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Distribution of the genus *Pseudomonas* bacteria in oil-polluted soil, water, polymeric materials, plant remnants and food products

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Samples of oil-polluted soil, water, plant remnants, polymeric materials and food products were taken in 1996–2001. The samples were examined by dilution plating on cetrimide agar for gram-negative bacteria of the genus *Pseudomonas.* There were 191 bacterial isolates: 40 from soil, 11 from water, 56 from plant remnants, 28 from polymeric materials and 56 from food products.

Bacteria of the genus *Pseudomonas* were most widely spread in food products and less abundant in oil-polluted soil and water. *Pseudomonas fluorescens* dominated on a variety of substrates. It was found in water, on trees and wood, on polymeric materials and in food products. *Pseudomonas putida* was found on all substrates. *Pseudomonas alcaligenes* was found only in oil-polluted soil, *P. spinosa* – in water, *P. syringae* – on wood. *P. aureofaciens, P. denitrificans, P. boreopolis, P. cruciviae* were recorded only on polymeric materials. *P. cichorii, P. fragi, P. gladioli, P. straminea, P. facilis, P. delafieldii* were detected in food products.

Key words: bacteria of the genus *Pseudomonas*, oil-polluted soil, water, plant remnants, polymeric materials, food products

INTRODUCTION

Bacteria of the genus *Pseudomonas* Migula function in a variety of ecological conditions: soil, oil-polluted soils, oilfields, seas, freshwater and in the digestive tract of water animals (Zdanavičiūtė, 1998; Ringø, Birkberk, 1999; Ringø, Olsen, 1999; Bruins et al., 2000; Киреева и др., 2001; Chythanya et al., 2002; Voverienė et al., 2002; Tanaka et al., 2003). Bacteria of this genus are frequent on plant surfaces and on substrates of plant origin (Пасичник, 1995; Spiewak et al., 1996; Положенец и др., 1997; El-Hendawy et al., 2002). Bacteria of the genus *Pseudomonas* get on food products from air, soil, packaging, people's hands, thus contaminating the products (Desmasures et al., 1997; Šarkinas, 1999). For their nutrition, bacteria of this genus are able to use various organic and inorganic substrates, various synthetic fabrics, oil products (Lugauskas et al., 1997; Zdanavičiūtė, 1998; Киреева и др., 2001), cellulose, lignin (Betts, Dart, 1988).

Oil is a compound substrate comprising 200–300 chemical compounds: hydrocarbons, oxygen, sulphur, nitrogen compounds, fatty acids, vanadium and nickel (Zdanavičiūtė, 1998). Due to its specific properties (rapid vaporization, solubility) oil makes a good nutritive medium for various microorganisms that use these compounds as a source of energy and carbon. Alongside with other microorganisms, bacteria of the genus *Pseudomonas* (*Pseudomonas aeruginosa, P. aureofaciens, P. putida, P. pseudoalcaligenes, P. acidovorans*) actively decompose oil and its products (Рубан, 1986).

Water, as well as soil, is a natural medium for microorganism development. Water, alongside oxygen, always contains various inorganic salts and plenty of organic compounds (Pečiulis, 1987; Demnerova et al., 2005; Karafstan, Ark-Colakoglu, 2005). Among many microorganisms inhabiting water, bacteria of the genus *Pseudomonas* are also recorded. They function in seas: *Pseudomonas marina, P. nautica, P. daudoroffii* (Baumann et al., 1983), fresh water: *P. aeruginosa, P. putida, P. fluorescens* (Fuentes et al., 1998), in distilled water (*P. huthiensis, P. lanceolata*), even in very salty lakes (*P. halophila*) where the concentration of NaCl reaches 0.02–3.3 M (Хоулт и др., 1997).

