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# FLUORESCENT PSEUDOMONADS ASSOCIATED WITH ASIAN PEAR BLOSSOM BUDS[1]

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## Abstract

Bacteria were isolated in 2006–2007 from blossom buds of some Asian pear trees grown in two localities near Warsaw. In all tested samples fluorescent pseudomonads were detected. Pear cultivars differed in number of buds associated with bacteria and bacteria population in the buds. Among the bacteria isolated *Pseudomonas syringae* pv. *syringae* dominated, accompanied by *P. viridiflava*. Some strains of both species were ice-nucleation active (INA).

**Key words:** *Pseudomonas* spp., Asian pear, buds, INA

## Introduction

Bacterial blossom blast of pear and dieback of young pear shoot tips are the symptoms caused by bacterium *Pseudomonas syringae* pv. *syringae* (McKeen 1955, Jones and Aldwinckle 1990). The potential source of the bacteria inoculum, which can develop not only as a pathogen but also as an epiphyte, comes from the surface of pear trees, both with symptoms and symptomless. The same role is played by buds, whose inner parts are inhabited by bacteria, and by weeds commonly growing in orchards (Mansvelt and Hattingh 1986, 1987). In Europe the disease was noted in many countries and recent information on losses caused by the blossom blast disease comes from Spain (Montesinos and Vilardell 1991).

In new regions of Asian pear (*Pyrus serotina*) commercial production dieback of buds is also observed. The phenomenon occurred in a great extent in New Zealand, where in 'Hosui' cultivar significant fruit yield decrease resulted from dieback and bud fall (Klinac and Geddes 1995). The authors stated, basing on field observa-

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tions, that the cause of bud fall were physiological disorders. It is known that in regions with cold spring where the Asian pears are grown, bacterial canker caused by *P. syringae* pv. *syringae* may be a threat (Beutel 1990). Apart from its pathogenicity, the bacteria may also affect the plants being ice-nucleation active (Lindow 1983). It can take place both on the surface and inside the plant, and forming ice crystals results in mechanical injury of tissue, which happens at temperature higher that in the case of bacteria-free tissues.

The aim of the work was recognition of bacteria associated with Asian pear buds and defining their role in blossom bud freezing.

## Materials and methods

For experiments in 2006–2007 spurs with buds were taken from trees grown in two locations. In 2006 the samples were taken in spring, at the break of February and March. In 2007 the samples were also taken in autumn, at the beginning of November. In the orchard in Wilanów (near Warsaw) buds from six Asian pear cultivars were considered: 'Chojuro', 'Early Shu', 'Hosui', 'Kosui', 'Nijiseiki' and 'Shinseiki', while in the orchard in Ursynów (surroundings of Warsaw) three cultivars were considered: 'Chojuro', 'Hosui' and 'Shinseiki'. In the first year of investigations an average of 10 buds were sampled from each cultivar, and in the second year – five buds per tree from every of three replications. The reference cultivar for both localizations was 'Konferencja'.

Before isolation the buds were washed under running tap water and then each bud was cut lengthwise and blossom parts were ground in a mortar together with 1 ml of 0.9% NaCl. From the homogenate obtained, 0.1 ml of suspension was streaked on Petri dishes with King B medium (King et al. 1954). After 72 h the growing bacterial colonies producing greenish pigment (fluorescing in UV light), characteristic of fluorescent pseudomonads, were noticed.

In order to identify the isolates obtained the following tests were performed: disruption of bacterial cell walls in 3% KOH (Suslov et al. 1982), presence of catalase (Lelliott and Stead 1987), levan production (Lelliott and Stead 1987), presence of oxidase (Kovacs 1956), causing potato soft rot, test for arginine dihydrolase (Thornley 1960), nitrate reduction (Kado 1979), hydrolysis of esculin (Lelliott et al. 1966), oxidation of saccharose and gelatine hydrolysis (Lelliott and Stead 1987) and hydrolysis of arbutin (Schaad et al. 2001). Pathogenicity of the isolates was tested with the hypersensitivity test on tobacco leaves, according to the procedure of Klement (1963).

Ice-nucleation activity (INA) was checked with a modified method of Paulin and Luisetti (1978): 20 µl suspension of three-day-old bacterial cultures ($10^9$ cfu; colony forming units) were placed on aluminium foil and kept at –6°C for 7 min. After the time the presence of ice microcrystals in the drop was checked with an entomological needle.

## Results and discussion

In all the trees of various Asian pear cultivars investigated, as well as in the 'Konferencja' trees, the fluorescent *Pseudomonas* genus bacteria were present in blossom buds. The cultivars in question differed from the point of view of both the number of buds inhabited by bacteria and the density of bacterial population in the buds. Yet, the predisposition of the cultivars to have buds inhabited by the bacteria can not be defined, as the localisation of orchard seems to be of importance. This was visible in the case of 'Chojuro', or 'Hosui' and 'Nijiseiki' (Table 1). The former cultivar grown in Ursynów had bacteria only in some buds tested, while in the Wilanów orchard all the buds of the cultivar trees were inhabited by bacteria. The situation was opposite with respect to 'Hosui' and 'Nijiseiki' – a part of buds from trees in Wilanów were bacteria free, while all the buds from Ursynów were inhabited by bacteria. In the course of two years no cultivar tested proved free from bacteria. The situation was described earlier for European pears and apples (Mansvelt and Hattingh 1988).

