Diversity of *Pseudomonas syringae* isolates from sweet and wild cherry

JOANA G. VICENTE and STEVEN J. ROBERTS
Department of Plant Pathology and Microbiology, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Summary. Representative isolates of *Pseudomonas syringae* from sweet and wild cherry (*Prunus avium* L.) were characterised by classic physiological and biochemical tests, serology and pathogenicity tests. The isolates from cherry were confirmed as being *P. syringae* pv. *syringae* (*Pss*), *P. syringae* pv. *morsprunorum* (*Psm*) race 1 and 2, and intermediate forms. Most isolates showed a positive reaction in indirect-ELISA and slide agglutination tests with at least one of two antisera produced against wild cherry isolates. RAPD and rep-PCR-based fingerprinting of nine *Psm* and *Pss* isolates from cherry with a number of primers showed clear differences between isolates. Pathogenicity tests in micropropagated plants of lilac and two clones of wild cherry differentiated *Pss* and *Psm* isolates.

Keywords: *Prunus avium*, bacterial canker, leaf spot disease, molecular fingerprinting.

Introduction

Bacterial canker of cherry (*Prunus avium* L.) caused by *Pseudomonas syringae* is a major limitation in the use of cherry for timber production in farm woodlands and it is a major disease associated with sweet cherry production causing losses in fruit yield and tree death. Two pathovars of *Pseudomonas syringae* can cause this disease: pv. *syringae* (*Pss*) and pv. *morsprunorum* (*Psm*) (Garrett *et al*., 1966; Roos and Hattingh, 1987). Copper compounds are commonly used to minimise the spread of canker in sweet cherry orchards, but these compounds have limited efficacy and may also have phytotoxic effects. It is not practical or economic to spray farm woodland tree plantations. Therefore, the only two practical approaches to control bacterial canker in farm woodland cherries are disease avoidance and disease resistance. Both of these approaches have been limited by lack of understanding of the pathogen and lack of reliable and consistent methods. The objectives of this work are to improve understanding of the pathogen, its taxonomy and variation, and to develop improved methods for detection and discrimination.

Materials and methods

Bacterial isolates

Thirty-five isolates of *Pseudomonas syringae* pv. *syringae* (*Pss*) and *P. syringae* pv. *morsprunorum* (*Psm*) from wild and sweet cherry mainly from the UK, together with 11 representative isolates of *P. syringae* from other hosts including lilac (*Syringa vulgaris*), pear (*Pyrus communis*), plum (*Prunus domestica*), cherry laurel (*P. laurocerasus*), peach (*P. persica*) and myrobalan (*P. cerasifera*) were used in this study. All isolates were preserved at –76°C in the HRI culture collection and were recovered from the freezer and grown on King's medium B (KB) at 25°C.

Physiological and biochemical tests

Isolates were tested for oxidase activity, fluorescence under UV light on KB medium, production of brown pigment on KB, levan production on nutrient sucrose agar (NSA) and colour of growth in nutrient broth (NB). The GATTa tests (Latorre and Jones, 1979) were also performed: (G) gelatin liquefaction in tubes containing 12% (w/v) gelatin; (A) ß-glucosidase activity on 0.1% (w/v) aesculin agar medium; (T) tyrosinase activity on agar medium containing 0.1% (w/v) L-tyrosine; (Ta) utilisation of tartrate as a sole carbon source tested on basal medium with 0.2% (w/v) sodium tartrate.

Serological tests

Isolates were tested in indirect-ELISA with 11 polyclonal antisera produced previously against different *P. syringae* isolates from non-cherry hosts and two new antisera raised against *Psm* (00/8/3) and *Pss* (00/9/3) isolates from wild cherry. Isolates were also tested using the *Staphylococcus aureus* slide agglutination technique (Lyons and Taylor, 1990) with selected antisera.

RAPD and rep-PCR

DNA of nine representative isolates of *Psm* and *Pss* from cherry were used in random amplification of polymorphic DNA (RAPD) with sixty primers (Operon kits A, G, and H). Isolates were also tested in polymerase chain reaction (PCR) with three sets of primers (REP, ERIC and BOX) based on repetitive extragenic palindromic sequences (Louws *et al*., 1994).

Pathogenicity tests

Micropropagated plants of two wild cherry clones (Charger and 1912) and lilac cv. Sensation were inoculated by dipping the plants in a bacterial suspension (10^8 to 10^9 cfu/ml). For each clone, three plants in a pot were inoculated per isolate. The plants were maintained at 24.6±2°C with a 16 h photoperiod.

Results

Physiological and biochemical tests

The results of the classical tests are presented in Table 1. The isolates from cherry were divided into four main groups.

Table 1. Results of physiological and biochemical tests for 46 isolates.

