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OPPORTUNISTIC BACTERIA IN AGROECOSYSTEMS OF UKRAINE

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Background. *Recently, the number of diseases caused by opportunistic bacteria has been increasing all over the world. Opportunistic microorganisms are characterized by ubiquitous proliferation, flexibility in adaptation to the conditions of the environment, lack of specific relationship with the macroorganism. Phytopathogenic bacteria are also able to infect both plants and animals.*

Objective. *The purpose of the research was to detect the Pantoea agglomerans and Pseudomonas fluorescens bacteria in various ecological niches and establish their virulence.*

Methods. *Classical microbiological, biochemical, serological methods were used in the research. The identification of bacteria was carried out according to their phenotypic properties.*

Results. *It has been established that opportunistic bacteria species P. agglomerans and P. fluorescens are present in wheat agrophytocenoses. The bacteria isolated from the agrophytocenoses are virulent for wheat, rye and weed plants. Antibodies to opportunistic bacteria, which are spread in agrophytocenoses of cereals crops, have been found in the blood of healthy rabbits.*

Conclusions. *Thus, we have established that virulent strains of opportunistic bacteria P. agglomerans and P. fluorescens are spread in agrophytocenoses of cereals. The presence of antibodies to these bacteria in the blood of healthy rabbits proves that opportunistic bacteria from plant material get into animals and humans. Knowledge of biology and the spread of opportunistic pathogens in agrophytocenoses is necessary for prevention of infections that these bacteria cause in humans.*

KEY WORDS: **opportunistic bacteria; *Pantoea agglomerans*; human health.**

Introduction

Recently, the number of diseases caused by opportunistic bacteria has been increasing all over the world. Opportunistic microorganisms are characterized by ubiquitous proliferation, flexibility in adaptation to the conditions of the environment, lack of specific relationship with the macroorganism. Such bacteria are characterized by the ability to cause nonspecific toxic infections in weakened people and animals.

It has been established that strains of certain species of microorganisms can cause damage to plants, insects, animals and humans [1]. This phenomenon is known as polybiotrophy [2], which is particularly spread among opportunistic microorganisms. For example, conditionally pathogenic bacteria for humans *Pseudomonas aeruginosa* cause an internal of putrefaction onion during storage [3]. Strains *P. aeruginosa*, isolated from sick people, under experimental conditions affect plants, nematodes and insects [4]. Phytopathogenic bacteria are also able to infect both plants and animals [1, 2]. Bacteria of the genus *Erwinia,* which are well known exclusively as pathogenic to plants, are often isolated in pathological processes in humans and animals [5]. In this case isolates isolated from humans and animals are pathogenic to plants [2]. The causative agent of vascular bacteriosis of *Erwinia toxica* cucumbers, in intraperitoneal administration to mice, leads to sepsis in animals. Infected with these bacteria fruits of cucumbers cause poisoning in people [6].

A high degree of similarity established by DNA hybridization analyses and phenotypic data between strains of *Erwinia herbicola*, *Enterobacter agglomerans* and *Erwinia milletiae* led Gavini *et al*. [7] to unite them as a single species, namely *Pantoea agglomerans* (Beijerinck 1888) comb. nov. *P. agglomerans* is widespread in numerous diverse natural habitats and is particularly associated with many different plants as a common epiphyte and endophyte [8]. Additionally, it has been also isolated from seeds, water, humans (e.g., wounds, blood,

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urine and internal organs) as well as from animals [7, 9-12].

Pantoea species are ubiquitous in nature and occasionally are associated with infections caused by contaminated clinical material. Hence, *Pantoea agglomerans* is considered as an opportunistic pathogen of humans [13].

The *Pseudomonas fluorescens* bacteria, under conditions favorable to their development, can cause diseases of agricultural crops [14]. For some human diseases, antibodies to *P. fluorescens* lipopolysaccharide have been identified in the patients' blood.

Therefore, the purpose of the research was to detect the *Pantoea agglomerans* and *Pseudomonas fluorescens* bacteria in various ecological niches and establish their virulence.

Methods

Materials for research were the plants with symptoms of damage of rye, wheat and weeds that grew in wheat agrocenoses. Bacteriological analysis and isolation of bacteria were carried out using generally accepted methods [15]. Virulent properties were investigated on the host: plant, aggressiveness of the pathogen was determined by a 4-point scale. The biological properties were investigated by the methods described by Klement et al [15]. The bacteria were identified by comparing their properties with the characteristics of strains collection, and according to the Bergey's Manual of Systematic Bacteriology [16].

