. s.* pv. *actinidiae* NCPPB 3739, pathotype strain, isolated from *A. deliciosa* in Japan. For inoculation, colonies grown on NSA for 24 h, at 25–27°C were used. Bacterial suspensions corresponding to $1–2 \times 10^6$ cfu $ml^{-1}$ were prepared in SS. Pot-cultivated *A. chinensis* Hort 16A, *A. deliciosa* cv. Hayward, hazelnut (*Corylus avellana* L.), walnut (*Juglans regia* L.), pear (*Pyrus communis* L.), apple (*Malus pumila* Mill.), peach (*Prunus persica* Batsch.), tomato (*Lycoperscoin esculentum* Miller) and lilac (*Syringa vulgaris* L.) were inoculated at the end of spring. Inoculations were performed on young shoots gently wounded with a sterile scalpel. Immediately after inoculation, two drops of the suspensions were placed on the wound. In addition, the leaf blade was also inoculated by gently pricking the tissue with a sterile syringe containing the bacterial suspension. Control plants were wounded in the same way and treated with sterile SS. For each isolate, three shoots and three leaves were inoculated. Re-isolations were performed after symptoms appeared using techniques previously described.

#### DNA extraction and ERIC-PCR analysis

The isolates obtained from yellow kiwi fruit were compared by means of enterobacterial repetitive intergenic consensus (ERIC-PCR) analysis with *P. s.* pv. *actinidiae* NCPPB 3739 (KW11), type-strain, *P. avellanae* BPIC 631 and ISF NOC111, *P. s.* pv. *coryli* NCPPB 4273, *P. s.* pv. *syringae* ISF 1A, *P. s.* pv. *tomato* ISF 615 and *P. s.* pv. *phaseolicola* 1448a. For each isolate and strain, a loop full (diameter, approximately 2 mm) of a single colony that had been grown for 24 h on NSA at 25 to 27°C, was suspended in SS and centrifuged at $12\,000 \times \boldsymbol{g}$ for 2 min. Then, the supernatant was discarded and the pellet was suspended in 100 μl of NaOH 0.05M to an optical density corresponding to $1–2 \times 10^8$ cfu $ml^{-1}$. The suspension was placed in water at 95°C for 15 min and then centrifuged at $12\,000 \times \boldsymbol{g}$, for 2 min. Subsequently, the supernatant was stored at −20°C for ERIC-PCR analysis. The ERIC primer sets were synthesized by Primm (Milano, Italy). The repetitive-sequence PCR (ERIC-PCR) method used was that of Louws et al. (1994). The PCR amplifications were performed in duplicate. PCR products were separated by gel electrophoresis on 1.5% agarose (Seakem, Rockland, ME, USA) in 0.5× TAE buffer at 5 V/cm over 5 h, stained with ethidium bromide, visualized under a UV transilluminator Spectroline (Spectronic Corporation, Westburg, NY, USA) and photographed with a Kodak Gel Logic 100 Imaging System apparatus (Eastman Kodak Company, Rochester, NY, USA).

## Results and Discussion

Symptoms of a hitherto new disease were observed on *A. chinensis* cv. Hort 16A cultivated in the province of Latina (Latium region), central Italy. Angular necrotic spots were observed on the leaves that in some cases coalesced resulting in further necrosis and wilting of the leaf. Elongated cracks along the leaf petiole were frequently observed. In addition, in some cases, oozing was noticed from the main trunk and branches. A discolouration of the bark tissue was also observed. From infected tissues, bacterial colonies were isolated on NSA. From these isolations, ten representative colonies were chosen on the basis of the colour and morphology (i.e. round edge, white-creamy colour, levan-positive) that were used in biochemical tests. All ten colonies showed characteristics of *Pseudomonas* LOPAT group Ia: levan-positive, oxidase-negative, potato soft rot-negative and arginine dehydrolase-negative, tobacco hypersensitivity-positive. These colonies were selected for further biochemical tests, a pathogenicity test, as well as for ERIC-PCR analysis. The results of biochemical and nutritional tests are reported in Table 1.

All ten isolates and *P. s.* pv. *actinidiae* NCPPB 3739 were pathogenic to both *A. chinensis* and *A. deliciosa* plants. All of them caused complete wilting of the plant inoculated into the shoots within 10 days after inoculation. Isolate ISF ACT3, which showed some differences in biochemical tests (Table 1), but not in the ERIC-PCR banding pattern reacted very aggressive, killing the plant after 4–5 days after the inoculation. Leaves showed extensive necrosis 1 week after the inoculation. Control plants and those of the other