**Table 1**

Results of fluorescent pseudomonads isolation from Asian pear buds

| Locality/cultivar | Number of buds/number of positive isolation | | |
|---|---|---|---|
| | 2006 – spring | 2007 – spring | 2007 – autumn |
| Ursynów | | | |
| 'Chojuro' | 10/2 | 5/3 | 5/2 |
| 'Hosui' | 10/10 | 5/5 | 5/5 |
| 'Shinseiki' | 9/6 | 5/5 | 5/4 |
| 'Konferencja' | 10/8 | 5/3 | 5/3 |
| Wilanów | | | |
| 'Chojuro' | 10/10 | 5/5 | 5/5 |
| 'Early Shu' | 10/7 | 5/5 | 5/5 |
| 'Hosui' | 10/4 | 5/4 | 5/3 |
| 'Kosui' | 11/10 | 5/5 | 5/5 |
| 'Nijiseiki' | 12/4 | 5/3 | 5/4 |
| 'Shinseiki' | 12/4 | 5/4 | 5/4 |
| 'Konferencja' | 10/7 | 5/3 | 5/3 |

Among the fluorescent pseudomonads isolated, *P. syringae* pv. *syringae* predominated, accompanied by *P. viridiflava* (Table 2). Within both species there were ice-nucleation active strains, rare in the case of *P. viridiflava*. In the second year of investigations also saprotrophic *P. fluorescens* and fluorescent *Pseudomonas* spp. (not identified to the species with conventional methods) were found. The domination of *P. syringae* pv. *syringae* among bacteria isolated from healthy and dying buds of pears was also found by Mansvelt and Hattingh (1986) and Montesinos and Vilardell (1991). The latter authors noted also fairly abundant population of *P.*

**Table 2**

Ice-nucleation activity in isolates of *Pseudomonas* spp. obtained from Asian pear buds

| Year/bacteria species | Number of isolates tested | Number of INA+ strains |
|---|---|---|
| 2006 | | |
| *Pseudomonas syringae* pv. *syringae* | 32 | 14 |
| *Pseudomonas viridiflava* | 17 | 2 |
| *Pseudomonas putida* | 6 | 0 |
| 2007 | | |
| *Pseudomonas syringae* pv. *syringae* | 147 | 36 |
| *Pseudomonas viridiflava* | 48 | 5 |
| *Pseudomonas fluorescens* | 26 | 0 |
| Fluorescent *Pseudomonas* spp. | 31 | 0 |

*fluorescens* and less abundant population of *P. viridiflava*, similarly as it was found in 2007 in buds of Asian pear cultivars considered here.

## Streszczenie

FLUORYZUJĄCE PSEUDOMONADY TOWARZYSZĄCE PĄKOM GRUSZY AZJATYCKIEJ

W latach 2006–2007 izolowano bakterie z pąków kwiatowych kilku odmian gruszy azjatyckiej rosnących w dwóch sadach w pobliżu Warszawy. We wszystkich badanych próbach stwierdzono obecność fluoryzujących bakterii rodzaju *Pseudomonas*. Testowane odmiany różniły się zarówno liczbą zasiedlonych przez bakterie pąków, jak i populacją bakterii w nich obecnych. Wśród wyizolowanych bakterii dominował gatunek *P. syringae* pv. *syringae,* mniej liczne były bakterie *P. viridiflava*. W obrębie obu gatunków odnotowano szczepy zdolne do tworzenia ośrodków krystalizacji lodu.

### Literature

Beutel J.A., 1990: Asian pears. In: Advances in new crops. Eds. J. Janick, J.E. Simon. Timber Press, Portland, OR: 304–309.

Jones A.L., Aldwinckle H.S., 1990: Compendium of apple and pear diseases. APS Press, St. Paul, MN.

Kado C.J., 1979: Methods in plant pathology. Plant Pathol. 228.

King E.O., Ward M.K., Reney D.E., 1954: Two simple media for demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301–307.

Klement Z., 1963: Rapid detection of pathogenicity of phytopathogenic pseudomonads. Nature (Lond.) 199: 299–300.

Klinac D.J., Geddes B., 1995: Incidence and severity of the floral bud "budjump" on nashi (*Pyrus serotina*) grown in the Waikato region of New Zealand. N. Z. J. Crop Hortic. Sci. 23: 185–190.
Kovacs N., 1956: Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature (Lond.) 178: 703.
Lelliott R.A., Billing E., Hayward A.C., 1966: A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29: 470–489.
Lelliott R.A., Stead D.F., 1987: Methods for the diagnosis of bacterial diseases of plants. Blackwell, Oxford.
Lindow S.E., 1983: Methods of preventing frost injury caused by epiphytic ice-nucleation-active bacteria. Plant Dis. 67: 327–333.
Mansvelt E.L., Hattingh M.J., 1986: Pear blossom blast in South Africa caused by *Pseudomonas syringae* pv. *syringae*. Plant Pathol. 35: 337–343.
Mansvelt E.L., Hattingh M.J., 1987: *Pseudomonas syringae* pv. *syringae* associated with apple and pear buds in South Africa. Plant Dis. 71: 789–792.
Mansvelt E.L., Hattingh M.J., 1988: Resident population of *Pseudomonas syringae* pv. *syringae* on leaves, blossoms and fruits of apple and pear trees. J. Phytopathol. 121: 135–142.
McKeen W.E., 1955: Pear blast on Vancouver Island. Phytopathology 45: 629–632.
Montesinos E., Vilardell P., 1991: Relationships among population levels of *Pseudomonas syringae*, amount of ice nuclei, and incidence of blast of dormant flower buds in commercial pear orchards in Catalunya, Spain. Phytopathology 81: 113–119.
Paulin J.P., Luisetti J., 1978: Ice nucleation activity among phytopathogenic bacteria. Proc. 4th Int. Conf. Plant Pathog. Bact. (Angers): 725–729.
Schaad N.W., Jones J.B., Chun W., 2001: Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, MN.
Suslov T., Schroth M.N., Isaka M., 1982: Application of rapid method for Gram-differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology 72: 917–918.
Thornley M.J., 1960: The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23: 37–52.

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