In technical and other spheres, various polymeric – natural (caoutchouc, leather, textile) and synthetic (rubber, wrap, polish, paint, glue, sealant) – materials of diverse chemical composition and structure are used. Every polymer is composed of various inorganic and organic components that can be used for the nutrition of microorganisms, termites and rodents (Lugauskas et al., 1997). Kapron fabric saturated with antistatic substances and containing aromatic polymeric substances, which is widely used for technical purposes, can be decomposed by *P. aeruginosa, P. dehalogenens, P. putida, P. fluorescens* bacteria. *P. cruciviae* is able to fully utilize polychloric biphenyles which were earlier used as greases, herbicides, medicinal preparations or antimicrobial reagents. Because of high toxicity these compounds are no longer in use, but they remain for a long time in the environment (Chandhry, Chapalamadugu, 1991). *P. cepacia* and *P. putida* bacteria utilize hardly decomposing chlorine aromatic compounds with one aromatic bond (dichlorbenzole,

pentachlorphenol, dichlorbenzoat, chlortoluol, etc.) (Lugauskas ir kt., 1997).

A high diversity of saprotrophic and pathogenic microorganisms is recorded in food raw materials and in processed food products. Microorganisms get on vegetables and fruit from air, soil, packaging materials, people's hands, during harvesting and storage. Plenty of vegetables and fruit are mechanically damaged. In this way, microorganisms from the surface easily penetrate into inner tissues and start functioning there. Here, conditions favour the development of various microorganisms causing rots of fruit and vegetables. Alongside with other microorganisms, bacteria of the genus *Pseudomonas* (*P. fluorescens, P. aeruginosa, P. putida*) are detected on vegetables (Положенец и др., 1997). Under favourable conditions, saprotrophic bacteria of these species can start to multiply profusely thus forming favourable conditions for the spread and development of rot-causing bacterial species. On the vegetable surface, particularly harmful *P. mallei, P. pseudomallei, P. aeruginosa, P. cepacia* species have been recorded (Suntres et al., 2002; Nasser et al., 2003). *P. fluorescens, P. azotoformans, P. fulva* are most widely spread on grain (Пасичник, 1995). Fresh carcass meat is free from microorganism contamination, however, they can get on meat from the equipment, people, air (Wirtanen et al., 2000). *P. fragi* are saprotrophic bacteria isolated from meat. However, inappropriately stored meat gets spoiled. The spoilage is caused by *P. fluorescens* and *P. aeruginosa*. The processed meat and fish could be contaminated by microorganisms, including *P. fluorescens* (Langsrud, Sundheim, 1997).

Pseudomonas bacteria are the object of intensive investigation in the world. In Lithuania, attention to these bacteria is insufficient. There are not enough data about their distribution in different substrates and the species variety of these bacteria. So, the aim of the present research was to isolate *Pseudomonas* bacteria from oil-polluted soil, water, plant remnants, polymeric materials, food products and to compare the distribution of individual species in these substrates.

MATERIALS AND METHODS

Different substrates were chosen for ecological investigations: oil-polluted soil, water, polymeric materials of various composition and application purpose, wood and various food products. In total, 98 samples were analysed: 1) twelve samples of oil-polluted soil after a tank from the oil fuel base territory in Vilnius (diesel lubricant, emulsol, nigrol, turbine lubricant TP, industrial lubricants I30A, I20A, transmision lubricant TEP, cooling MR 7 maschine) cooler. The control samples were taken beyond the base territory); 2) six samples of water (the Nemunas river near Uostadvaris settlement in Lithuania, the Baltic Sea near Juodkrantė in Lithuania and wells in Juodkrantė in Lithuania); 3) 30 samples of polymeric materials from Mycological Station of Institute of Botany, where materials were exposed to open air, under a shelter and in a cellar; 4) ten samples of wood and trees of oak, fir, pine, hornbeam from the Karoliniškės preserve and the Regional Park of Verkiai in Vilnius (Lithuania); 5) 40 samples of food products: fresh, treat meat, barley, beans, fruit (pears, pomegranates, figs, peashes), vegetables (potatoes, carrots, onions, beetroots, cabbages, leeksradishes, cucumbers) from the Kalvarijos market (Vilnius) and from farms near Vilnius.

