ACTA SCIENTIARUM POLONORUM *Hortorum Cultus 2(2) 2003, 153-160*

BACTERIA OF THE *PSEUDOMONAS* MIGULA GENUS ON STORED VEGETABLES

Rūta Tekorienė, Albinas Lugauskas, Milda Vasinauskienė

Abstract. For the study on *Pseudomonas* bacteria, samples of vegetables with soft rot symptoms were collected in a market place and storage-rooms. The samples were examined on selective media for Gram-negative bacteria of the genus *Pseudomonas*. Forty *Pseudomonas* strains were isolated from injured carrot roots, potato tubers, onions, leek leaves, beetroots, cabbages, radish. On the basis of biological tests the bacteria were ascribe to 10 *Pseudomonas* species: *P. fluorescens, P. marginalis, P. putida*, *P. facilis, P. aeruginosa, P. delafieldii, P. cichorii , P. pseudoalcaligenes, P. cepacia* (=*Burkholderia cepacia*), *P. gladioli* (=*Burkholderia gladioli* pv. *aliicola*). Five species isolated from potato tubes, carrot roots, onions, and beetroots – *P. fluorescens, P. marginalis, P. cichorii, P. cepacia* (= *Burkholderia cepacia*), and *P. gladioli* (=*Burkholderia gladioli* pv. *aliicola*) – were able to cause soft rot symptoms of various vegetables.

Key words: carrot, potato, onions, leek, beetroot, cabbage, radish, *Pseudomonas* spp.

INTRODUCTION

Human diet includes a lot of products of plant origin; they supplement and enrich the nutrition, make it wholesome. Vegetables intended for food are grown under different conditions, in various soils, applying several means of agricultural engineering. Therefore, favourable conditions for the development of different groups of microorganisms form in soil. Certain microorganisms are also found on the harvested, stored vegetables and could make serious loses in vegetable-growing farms and storage-rooms. Due to their activities up to 50% of the stored harvest can be lost. Among many microorganisms detected in foodstuff of plant origin, bacteria of the *Pseudomonas* genus are also found [Petchiulis 1983]. Some species of this genus are considered as saprotrophic – *Pseudomonas fluorescens*, other species – pathogenic, hazardous to people and animals, – *Pseudomonas aeruginosa*, *Pseudomonas mallei*, or other phytopathogenic – *Pseudomonas syringae.* The species of this genus that is known as pathogenic to people, animals, and plants is *Pseudomonas cepacia*. It is determined that bacteria of this genus cause certain plant diseases: wiltering, plant cancer, rots, leaf spotting, malignant tumours, diseases of flowers and shoots [Hildelbrand et al. 1988].

Bacteria of the *Pseudomonas* genus are subjected to intensive research in many countries. However, in Lithuania these bacteria have not received the sufficient attention. The data on bacteria of the *Pseudomonas* genus, injuring vegetables grown in different regions of Lithuania and bacteria functioning on stored and sold vegetables, are very scarce.

The aim of the present work is to isolate and identify bacteria of the *Pseudomonas* genus from the stored and sold vegetables, to determine their detection frequency and the level of damage.

MATERIAL AND METHODS

The condition of the stored and sold vegetables was studied in 2001–2002. For bacteriological investigations the vegetables were taken from the following sites:

1. In private vegetable storeroom of a large farm in Vilnius district the samples were taken in early spring, and the assortment of the stored vegetables was not large. Plenty of vegetables were injured by microorganisms.

2. In the Kalvarijos open market place in Vilnius the vegetable samples were taken at the end of February, at the air temperature of +3°C, immediately after stronger frosts; therefore, a part of vegetables were damaged by frost. The vegetables were brought to the market via different ways and kept on the counters, in bags, often together with other food products.

Altogether 26 vegetable samples were taken into sterile plastic bags. Microbiological analysis of the samples was performed according to the accepted methods described by Gerhardt [1984], Ruban [1986], Smirnov et Kiprianova [1990], Choult et al. [1997].