Number of isolates	Hosts of origin	Fluorescence on KB	Colour of growth in NB	GATTa[a]	Classification
14	lilac, cherry laurel, pear, sweet and wild cherry	blue	yellow	++--	*Pss*
6	wild cherry	variable	variable	++--	*Ps*-intermediate
1	sweet cherry	blue	yellow	-+--	*Ps* type?
16	plum, sweet and wild cherry	variable	white or intermediate	--++	*Psm* Race 1
7	sweet and wild cherry	negative	white	+--- or +-(+)-	*Psm* Race 2
1	myrobalan	negative	white	+-+-	*Psm* Race 2?
1	peach	negative	yellow	----	*Ps* pv. *persicae*

[a] Results of four tests: G, gelatin liquefaction; A, aesculin hydrolysis; T, tyrosinase activity; Ta, tartrate utilisation.

Serological tests

The results indicated that none of the existing antisera raised against isolates from non-cherry hosts was sufficiently specific for the wild cherry isolates. Most cherry isolates reacted well with at least one of the new antisera raised against isolates from wild cherry. In general, one antiserum (00/8/3) is more specific for *Psm* isolates, while the other (00/9/3) reacts better with *Pss* isolates. Several isolates from cherry reacted well in agglutination tests with both antisera.

RAPD and rep-PCR

RAPDs with a large number of primers allowed the separation of *Psm* race 1, *Psm* race 2 and *Pss* isolates. Fig. 1 presents an example of the results obtained. rep-PCR also clearly separated the isolates.

Pathogenicity tests

Ten isolates from the *Pss* group were strongly pathogenic on lilac, Charger and 1912, killing the plants in less than 4 weeks. One *Pss* isolate from wild cherry was not aggressive on clone 1912; one isolate was weak on lilac and two isolates were weak or not pathogenic. Three *Ps*-intermediate isolates from wild cherry were strongly pathogenic on lilac, Charger and 1912; three other isolates were not pathogenic. The *Psm* isolates were generally not pathogenic on lilac and were weaker on the wild cherry clones than the *Pss* isolates. Many of these isolates caused leaf spots in the wild cherry clones. The *Ps*-type isolate from wild cherry and the isolates from *P. cerasifera* and *P. persica* were not pathogenic in the clones tested.

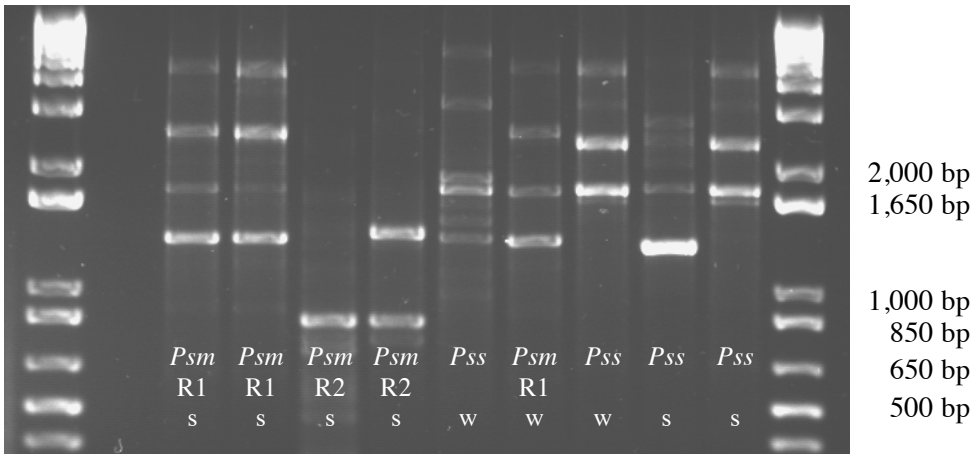

Fig. 1. RAPD banding patterns of isolates from sweet (s) and wild cherry (w) generated with primer OPG-08. First and last lanes: 1Kb Plus DNA ladder. Second lane: blank control.

Discussion

The *P. syringae* isolates from cherry were differentiated into two pathovars (*syringae* and *morsprunorum*) and intermediate forms using classic physiological and biochemical tests. These tests have the disadvantage of being time consuming. Serological tests with two antisera produced against cherry isolates could be used in addition or as an alternative to the classical tests to increase the speed of detection and discrimination of isolates obtained from plant material. Isolates of the different types could also be distinguished using RAPD and rep-PCR. Selected primers will be used to perform molecular fingerprinting of a larger number of strains obtained from plantations and nurseries. The consistent results obtained with inoculation of micropropagated plants showed a range of pathogenic variation amongst isolates from cherry and confirmed the distinction between *Psm* and *Pss* which produce different symptoms on cherry. Based on pathogenicity, three *Ps*-intermediate strains should be assigned to *Pss*. The results will allow a rational selection of isolates for use in future resistance screenings.

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