For isolation of *Pseudomonas* bacteria from different substrates, an accumulative medium of the following composition was prepared (g/l): peptone – 6.0; Na_2HPO_4 – 9.8; KH_2PO_4 – 3.0; NaCl – 1.0; glucose – 4.0; $H_2O_{(dist.)}$ – 1.0l (Смирнов, Киприанова, 1990). 100 ml of the medium was poured into 250 ml bulbs, and 0.25 g of the test soil, 2.5 ml of water, 2.5 g of wood, 2.5 g of sterile ground food product or 2.5 g of seeds were added. The samples were incubated at a temperature of 28 °C for 3–5 days in a shaker at 105 turn/min (Pečiulis, 1987).

Pure bacterial isolates were isolated from accumulative media onto selective cetrimide agar medium (*Pseudomonas* (cetrimide) agar, Liofilchem s.r.l., Italy) of the following composition (g/l): peptogelatin – 20, $MgCl_2$ – 1.4; K_2SO_4 – 10; cetrimide – 0.3; agar – 15. pH of the medium – 7.3 ± 0.2 (Pečiulis, 1987). Cetrimides present in the medium inhibit the growth of gram-positive and gram-negative bacteria, but do not affect the bacteria of the genus *Pseudomonas*. The inoculum was incubated for 2–5 days in Petri dishes at 28 °C. The primary selection of *Pseudomonas* bacteria was performed cultivating them on the surface of agar certimide medium. The morphological features and pigmentation of the colonies were evaluated: shape, colour, consistence, peculiarities of the colony edge, the colour of the isolated pigments. Simultaneously, the morphological, physiological and biochemical properties of the isolates were investigated (Рубан, 1986). In total, 191 isolates were characterised by typical features of the genus *Pseudomonas* – mobile rod-shaped, straight of slightly curved gram-negative aerobic bacteria. The physiological properties of the bacterial cells were investigated: ability to oxidate and ferment carbohydrates, synthesize fluorescent and non-fluorescent pigments, grow at 42 °C an 5 °C, assimilate mineral forms of nitrogen and various carbon sources; the denitrificating reaction of bacteria was also tested (Смирнов, Киприанова, 1990; Хоулт и др., 1997). There were examined the biochemical properties of bacterial cells: oxidative activity, formation of arginine dehydrolase, formation of saccharose, gelatin, starch hydrolysis, formation of lecithinase and lipases, accumulation of poly-β-hydroxybutyrate (Смирнов, Киприанова, 1990; Хоулт и др., 1997). Bacteria were identified by conventional methods (Ruban, 1986, Pečiulis, 1987; Смирнов, Киприанова, 1990; Хоулт и др., 1997).

Statistical analysis. The obtained data were analysed employing Microsoft Excel. The prevailing bacterial species were determined according to the LSD and t tests.

RESULTS AND DISCUSSION

Pseudomonas bacteria of 19 strains were identified: *Pseudomonas aeruginosa, P. alcaligenes, P. putida* biovar. A and biovar. B, *P. fluorescens* biovar. I, III, V, *P. marginalis* (=*P. fluorescens* biovar. II), *P. spinosa, P. pseudoalcaligenes, P. aureofaciens, P. denitrificans, P. boreopolis, P. cruciviae, P. syringae, P. cichorii, P. facilis, P. delafieldii, P. straminea, P. fragi, P. cepacia* (= *Burkholderia cepacia*), *P. gladioli* pv. *aliicola* (= *Burkholderia gladioli* pv. *aliicola*) (Table 1).

Bacteria of the genus *Pseudomonas* were recorded in all the substrates. This agrees with the literature data that bac-

Table 1. **Detection of the genus *Pseudomonas* bacteria in various substrates**

Substrate	Number of samples	Number of samples with isolated *Pseudomonas* bacteria	Number of isolates	Number of species
Soil	12	5	40	3
Water	6	4	11	3
Trees and wood	10	8	56	4
Polymeric materials	30	12	28	8
Food products	40	24	56	12

teria of this genus are widely spread in various substrates (Положенец, 1997; Desmasures et al., 1997; Lugauskas ir kt., 1997; Zdanavičiūtė, 1998, Šarkinas, 1999; Bruins, 2000; Киреева и др., 2001; Chythanya et al., 2002; El-Hendawy et al., 2002).