For isolation of the *Pseudomonas* bacteria the selective agar cetrimide medium (*Pseudomonas* (cetrimide) agar – Liofilchem s. r. l., Italy) was prepared; the cetrimides, present in the medium, inhibit the growth of other Gram-positive and Gram-negative bacteria. In such medium only bacteria of the genus *Pseudomonas* grow.

Identification of bacteria was performed basing on the analysis of their biological properties [Palleroni Norberto J. 1984, Choult et al. 1997].

Pathogenic properties of 31 bacterial strain isolated from the injured vegetables were tested. Pathogenicity of strains was investigated employing the methods suggested by Lelliott et Stead [1987] and Hildelbrand et. al. [1988]. Morphology of colonies was investigated by cultivating bacteria in three agar media: 5% of saccharose, King B, and dextrose. Pathogenic properties of the isolates were tested artificially infecting tobacco leaves with bacterial suspension of one day (10^{-8} cfu·ml). The results were checked after 24 hours. Pathogenic properties of the isolates were also investigated using pieces of potato tubers. In that case potato tubers were placed in Petri dishes and infected with bacteria, previously cultivated for 24 hours, sowing them in ca. 1.0 cm long and 0.8 cm wide line on the surface of tubers. The infected tubers were incubated for three days at a temperature of +28°C. The results were evaluated applying the five-point system [Pasichnik 1995, Poloszenec et. al. 1997, El-Hendawy et al. 2002].

RESULTS

The investigated bacteria were isolated from the rotten vegetables stored in the storehouse and sold in the open market (tab. 1).

After investigations of biological properties of the isolated bacteria, 10 species of the *Pseudomonas* genus were identified: *Pseudomonas fluorescens, Pseudomonas marginalis, Pseudomonas putida, Pseudomonas facilis, Pseudomonas aeruginosa, Pseudomonas delafieldii, Pseudomonas cepacia* (=*Burkholderia cepacia*), *Pseudomonas cichorii, Pseudomonas pseudoalcaligenes,* and *Pseudomonas gladioli* pv. *aliicola* (=*Burkholderia gladioli* pv. *aliicola*).

Table 1. Species of the *Pseudomonas* genus isolated of rotten vegetables
Tabela 1. *Pseudomonas* spp. izolowane z gnijących warzyw

Samples Próby	Sampling place Miejsce pobrania próby	Species Gatunek bakterii
Potato tubers Bulwy ziemniaka	Storeroom	*P. facilis, P. marginalis, P.putida, P. fluorescens*
Carrot roots Korzenie marchwi	Storeroom	*P. fluorescens*, *P. aeruginosa, P. delafieldii, P. cepacia, P. cichorii, P. facilis*
	Market place	*P. pseudoalcaligenes, P. cichorii*
Onions Cebule	Storeroom	*P. cepacia, P. fluorescens*, *P. gladioli* pv. *aliicola*
Leek leaves Liście pora	Market place	*P. fluorescens*
Beetroots Buraki ćwikłowe	Market place	*P. fluorescens*
Cabbages Kapusta	Market place Storeroom	*P. pseudoalcaligenes* not isolated
Radishes Rzodkiewki	Market place	*P. marginalis*
Pickled cucumbers Ogórki konserwowe	Market place	not isolated

Four species of the *Pseudomonas* genus were isolated from potatoes: *P. fluorescens, P. marginalis, P. putida,* and *P. facilis;* 6 species – from carrots: *P. fluorescens, P. aeruginosa, P. delafieldii, P. cepacia, P. facilis,* and *P. cichorii*. In onions *P. cepacia*, *P. gladioli* pv. *aliicola,* and *P. fluorescens* were identified. In leek leaves and in beetroots only one bacterium species *P. fluorescens* was identified, in cabbage leaves – *P. pseudoalcaligenes*, in radish – *P. marginalis* (tab. 1).

Pathogenic properties of the isolated and identified bacteria of the *Pseudomonas* genus were determined. The obtained data are presented in table 2.