Results

We have found out that yellow-pigmented bacteria, flat or with a conical center, are opaque, wavy edges; and the oxidase-positive gray-colored isolates with wavy edges were isolated from all investigated plant materials.

It was established that a part of the investigated isolates of bacteria was avirulent for plants. In all other cases, the isolated bacteria caused diseases of rye, wheat and weeds.

The yellow pigmented bacteria are polymorphic, short rods, single arranged, sometimes in pairs in the form of short chains. Bacteria are mobile, Gram negative, spores are not formed, oxidase negative. Facultative anaerobes (Table 1). On the meat-peptone broth they grow with the formation of uniform turbidity, ring, film and sediment. The bacteria are utilized glucose (anaerobic), reduce nitrates, acidify litmus serum. Strains differ in the use of rafinose, sorbitol and inulin. All investigated strains do not use dulcitol, cause coagulation or peptonization of milk.

The isolates obtained from the affected plants according to the physiological and

Table 1. Physiological and biochemical properties of isolates

Test	Yellow-pigmented isolates	*P.agglomerans* [16]	Unpigmented isolates	*P.fluorescens* [16]
Gram's staining	–	–	–	–
Motility	+	+	+	+
Nitrate reduction	+	+	–	–
Oxidase	–	n/i	+	+
Formation of H_2S	–/+	n/i	–	n/i
Formation of indole	–	–	–	n/i
Gelatinase	+/-	+	+	n/i
OF-test	Facultative anaerob	Facultative anaerob	Aerob	Aerob
Utilization: D-glucose, L-arabinose, D-mannitol, D- xylose	+	+	+	+
Fructose, galactose	+	+	+	n/i
Salicin	+	+	–	–
Lactose	+	d	–	–
Inositol	+	+	+/–	+
Raphinose	+/–	d	+/–	n/i
Dulcitol	–	–	+/–	n/i
Sorbitol	+/–	–	+/–	+
Inulin	+/–	n/i		

Note: n/i – not investigated.

biochemical properties are similar to each other and do not differ from the characteristics of the described *P. agglomerans* species [16]. According to this they were identified as *P. agglomerans*.

Gray colored oxidase-negative bacteria grow on the meat-peptone broth, form a film, ring and precipitate, and use glucose (aerobic), mannitol, xylose, fructose, arabinose, and do not utilize lactose, inulin, salicin, as a single source of carbon.

Most of the isolates utilize raffinose, maltose and dulcitol. All isolates hydrolyze gelatin, alkalinize the litmus serum, and do not reduce nitrates (Table 1).The isolates of bacteria obtained from the affected cereals and weeds on the morphological and cultural-biochemical properties did not differ from the described strains of *P. fluorescens* and from the *P. fluorescens* characteristic given in Bergey's Manual of Systematic Bacteriology [16].

All bacterial strains tested were virulent for wheat, rye and weed plants (Table 2). It was established that the strains of the isolated bacteria are more aggressive on weeds than on agricultural crops.

Consequently, pathogenic bacteria for plants of the species *Pantoea agglomerans* and *Pseudomonas fluorescens* are able to come into contact with humans and animals and cause their diseases. This is evidenced by the fact that we detected antigens to the strains *Pantoea agglomerans* and *Pseudomonas syringae* pv. *syringae* in the serum of non-immunized rabbits (true phytopathogen) (Table 3).

Thus, the study of phytopathogenic and opportunistic bacteria is important not only for the development of plant protection methods, but also from the point of view of studying their effects on human health, since these bacteria are widespread in nature and can be ingested by humans.

Discussion

Our research has found that rye, wheat and weeds that grow in the agroecosystem of wheat are affected by opportunistic bacteria *Pantoea agglomerans* and *Pseudomonas fluorescens*. Our previous studies proved that strains of *P. agglomerans* and *P. fluorescens* were more commonly found in agricultural crops as epiphytes and did not cause plant diseases [17]. With the change of environmental factors and the influence of agronomic techniques, these opportunistic bacteria in recent years increasingly acquire virulent properties. For example, the bacteria of *P. agglomerans* were isolated from the affected locales of cotton bolls collec_ ted in a field in the USA and were able to cause comparable disease symptoms in greenhouse grown cotton fruit [18].