The highest number of *Pseudomonas* bacteria was isolated from various food products: *Pseudomonas fluorescens* biovar. I, III, V, *P. marginalis* (= *P. fluorescens* biovar. II), *P. cepacia* (= *Burgholderia cepacia*), *P. gladioli* pv. *aliicola* (= *Burgholderia gladioli* pv. *aliicola*), *P. cichorii, P. facilis, P. putida* biovar. A and B, *P. aeruginosa, P. delafieldii, P. pseudoalcaligenes, P. straminea* and *P. fragi*. All these bacteria, except for *P. delafieldii* and *P. pseudoalcaligenes*, are found in vegetables, meat, milk, grain (Пасичник, 1995; Langsrud and Sundheim, 1997; Положенец и др, 1997; Wirtanen et al., 2000). The mentioned bacteria were isolated from the food products. *P. delafieldii* and *P. pseudoalcaligenes* bacteria can function in soil and water, so they could easily get from these substrates onto food products. *Pseudomonas fluorescens* biovar. I, III, V prevailed in food products with the detection frequency 27.2% (Table 2). The detection frequency of *P. fragi* and *P. straminea* bacteria was the same, i. e. 16.7%. The detection frequency of other bacterial species is presented in Table 2. The lowest numbers of *Pseudomonas* bacteria were recorded in soil and water.

In the oil-polluted soil, *P. aeruginosa* bacteria prevailed; their detection frequency reaching 62.7%. Less abundant were *P. alcaligenes* and *P. putida* biovar. A and biovar. B bacteria; their detection frequency was 16.6% and 16.7%, respectively (Table 2). Literature sources indicate that *Pseudomonas aeruginosa, P. aureofaciens, P. putida, P. pseudoalcaligenes, P. acidovorans* are particularly active decomposers of oil products; these bacteria perform the processes of oxidation-reduction, actively decompose plant remnants and other hydrocarbon-rich substrates, clean water bodies from oil pollutants (Рубан, 1986; Kastner et al., 1990; Киреева и др., 2001). It is known that the amount of microorganisms in oil-polluted soils depends upon the season: in spring and summer, the amounts of bacteria considerably increase, while in autumn and winter they decrease. The soil samples were taken in autumn, therefore, we supposed that the species composition of *Pseudomonas* bacteria in oi-polluted soil was not abundant.

P. putida, P. fluorescens and *P. spinosa* bacteria were isolated from water, in agreement with the literature data. *P. aeruginosa, P. putida, P. fluorescens*, *P. spinosa* bacteria are listed among constant inhabitants of water (Fuentes et al., 1998). *P. fluorescens* biovar. II, III, V prevailed in water; their detection frequency was 64.9% (Table 2). The detection frequency of

Table 2. **Detection frequency of bacterial species in the substrates, %. GDFS – general detection frequency of a species, S – soil, W – water, T – trees and wood, PM – polymeric materials, FP – food products, N – unidentified**

Bacterial species	GDFS	S	W	T	PM	FP
Pseudomonas aeruginosa	7.8	62.7	N	25.0	N	2.5
P. fluorescens biovar. I, III, V	25.5	N	64.9	59.0	23.0	27.2
P. marginalis (= *P. fluorescens* biovar. II)	5.8	N	N	N	N	7.5
P. putida biovar. A and B	6.8	16.7	8.4	7.0	3.3	5.0
P. pseudoalcaligenes	3.9	N	N	N	20.0	2.9
P. aureofaciens	0.9	N	N	N	2.5	N
P. cepacia (= *Burkholderia cepacia*)	3.9	N	N	N	3.3	7.5
P. denitrificans	2.9	N	N	N	32.0	N
P. cichorii	1.9	N	N	N	N	5.0
P. alcaligenes	1.9	16.6	N	N	N	N
P. fragi	2.0	N	N	N	N	16.7
P. gladioli (= *Burkholderia gladioli* pv. *aliicola*)	0.9	N	N	N	N	2.5
P. spinosa	0.9	N	16.7	N	N	N
P. straminea	0.9	N	N	N	N	16.7
P. facilis	1.9	N	N	N	N	8.0
P. delafieldii	1.0	N	N	N	N	2.5
P. syringae	0.9	N	N	7.0	N	N
P. boreopolis	1.0	N	N	N	3.2	N
P. cruciviae	0.9	N	N	N	3.2	N
Unidentified bacteria	19.4	4.0	9.4	2.0	6.0	4.0

