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PSEUDOMONAS SPP. ISOLATED FROM STONE FRUIT TREES IN POLAND

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Abstract

Samples of symptomatic tissue originating from 4 species of stone fruits were collected in 2007 from orchards located in various regions of Poland. Nearly 130 isolates of fluorescent *Pseudomonas* were obtained. Biochemical and physiological tests used for identification of isolates showed that 110 of them belonged to *P. syringae*. Five isolates did not induce HR on tobacco and 10, although showed this ability, appeared to be not *P. syringae*. Using the GATT'a tests it was found that 31 isolates were classified to race 1 of *P. syringae* pv. *morsprunorum* (*Psm* race 1) and 37 isolates to race 2 of this pathovar (*Psm* race 2). Remaining 42 isolates were identified as pathovar *syringae* (*Pss*).

Keywords: *Pseudomonas syringae*, phenotypic identification, races, copper resistance.

Introduction

Pseudomonas syringae is polyphagous bacterium causing diseases of over 180 plant species /Bradbury, 1986; Agrios, 1997/. Depending on pathogenic abilities this species consists of over 50 pathovars /Bradbury, 1986; Young et al., 1996/. On stone fruit trees mainly two pathovars cause bacterial canker and gummosis: *P. s.* pv. *syringae* (*Pss*) and *P. s.* pv. *morsprunorum* (*Psm*). They belong to genomospecies 1 and 2, respectively /Young et al., 1991; Gardan et al., 1999/. Because of different way of tree infection by both bacteria they have different epidemiological importance /Cameron 1962; Crosse 1966/. The disease occurs in all regions of stone fruit production of the world /Agrios, 1997/. It attacks branches and main trunk of the trees as well as buds, flowers, leaves and fruits. Bacterial canker reduces yield and can cause death of the trees. On pome fruits, especially on pear trees, *P. s.* pv. *syringae* cause in some years considerable damages due to synergism with low temperatures during blooming period /Whitesides, Spotts, 1991/.

The studies on gummosis and cankers occurring on stone fruits in Poland, performed for the first time at the beginning of 20th, revealed their bacterial origin /Brzeziński, 1902/. Next, Łyskanowska (1976, 1979) and Burkowicz (1981) proved that the main causal agent of this disease on sweet cherry is *Pseudomonas morsprunorum* (*Psm*) while Sobiczewski (1984) reported that bacterial canker on sour cherries is caused mainly by *Pseudomonas syringae* (*Pss*). The diagnostic of bacterial canker is commonly based on phenotypic characterization of its causal agent /Bultreys, Gheysen, 1999; Schaad et al., 2001, Vincente et al., 2004, Vincente, Roberts, 2004/.

The aim of the present study was identification of bacteria isolated from the bacterial canker or similar symptoms on different organs of stone fruit trees in Poland using conventional methods.

Materials and Methods

Small fragments at the margin between healthy and diseased tissue (leaves, fruitlets, blossoms, shoots) originating from stone fruit trees including sweet and sour cherries, plums and peaches from 5 geographical regions: Central, North, South, North-West, and South-East part of Poland were macerated in sterile distilled water. After 20 min., the suspensions were plated onto King B medium /King et al., 1954/. Characteristic colonies which produced fluorescent pigment visible under UV light were selected for further study.

All fluorescent isolates were characterized using LOPAT tests according to Lelliot and Stead (1987). The following properties of bacteria were determined: levan production from sucrose (L); presence of oxidase (O), ability to cause rot on potato tubers (P); presence of arginine dihydrolase (A) and ability of inducing hypersensitivity reaction (HR) on tobacco leaves (T) according to the method of Klement (1963). Bacteria identified as *P. syringae* were next discriminated into pathovars using GATTa tests: gelatin hydrolysis (G), aesculin hydrolysis (A), tyrosinase activity (T) and utilization of tartate (Ta) tests /Lelliot et al., 1966; Lattore, Jones, 1979; Lelliot, Stead, 1987/. *Pss* 2905 from own collection was used as a reference strain.

All *Pseudomonas* isolates were screened for sensitivity to copper sulphate $CuSO_4$ x $5H_2O$ using Casitone Yeast Extract (CYE) /Loper et al., 1991/ and the Mannitol-Glutamate (MG) media /Keane et al., 1970/. 10µl of water suspension of each isolate was spotted on these media containing the following final concentrations of copper: 0, 0.04, 0.08, 0.16, 0.32, 0.72, 0.96, 1.44, 1.6, 2.0 mM. The plates were kept at 28ºC for 72 h. Isolates which were able to grow on media containing 0.36 mM or more $CuSO_4$ x $5H_2O$ were scored as copper resistant.