Table 1
Results of biochemical and nutritional tests showed by the isolates obtained from *Actinidia chinensis* and by *Pseudomonas syringae* pv. *actinidiae* NCPPB 3739 isolated from *A. deliciosa*

| | Isolated from *A. chinensis* | *P. syringae* pv. *actinidiae* NCPPB 3739 |
|---|---|---|
| Levan | + | + |
| Oxidase | − | − |
| Potato soft-rot | − | − |
| Arginine dehydrolase | − | − |
| Tobacco hypersensitivity | + | + |
| Fluorescence on KB | − | − |
| Glucose metabolism | O | O |
| Aesculin hydrolysis | − | − |
| Gelatin hydrolysis | − | −[a] |
| Utilization of: | | |
| Adonitol | − | −[a] |
| L-alanine | + | + |
| Arabinose | − | − |
| Erythritol | − | −[a] |
| D-fructose | + | + |
| Glycerol | + | + |
| Inositol | + | + |
| Mannitol | + | + |
| Sorbitol | + | + |
| L-tyrosine | + | + |
| D-xylose | − | − |

O, Oxidative metabolism.
[a]The isolate ISF ACT3 showed a positive response.

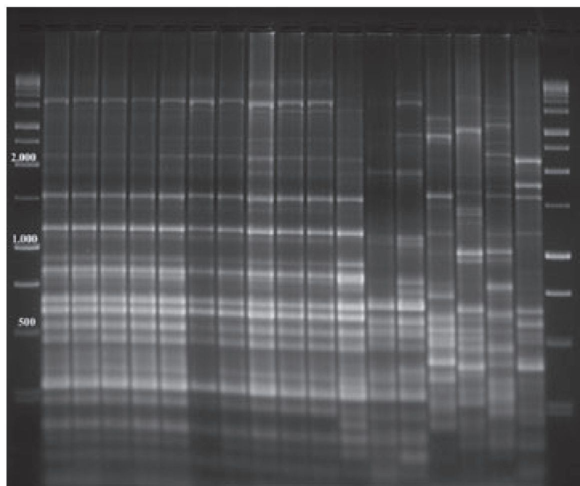


Fig. 1 Repetitive ERIC-PCR fingerprinting patterns from genomic DNA of bacterial isolates obtained from *Actinidia chinensis* (lanes 1–10) and, from left to right, *Pseudomonas syringae* pv. *actinidiae* NCPPB 3739 (KW11), *P. avellanae* BPIC 631, *P. avellanae* ISF NOC111, *P. syringae* pv. *coryli* NCPPB 4273, *P. s.* pv. *syringae* ISF 1A, *P. s.* pv. *tomato* ISF 615, *P. s.* pv. *phaseolicola* 1448a. M, molecular size marker (1 kb DNA ladder, Promega, Madison, WI, USA); the size of bands are indicated in base pairs. Note the relevant similarity between 10 isolates obtained from yellow kiwifruit with *P. s.* pv. *actinidiae* NCPPB 3739, type-strain of the pathovar (lanes 1–11)

plant species did not show any symptoms upon inoculation. Re-isolations yielded typical colonies on NSA that were confirmed by LOPAT tests and ERIC-PCR analysis, fulfilling Koch's postulates.

A representative gel of ERIC-PCR analysis is shown in Fig. 1. Patterns of all ten isolates pathogenic to *A. chinensis* Hort 16A were remarkably similar to that of *P. s.* pv. *actinidiae* NCPPB 3739 (KW 11), the type-strain of the pathovar. A certain degree of similarity was also noticed with *P. avellanae*, another bacterium that, like *P. s.* pv. *actinidiae*, belongs to genomospecies 8 *sensu* Gardan et al. (1999) (Scortichini et al., 2002). All of the other *P. syringae* pathovars showed a distinct and diverse DNA fingerprinting.

On the basis of biochemical, pathogenicity and host range tests as well as for the remarkable similarity with the *P. s.* pv. *actinidiae* type-strain upon ERIC-PCR analysis, the isolates obtained from *A. chinensis* cv. Hort 16A grown in central Italy are identified as *P. s.* pv. *actinidiae*. This is the first report of this pathogen on *A. chinensis* in Italy and, as far as we currently know, in the world. In the same geographic area, *P. s.* pv. *actinidiae* was isolated from *A. deliciosa* cv. Hayward in 1994. After the record, the pathogen was sporadically noticed and never caused significant losses. The outbreak here reported on *A. chinensis* appears to be more dangerous as it was observed in different orchards where it caused the wilting of branches. Interestingly, one isolate that proved to be more aggressive upon artificial inoculation showed some differences in biochemical tests, too. Variability in the biochemical features among strains of this pathovar was already recorded (Takikawa et al., 1989; Scortichini et al., 2002; Lee et al., 2005). This study shows that pathovar *actinidiae* of *P. syringae* is capable to incite disease symptoms not only on *A. deliciosa* but also to another species, namely *A. chinensis*.

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