P. putida biovar. B and *P. spinosa* isolated from water was 16.7% each (Table 2).

In trees and wood, *Pseudomonas fluorescens* biovar. III, V bacteria prevailed; their distribution frequency reached 59.0% (Table 2) in good agreement with literature data stating that the main decomposers of the lignin–cellulose complex in wood are bacteria of the genus *Pseudomonas, P. fluorescens* var. *cellulosa* being most active (Dutkiewicz et al., 1991; Lugauskas ir kt., 1997). *P. aeruginosa* bacteria also actively participate in lignin decomposition (Erikson et al., 1990; Lugauskas ir kt., 1997). Bacteria of these species were also isolated from the wood samples, but their detection frequency was lower – 25.0% (Table 2). *P. syringae* and *P. putida* biovar. A bacteria were also recorded on wood, although no data concerning their direct participation in the processes of wood decomposition are available. It is known that *P. putida* bacteria are typical inhabitants of water and soil (Смирнов, Киприанова, 1990). They could get on wood from the environment. *P. syringae* are regarded as phytopathogenic bacteria able to damage a wide spectrum of plants (Hildelbrand et al., 1988). Their detection on trees could be explained by their wide distribution on grasses growing nearby.

From polymer materials exposed to different environmental conditions, *Pseudomonas fluorescens* biovar. I bacteria were most frequently isolated; their detection frequency was 23.0% (Table 2). According to the literature data, *P. fluorescens* var. *cellulomonas* bacteria actively participate in the decomposition of materials containing cellulose and cellulose–lignin complexes (Erikson et al., 1990; Lugauskas ir kt., 1997). Here, *P. fluorescens* biovar. I bacteria were recorded on synthetic fabric where favourable conditions for their growth had formed. *P. dehalogenens* bacteria participate in the dehalogenisation of various synthetic aromatic compounds (Chandhry, Chapalamadugu, 1991). Bacteria of this species were not traced in contemporary bacterial systematics; still, according to their characteristics, they mostly resemble *P. denitrificans* bacteria isolated from synthetic materials. The detection frequency of these bacteria was 8.0% (Table 2). On polymer materials, *P. cepacia*, *P. boreopolis*, *P. putida* biovar. B and *P. cruciviae* bacteria were recorded (Table 2). Although *P. pseudoalcaligenes* bacteria are rarely detected on polymer materials and their impact upon these materials is not defined, their detection frequency was rather high – 20.0% (Table 2). Bacteria of this species might have got on the test substrates with rain and moist. *P. aureofaciens* bacteria are usually recorded in water and soil (Хоулт и др., 1997). In the present research, they were isolated from the polymers samples, and their detection frequency reached only 2.5% (Table 2).

The general detection frequency of bacterial species (GDFS) was also determined. In all the substrates studied, *Pseudomonas fluorescens* bacteria prevailed. Their GDFS in all substrates was 25.5%. Less abundant were *P. aeruginosa, P. putida* and *P. marginalis* bacteria; their detection frequency was 7.8%, 6.8%, and 5.8%, respectively. *P. gladioli, P. spinosa, P. straminea, P. syringae* and *P. cruciviae* were even rarer; their detection frequency reached only 0.9% (Table 2).

The substrates were compared according to the average number of bacterial species per one sample (t test) (Fig. 1).