P. agglomerans, gram negative bacteria of *Enterobacteriaceae* family, were isolated from feculent material, plants and soil. Soft tissue and bone-joint infections due to *P. agglomerans* following penetrating trauma by vegetation and bacteraemia in association with intravenous fluid, total parenteral nutrition, blood products and anesthetic agent contamination were reported [19]. Some authors on the basis of their studies suggested that, independent of their origin, all *P. agglomerans* strains might possess indistinguishable virulence potential [13]. *P. agglomerans* was also proved to be an antibiotics producer [20].

Some researchers reported on isolation of *P. agglomerans* in two cases of septic monoarthritis after plant thorn and wood sliver injuries [21]. This indicated the transfer of *P. agglomerans* from the infected plant material to humans.

Table 2. Virulence properties

Species	Aggressiveness (marks) on plant:		
	Rye	Wheat	Weed
Pantoea agglomerans	1–2	1–3	2–3
Pseudomonas fluorescens	1–2	2	2–3

Table 3. Results of agglutination reaction

Species, strains	Titres of agglutination reaction with sera of non-immunized rabbits	
	Serum 1	Serum 2
Pantoea agglomerans, 116	50	100
Pseudomonas syringae pv. *syringae, NCPPB* 281	200	200
Erwinia amylovora, 2024	0	0

When studying the effect of bacteria of the genus *Klebsiella* (*K. pneumoniae, K. hinoscleromatis, K. ozaenae*), which, like the *Erwinia* phytopathogenic genus, belonged to the *Enterobacteriaceae* family, on leaves of potatoes, horse beans, beans, cabbage, cucumber, pumpkin and apple fruits Jonathan found out that four strains of *K. pneumoniae* were capable to affect horse beans and potatoes. The phytopathogenic properties of *Klebsiell*a species did not correlate with their ability to produce pectinases [22].

At the same time, phytopathogenic properties can be presented by bacteria, which are traditionally pathogens of animals and humans. It is proved that 15% of the strains of bacteria of genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Proteus*, *Pseudomonas*, isolated from urological patients, have phytopathogenic properties. The most pronounced these properties were on fruits of tomatoes [23].

Among the species of the genus *Pseudomonas*, which produce pigments (*P. aeruginosa*, *P. fluorescens*, *P. aureofaciens*), it is proved that the strains have phytotoxic and entomocidal properties. The greatest number of such strains is found among the bacteria of *P.fluorescens* species. The cultural fluid of these strains suppresses the germination of seeds of radish, lettuce and, to a lesser extent, wheat, and also causes the death of 100% of mosquito larvae. Entomopathogenic strains of *P. fluorescens* have antagonistic effects on some saprophytic bacteria (*Bacillus subtilis*, *B. megaterium*, *Sarcina lutea*, *Escherichia coli*, *Mycobacterium* sp.). Thus, the toxins of pigmented strains of the genus *Pseudomonas* are not narrowly specific and affect a wide range of organisms [24]. In some human diseases antibodies to the lipopolysaccharide of *P. fluorescens* 7769, which were isolated from affected rye tissues, were identified [25].

The adaptation of *P. agglomerans* to diverse microenvironments might suggest that this species maintain high genetic plasticity. *P. agglomerans* appears to be readily accessible to horizontal gene transfer driven by plasmids and other mobile elements [26], a trait that may explain its flexibility in adapting to different life styles.

Some researchers noted that pathogenic microorganisms had a lot in common in mechanisms of pathogenicity, regardless of which macroorganism they were infected with [1, 27, 28].

Conclusions

Thus, in the agrophytocenosis of wheat, one of the most widespread agricultural crops in Ukraine, there are virulent strains of opportunistic bacteria of *P. agglomerans* and *P. fluorescens* species. Present agroecosystems, which are overloaded with chemical pollutants, create conditions for increasing the aggressiveness of opportunistic bacteria. It has been established that in the blood of healthy rabbits antibodies to opportunistic bacteria, which are spread in agrophytocenoses, are present. It proves the intake by animals of opportunistic bacteria together with plant food. Since opportunistic bacteria can cause the infections processes in humans, animals, insects and plants, the control of their spread and investigation of peculiarities of virulent strains circulation is necessary.

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