The investigations on biological properties of 40 isolates, belonging to various species of the *Pseudomonas* genus, revealed bacteria of 5 species as being pathogenic: *P. fluorescens* (isolated from potatoes, carrots, and onions), *P. marginalis* (from potatoes), *P. cepacia* (from carrots, onions), *P. cichorii* (from carrots), and *P. gladioli* (from onions). The results of the research are presented in table 3. They were compared with those presented in literature sources [Lelliott et Stead 1987, Hildelbrand et al. 1988, Smirnov et Kiprianova 1990, Poloszenec 1997]. Further investigation revealed different

Table 2. Pathogenicity *Pseudomonas* species isolated of different vegetables
Tabela 2. Patogeniczne *Pseudomonas* spp. izolowane z różnych warzyw

Pseudomonas spp.	Number of isolates Liczba izolatów			Samples Próby
	Total Ogółem	Pathogenic Patoge-niczne	Not pathogenic Niepatoge-niczne	
P. fluorescens (Trevisan 1889) Migula 1895)	9	5	4	potato tubers, carrot roots, onions, leek-leaves, beetroots
P. marginalis (Brown 1918) Stevens 1925	3	2	1	potato tubers, radish
P. cepacia (ex Burgholder 1950) Paleroni and Holmes 1981	3	2	1	carrot roots, onions
P. gladioli pv. *aliicola* Severini 1913	1	1	—	onions
P. cichori (Swingle 1925) Stapp 1928	2	2	—	carrot roots
P. facilis (Schatz and Bovell 1952) Davis, in Davis, Stanier, Doudoroff and Mendel 1969	3	—	3	potato tubers, carrots
P. putida (Trevisan 1889) Migula 1895	1	—	1	potato tubers
P. aeruginosa (Schroeter 1872) Migula 1990	1	—	1	carrot roots
P. delafieldii Davis, in Davis, Stanier, Dudorof and Mendel 1970	1	—	1	carrot roots
P. pseudoalcaligenes Stanier, in Stanier, Palleroni and Doudoroff 1966	3	—	3	cabbages

Table 3. Biological properties of bacteria isolated of rotten vegetables
Tabela 3. Biologiczne właściwości *Pseudomonas* spp. izolowanych z różnych warzyw

Medium Pożywka	Bacterial species – Gatunki bakterii				
	P. fluorescens	*P. marginalis*	*P. cepacia*	*P. cichorii*	*P. gladioli*
5% saccharose medium 5% sacharozy w pożywce	Whitish-grey, concave	Whitish, concave	Yellowish-brownish, concave	Whitish-grey, concave	Whitish-yellowish, concave
King B King B	Whitish-grey, concave, with greenish-yellow pigment penetrating into medium	Greyish, concave, with greenish-yellow pigment penetrating into medium	Grey, with yellow pigment penetrating into medium	Whitish-grey, concave, with greenish-yellow pigment penetrating into medium	Whitish-grey
Dextrose medium Pożywka glukozowa	Whitish-greyish, concave	Whitish-greyish, concave	Yellowish, concave	Whitish-grey, concave	Whitish-yellowish, concave
Pieces of potato tubers Fragmenty bulw ziemniaka	+	+	+	–	+
Reaction of tobacco leaves Reakcja liści tytoniu	–	+	–	+	–
Oxidase Oxydaza	+	+	+	+	+
Arginine dihydrolase Dehydrolaza argininowa	–	+	–	–	–

aggressiveness of the isolates towards the host-plant; it was evaluated from 1 to 4 points according to the system proposed by Pasichnic [1995], Poloszenec et al. [1997], El-Hendawy [2002]. Isolates of some bacteria were avirulent. Among all investigated cultures, only *P. cichorii* did not infect the potato tubers. Tobacco leaves were infected by all strains of *P. marginalis* and *P. cichorii*.

Investigation on biological properties revealed the most archetypal features enabling to identify the isolated bacteria, first of all to check their inclusion into the *Pseudomonas* genus, and consequently the species. Therefore, mobile, spore-less, Gram-negative, stick-shaped bacteria, functioning under aerobic conditions and characterised by oxidase activity were ascribed to the *Pseudomonas* genus. Bacteria of separate isolates unequally dissociated gelatine, with different intensity excreted pigments into King B medium, and synthesised levan. Bacteria of some isolates were characterised by lecithinaze activity, denitrificational features, and the growth at a temperature of +42ºC.