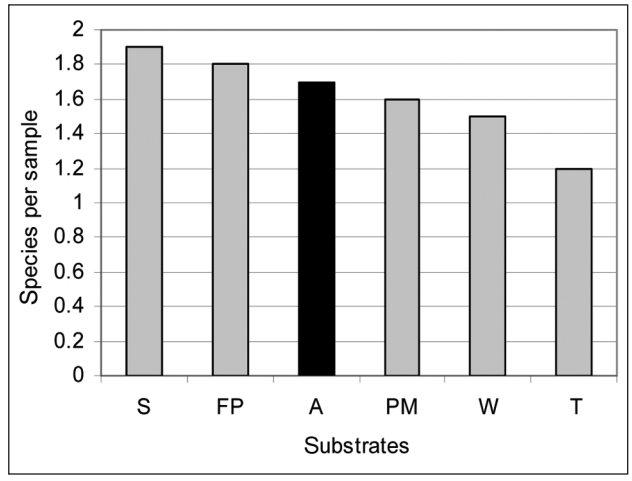

Fig. 1. Comparison of substrates according to the average number of bacterial species per sample. S – soil, FP– food products, A – average, PM – polymeric materials, W – water, T – trees and wood, black colour – significant difference from the average ($p \leq 0.005$)

In our experiment, all investigated substrates, according to the number of bacterial species per sample (1.2–1.9), were not strongly contaminated with bacteria of the genus *Pseudomonas* versus the average level (1.7) (Fig. 1).

The distribution of the *Pseudomonas* species in various substrates was determined (Fig. 2).

Investigations of the general distribution of the genus *Pseudomonas* bacteria in the substrates revealed that *P. fluorescens* (36.8% of the total number of species) and *P. aeruginosa*

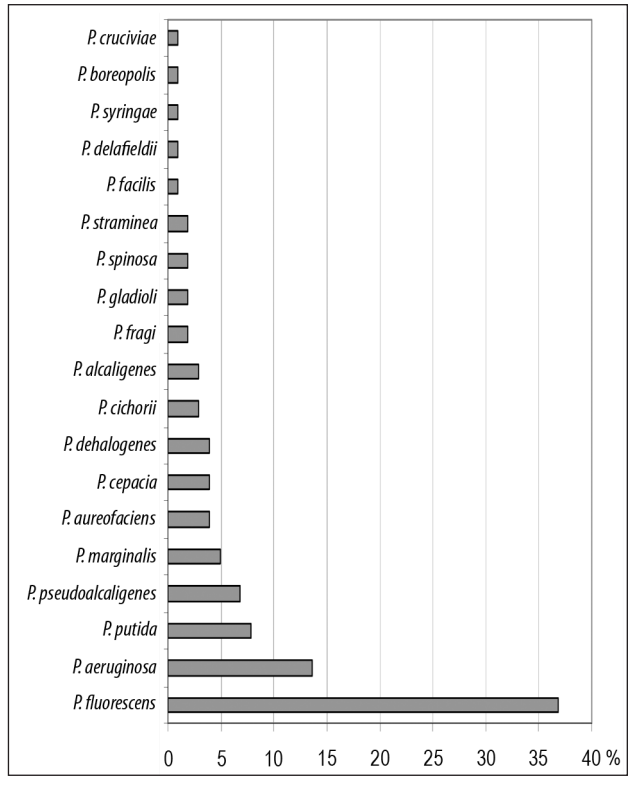

Fig. 2. Distribution of the genus *Pseudomonas* bacteria on investigated substrates (oil-polluted soil, water, wood, polymeric materials and food products), %

(13.9%) were most widespread. *P. putida* (7.8%), *P. pseudoalcaligenes* (6.8%), *P. marginalis* (4.9%), *P. cepacia, P. aureofaciens* and *P. denitrificans* (3.9% each), *P. cichorii, P. alcaligenes* (2.9% each), *P. gladioli, P. straminea, P. spinosa, P. fragi* (1.9% each) were less frequent. *P. facilis, P. delafieldii, P. syringae, P. boreopolis* and *P. cruciviae* bacteria were rarest – only 0.9% each (Fig. 2).