Results and Discussion

Of 280 samples of symptomatic tissue including 62 samples originated from sour cherry, 80 from sweet cherry, 94 from plums and 44 from peach, almost 130 isolates of fluorescent *Pseudomonas* were obtained. Using LOPAT tests 110 isolates were identified as *Pseudomonas syringae* – group Ia /Lelliot et al., 1966; Lelliot, Stead, 1987/. Five isolates did not induce HR on tobacco leaves and 10, which were HR positive appeared to be not *P. syringae*. Based on the results of GATTa tests of all studied *P. syringae* isolates 31 belonged to race 1 of *P. syringae* pv. *morsprunorum* (*Psm* race 1) (– – + +) and 37 to race 2 of this pathovar (*Psm* race 2) (+ – – –). Remaining 42 isolates were classified as pathovar *syringae* (*Pss*) (+ + – –). Six isolates which induced HR on tobacco leaves were characterized as *P. viridiflava* and one as *P. s.* subsp. *savanastoi*. Remaining 3 isolates which were HR positive as well as other bacteria which gave negative response on tobacco leaves were not characterized.

The comparison of obtained results with isolates origin showed that 18 isolates including 2 *Pss*, 14 *Psm* race 1 and 1 isolate of *Psm* race 2 were obtained from sweet cherry, 41 isolates including 14 of *Pss*, and 33 of *Psm* race 2 came from sour cherry, 40

isolates containing 21 of *Pss*, 16 of *Psm* race 1 and 3 *Psm* race 2 originated from plum and 5 isolates from peaches were classified to *Pss* (4) and *Psm* race 1 (1). Bacteria characterized as *P. viridiflava* were isolated from plum (4), sour cherry (1) and peach (1) from Central North and North West part of Poland but those identified as *P. s.* subsp. *savanastoi* originated from plum in Central Poland. In total 47 isolates were obtained from sour cherry, 18 from sweet cherry, 40 from plums and 5 from peach. It was found that *Pss* isolates dominated on sour cherry and plum, *Psm* race 1 on sweet cherry and plum and *Psm* race 2 on sour cherry. Similar results were also obtained by Burkowicz and Rudolph (1994) who found that *Pss* occurred most frequently on sour cherries. Moreover, Bultreys and Gheysen (2004) determined that *Psm* isolates in Belgium originated mainly from sweet cherry trees while isolates of *Pss* were found on sour cherry and plum and only sometimes on sweet cherry. Also in one of the first studies on etiology of bacterial canker on sour cherry Sobiczewski (1984) proved that almost all of over 600 isolates obtained in Poland were identified as pathovar *syringae.* This finding also corresponds to our researches.

The copper resistance tests showed that more isolates grew on MG than on CYE medium containing the same concentrations of $CuSO_4$ x $5H_2O$. Out of all *Pseudomonas syringae* isolates 46 appeared to be resistant to copper and grew on CYE medium containing 0.36 mM or more of copper, 39 were sensitive to these concentrations and 25 isolates showed a weak growth but at a little higher concentrations their growth was not observed at all. On MG medium bacteria showed higher resistance to the same concentrations of $CuSO_4$ x $5H_2O$ as compared to CYE medium: 68 isolates were resistant to copper and grew on MG medium containing copper sulphate at concentrations of 0.36 mM or higher, 4 were sensitive and 38 showed a weak growth on that medium at 0.36 mM or higher concentrations of $CuSO_4$ x $5H_2O$. The most resistant to copper on CYE medium appeared to be *Psm* race 2 isolates but the sensitive *Psm* race 1 as well as *Pss* isolates. Similarly on MG medium isolates belonging to *Psm* race 2 were also highly resistant. However, many of *Pss* isolates grew well on that medium containing 0.36 mM of $CuSO_4$ x $5H_2O$.

In the case of one isolate identified as *Psm* race 2 the production of brown pigment on CYE medium at concentrations of 0.16, 0.32 and 0.72 mM of copper as well as on MG at 0.72 mM or little higher, was observed. The intensity of that brown colour became weak at high concentrations of copper sulphate in media and gradually disappeared. The most resistant were isolates originated from Central part of Poland and the most sensitive from North-West Poland. Higher level of resistance showed isolates from sour cherry but the lower – from sweet cherry and plum.

Bacteria identified as *Pseudomonas viridiflava* grew on CYE medium very weakly or did not grow at all, but on MG medium they grew quite well or weak. Isolates which did not grow on CYE media grew weakly on MG medium, respectively. A few isolates were unable to grow at 0.16 mM of $CuSO_4$ x $5H_2O$. *P. s.* subsp. *savanastoi* grew very well on both media even at high concentrations of copper. None isolate grew on CYE and MG media at concentrations of 1.44 and 2.0 mM, respectively. The resistance of *Pseudomonas* isolates was various on both media used in our studies. On MG medium the bacteria were more tolerant to copper than on CYE which is commonly used in other studies for testing copper resistance of bacteria, e. g. *Erwinia amylovora* /Loper,

Henkels, 1991/ and *Pseudomonas syringae* /Spotts, Cervantes, 1995/. In our opinion, this medium is more suitable for such screenings.

The Pseudomonads isolated in Central part of Poland were more tolerant to copper than those from other regions. This is probably related to more common use of copper based preparations by growers in this part of the country. Andersen et al., (1991) pointed out that copper resistance may reflect ecological variations in the strains selected by the host plant.

Conclusions

1. The occurrence of various pathovars and races of *Pseudomonas syringae* on various species of stone fruits may reflect their epidemiological and economic importance.

2. The presence of considerable number of copper resistant isolates should help in reorientation the present program of bacterial canker control.

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