Not to all *Pseudomonas* bacteria isolated from vegetables were pathogenic. This feature was typical of 71% of *P. fluorescens* and *P. marginalis* isolates from potatoes (fig. 1). Bacteria of *P. putida* and *P. facilis* isolates from potatoes were saprotrophs.

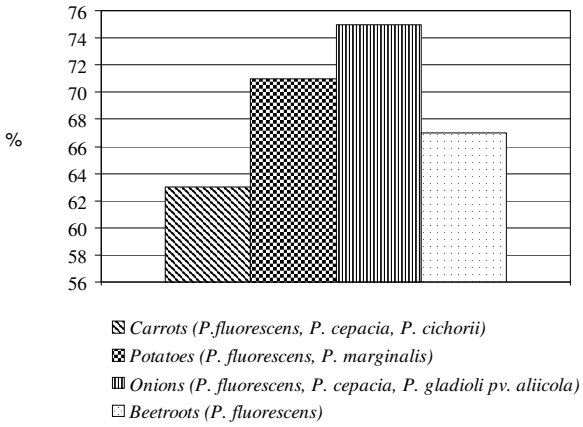

Fig. 1. Percentage of phytopathogenic bacteria of the *Pseudomonas* genus detected in rotten vegetables.

Rys. 1. Udział fitopatogenicznych bakterii z rodzaju *Pseudomonas* powodujących zgniliznę warzyw

Not all isolates of *Pseudomonas* from carrots were pathogenic. Basing on the research results, only 63% of isolates from carrots belonging to *P. fluorescens, P. cepacia,* and *P. cichorii* (fig. 1) could be treated as pathogenic. Meanwhile bacteria ascribed to *P. aeruginosa, P. delafieldii,* and *P. facilis* species could be considered as saprotrophic, as they did not cause carrot soft rot diseases.

From onions, *P. gladiolii* pv. *aliicola*, *P. cepacia,* and *P. fluorescens* were isolated and identified. Pathogenic properties were characteristic to 75% of these bacteria (tab. 1). Bacteria of *P. fluorescens* isolated from leek leaves were not pathogenic, while

the same bacterial species isolated from beetroots showed pathogenicity. *P. pseudoalcaligenes* was detected on cabbages. It is considered as water saprotroph and could possibly got on vegetables with water. *P. marginalis* bacteria, causing plant rots, were isolated from radishes.

It should be mentioned that bacteria of the genus *Pseudomonas* were detected in not all investigated vegetables. No bacteria were isolated from some sampled carrots, cabbages, and beetroot. No bacteria of the *Pseudomonas* genus were detected in pickled cucumbers.

DISCUSSION

The obtained results were compared with the literature data, and first of all were similar to the results presented by Poloszenec et al. [1997]. On the stored potato tubers, saprotrophic *Pseudomonas fluorescens* start to multiply first. Majority of bacteria from this species do not considerably cause soft rot, but while developing and functioning they excrete secondary metabolites and create favourable conditions for the development of phytopathogenic forms of the same *P. fluorescens* species. These bacteria are Gram-negative, mobile, stick-shaped, able to produce oxidase, not synthesising arginine dihydrolase; while growing on saccharose and dextrose media they form whitish-grey convex colonies. On King B medium the colonies are of the same appearance but excrete into surrounding greenish-yellow fluorescent pigment. *P. fluorescens* isolates has negative reaction on tobacco leaves and they has positive reaction on rot potato tubers. These results coincide with the data presented by Petchiulis [1983], Hildelbrand et al. [1988], Shlegel‘ [1988], Smirnov and Kiprianova [1990], Choult et al. [1997]. *Pseudomonas fluorescens* bacteria were also detected in carrot roots and onions. Together with the above-mentioned bacteria, *P. marginalis* was also detected on potato tubers. These bacteria of the *Pseudomonas* genus together with other microorganisms cause soft rot of potato tubers. By its biological properties *P. marginalis* strongly resemble *P. fluorescens*. According to the latest data, *P. marginalis* is ascribed to *P. fluorescens II* biovar [Palleroni 1984, Young et al. 1996]. It differs by some biochemical properties. The bacterial strains of the *Pseudomonas* genus detected in the samples during the present study exhibited pathogenicity, except for one strain of *P. fluorescens* and *P. marginalis.*