CONCLUSIONS

1. Nineteen species of the genus *Pseudomonas* were isolated and identified: *Pseudomonas aeruginosa* (in oil-polluted soil, water, plant remnants, food products), *P. alcaligenes* (in soil), *P. putida* biovar. A and B (in soil, water, plant remnants, polymeric materials, food products), *P. fluorescens* biovar. I, III, V (in water, plant remnants, polymeric materials, food products), *P. marginalis* (= *P. fluorescens* biovar. II) (in food products), *P. spinosa* (in water), *P. pseudoalcaligenes* (in polymeric materials, food products), *P. aureofacien*), *P. denitrificans, P. boreopolis, P. cruciviae* (all species in polymeric materials), *P. syringae* (in plant remnants), *P. cichorii, P. facilis, P. delafieldii, P. straminea, P. fragi, P. gladioli* pv. *aliicola* (= *Burkholderia gladioli* pv. *aliicola*) (all species in food products), *P. cepacia* (= *Burkholderia cepacia*) (in polymeric materials, food products).

2. *Pseudomonas fluorescens* bacteria comprised the largest part of the isolated bacterial species. They were recorded in four types of substrates: all water samples, all wood samples, polymeric materials and food products (in vegetables and fresh meat) ; their total detection frequency reached 25.5%.

3. *Pseudomonas denitrificans, P. boreopolis* and *P. cruciviae* bacteria were isolated only from polymeric materials of a particular composition. *Pseudomonas syringae* bacteria were detected only in wood (pines).

4. The highest diversity of the genus *Pseudomonas* species (12 species) was recorded in food products. Bacterial species not recorded in other substrates were detected in food products: *P. marginalis* (= *P. fluorescens* biovar. II) (in potatoes, radishes), *P. cichorii* (in carrots), *P. facilis* (in potatoes, carrots), *P. delafieldii* (in carrots), *P. straminea* (in barleys), *P. fragi* (in meat) and *P. gladioli* pv. *aliicola* (= *Burkholderia gladioli* pv. *aliicola*) (in onions).

5. Most species of *Pseudomonas* bacteria were detected in carrots (*P. fluorescens* biovar. I, *P. aeruginosa, P. cepacia* (= *Burkholderia cepacia*), *P. cichorii, P. putida* biovar. B, *P. delafieldii, P. facilis*).

6. *Pseudomonas* bacteria were not isolated from the test fruits: pears, pomegranates, figs, pearches, as well as from beans.

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Rūta Tekorienė

PSEUDOMONAS MIGULA GENTIES BAKTERIJŲ PAPLITIMAS GAMTINĖJE APLINKOJE

S a n t r a u k a

Ėminiai buvo paimti 1996–2001 m. iš nafta užteršto grunto, vandens, įvairių polimerinių medžiagų, augančių medžių bei pūvančios medienos, maisto produktų. *Pseudomonas* genties bakterijoms iš minėtų ėminių išskirti buvo naudojama selektyvinė cetrimido terpė. Taip buvo išskirtas 191 *Pseudomonas* genties bakterijų izoliatas: 40 izoliatų – iš dirvožemio, 11 – iš vandens, 56 – iš medžių ir medienos, 28 – iš polimerinių medžiagų, 56 – iš maisto produktų. Tirtuose substratuose vyravo *Pseudomonas fluorescens* bakterijos, kurios buvo aptiktos vandenyje, medienoje, polimerinėse medžiagose ir maisto produktuose. Visuose tirtuose substratuose buvo aptikta *P. putida* bakterijų. *P. alcaligenes* aptikta tik dirvožemyje, *P. spinosa* – tik vandenyje, *P. syringae* – tik medienoje, *P. aureofaciens, P. denitrificans, P. boreopolis, P. cruciviae* – tik polimerinėse medžiagose, *P. cichorii, P. fragi, P. gladioli, P. straminea, P. facilis, P. delafieldii* – maisto produktuose.

Raktažodžiai: *Pseudomonas* genties bakterijos, nafta užterštas dirvožemis, vanduo, mediena, polimerinės medžiagos