Bacteria of *Pseudomonas cepacia* (=*Burkholderia cepacia*), another species characterised by pathogenic properties, were isolated from onions. Already in 1950, Burkholder described these bacteria as causing onion rot. They are characterised by very specific cultural properties [Smirnov et Kiprianova 1990]. According to the above-mentioned authors, these bacteria can cause the diseases of people and animals; they are often isolated from the samples of pathologic material. Bacteria of *P. cepacia* species, isolated during this study, were Gram-negative, stick-shaped, not synthesising arginine dihydrolase and oxidase, grew at a temperature of +42°C, in the saccharose medium formed yellowish-brownish, concave colonies. In King B medium the colonies were of the same colour, but excreted yellow pigment into the medium, in dextrose medium they

formed concave, yellowish colonies, caused the rot of potato tubers, but did not affect the tobacco leaves. Bacteria of this species were also detected in carrots.

Another bacterial species *P. gladioli* pv. *aliicola* (=*Burkholderia gladioli* pv. *aliicola*) was detected in onions. They were Gram-negative, stick-shaped, producing oxidase, but not synthesising arginine dihydrolase, normally growing at a temperature of +42ºC, in saccharose and dextrose media forming concave, whitish-yellowish colonies. In King B medium whitish-grey colonies formed; the bacteria cause rot of potato tubers, but they has negative reaction on tobacco leaves [Lelliott 1987, Hildelbrand et al. 1988].

One more species of the *Pseudomonas* genus *P. cichorii,* characterised by pathogenic properties, was isolated from carrots. The bacteria of this species are Gram-negative, stick-shaped, characterised by oxidase synthesis, disability to produce arginine dihydrolase; in saccharose and dextrose media they form whitish-grey concave colonies; equal colonies form in King B medium, but here yellowish-green pigment is excreted into surrounding. Bacteria of this species did not cause the rot of potato tubers, but affected the tobacco leaves. The obtained results confirmed the results of other similar investigations performed under different conditions [Lelliott 1987, Hildelbrand et al. 1988].

In vegetables together with the above-mentioned bacterial species of the genus *Pseudomonas* saprotrophic bacterial species were also recorded. *P. facilis* and *P. putida* were isolated from potato tubers. These bacteria are usual in soil and possibly get into storehouse together with insufficiently dried potatoes covered with soil, and under favourable conditions they start to develop. *P. aeruginosa, P. delafieldii,* and *P. facilis,* isolated from carrots, are usually found in soil and water, so they get into storehouses together with wet soil. *P. pseudoalcaligenes*, isolated from cabbages, are water saprotrophs and possibly get on vegetables with water.

CONCLUSIONS

1. Basing on the obtained results it could be stated that a large part of stored vegetables, intended for food, are infected with bacteria of the *Pseudomonas* genus, which under favourable conditions can cause rot of vegetables, be the nidus of infection, and, therefore, be hazardous to the health of consumers.

2. Bacteria of the *Pseudomonas* genus, pathogenic to plants, were most abundant in onions and made 75% of all isolates, in potatoes – 71%, in beetroots – 67%, in carrots – 63%.

3. In the stored and sold vegetables 10 species of the *Pseudomonas* genus were isolated and identified: *Pseudomonas fluorescens, P. marginalis, P. putida, P. facilis, P. aeruginosa, P. delafieldii, P. cepacia, P. pseudoalcaligenes, P. cichorii,* and *P. gladiolii var. aliicola.*

4. Bacteria of 5 species ascribed to the *Pseudomonas* genus (*P. fluorescens, P. marginalis, P. cepacia, P. cichorii, P. gladiolii*) were characterised by pathogenic properties.

REFERENCES

Burkholder W., 1950. Sour skin, a bacterial rot of onion bulbs. Phytopatology 40 (1), 115–117.
Choult Dsz., Krieg N., Snit T., Steili Dž., Wiljams S., 1997. Opredelitel' bakterii Berdszi. Moskva 1, 157–174.
El-Hendawy, Osman M. E. and Ramadan H. A., 2002. Pectic Enzymes Produced *In vitro* and *In vivo* by *Erwinia* spp. Isolated from Carrot and Pepper in Egypt. J. Phytopathology 150, 431–438.
Gerhardt D. T., 1984. Mietody obščiai bakteriologii. Moskva.
Hildelbrand D. C., Schroth M. N., Sands D. C., 1988. Laboratory Gide for Identification of Plant Pathogenic Bacteria. In.: Schaad N. W. (ed.) 60–80.
Young J. M., Saddler G. S., Takikawa Y., De Boer S. H., Vauterin L., Gardan L., Gvozdyak R. I., Stead D. E., 1996. Review of Plant Pathology. Names of plant pathogenic bacteria 1864–1995. 75 (9), 721–762.
Lelliott R. A., Stead D. E., 1987. Methods for Diagnosis of Bacterial Diseases of Plant. Oxford, London.
Palleroni Norberto J., 1984. Bergey's manual systematic bacteriology. Baltimore: Williams and Wilkins Co, 1, 140–218.
Petchiulis J., 1983. Mikrobiologija. Vilnius.
Pasichnik L. A., 1995. *Pseudomonas fluorescens* – novy vozbuditel' zabolevanii rži. Mikrobiol. Journ. 57(2), 3–7.
Poloszenec V. M., Ivashchenko I. V., Kabashna L. V., 1997. Zbudniki gnilei kartopli v zonach Polissia ta lisostiepu Ukraini. Mikrobiol. journ. 59 (3), 28–33.
Ruban E. L., 1986. Fiziologija i biochimija predstavitelei roda *Pseudomonas.* Moskva.
Smirnov V. V., Kiprianova E. A., 1990. Bakterii roda *Pseudomonas.* Kiiev.
Shlegel' G., 1987. Obshchaja mikrobiologija. Moskva.

BAKTERIE Z RODZAJU *PSEUDMONAS* MIGULA USZKADZAJĄCE NIEKTÓRE WARZYWA

Streszczenie. Z warzyw przechowywanych w różnych warunkach na pożywkach sztucznych wydzielono 40 patogenów rodzaju *Pseudomonas.* Po sprawdzeniu właściwości biologicznych bakterii stwierdzono, że są to gatunki *P. fluorescens, P. marginalis, P. putida, P. facilis, P. aeruginosa, P. delafieldi, P. cepacia* (=*Burkholderia cepacia*), *P. cichorii, P. alcaligenes* ir *P. gladioli* pv. *aliicola* (=*Burkholderia gladioli* pv. *aliicola*). W wyniku przeprowadzonych testów na określenie patogeniczności bakterii stwierdzono, że pięć spośród wyizolowanych okazało się patogenicznymi: *P. fluorescens* wyizolowana z buw ziemniaka, korzeni marchwi i cebuli, *P. marginalis* wyizolowana z bulw ziemniaka, *P. cepacia,* z korzeni marchwi i cebuli, *P. cichorii,* wyizolowana z korzeni marchwi oraz *P. gladioli* wyizolowana z cebuli. Wykazano, że niektóre gatunki bakterii mogą być patogeniczne i powodować zgniliznę warzyw. Porażone warzywa mogą być pierwotnym źródłem zakażenia zdrowych warzyw. Nieliczne spośród tych bakterii są chorobotwórcze dla zwierząt i człowieka.

Słowa kluczowe: marchew, ziemniak, cebula, por, burak ćwikłowy kapusta, rzodkiewka, *Pseudomonas* spp.

Rūta Tekorienė, Albinas Lugauskas, Laboratory of Biodeterioration Reseach, Institute of Botany, Žaliųjų ežerų St. 49, LT-2021 Vilnius, Lithuania, e-mail: lugauskas@botanika.lt
Milda Vasinauskienė, Laboratory of Phytopathogenic microorganisms, Institute of Botany, Žaliųjų ežerų St. 49, LT-2021 Vilnius, Lithuania