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PHYTOPATHOGENIC BACTERIA IN THE SYSTEM OF MODERN AGRICULTURE

The stages of studying bacterial diseases of crops and weeds at various farming systems have been characterized, biological properties have been investigated and pathogens identified using traditional and modern molecular genetic methods of research.

Keywords: phytopathogenic bacteria, bacterial diseases of plants, agricultural crops, weeds, lipopolysaccharides

The Department of Phytopathogenic Bacteria (Department of Plant Bacterioses up to 1963) is one of the first in newly formed in the structure of the Zabolotny Institute of Microbiology and Immunology. Since that time (1933) and till the present day both Ukraine and the Department of Phytopathogenic Bacteria are recognized leaders in the field of knowledge [4].

Bacterial diseases of numerous plant species, including cereals, legumes**,** potato, carrot, tomato, pepper, cucumber, onion, cotton, tobacco, rubber, sugar beet, sudan grass, fruit, forest trees and ginseng, have been studied in Ukraine [5].

Study of bacterial plant diseases has become the major direction of researches during the whole period of the Department existence (Table).

The works which have been done by N.S. Novikova in serological methods for identification of plant pathogenic bacteria are widely used today. The strains with specific affinity of thermally stable antigenic composition for plant-host were detected for the first time [12]. On the basis of thermostable antigens *Pseudomonas syringae* pathovars were divided into serogroups. The chemical nature of O-specific chain and the core of lipopolysaccharides (LPS) of these serogroups and their biological activity were also determined for the first time [4].

Phytopathogenic and saprophytic bacterial microflora of the surface and internal tissues of wheat was investigated. It has been revealed that the quality composition of the dominant saprophytic epiphytic bacteria does not differ from the composition of endophytic bacteria. It was shown that endophytic and epiphytic strains of *Pantoea agglomerans* possess the same set of fatty acids, but differ in their proportions. The presence of branched fatty acids and the absence of hydroxy-acids [13] are typical of the strains of the genus *Bacillus*.

Mass lesion of stems by conventional soybean pathogen *P. agglomerans*, which did not reduce the harvest but reduced the development of diseases caused by other bacteria and fungi, was revealed for the first time in 2003. The effect of weather conditions on the expression of pathogenicity of *P. agglomerans* and other pathogens was noted [5, 9].

It was proved that couch and high ryegrass are affected by *P. syringae*, *P. viridiflava*, *Pseudomonas* sp. and *P. agglomerans*. Isolates from weeds in the experiment were highly aggressive for the wide range of farm crops [6, 16]. Bacterial diseases of aquatic plants (calamus, utricularia, pondweed, hornwort, lily white and yellow jugs) were detected for the first time, their agents were identified as *Pectobacterium carotovorum*, *Pseudomonas* sp. and *Bacillus* sp. [11].

According to the complex of phenotypic and genotypic characteristics (GC content couples nucleotide sequence of 16S rRNA gene), the bacterial brown spot agent of lupine was reclassified to *P. syringae* and *P. savastanoi*. The ability of bacteria isolated from lupine to damage the wide range of legumes as well as the dependence of lupine variety resistance level on the content of alkaloids have been were proved [7, 8].

The Ukrainian collection of plant pathogenic bacteria of IMV of NAS of Ukraine is the biggest and the most complete in all Eastern Europe. The collection consists of about 2 thousand strains of 200 species and pathovars of phytopathogenic bacteria which are constantly added by new strains from different countries. The availability of the collection of phytopathogenic bacteria allows achieving various objectives concerning the taxonomy and ecology of pathogens, studying their biological properties and solving the problem of biological control of bacterioses [4].

The possibility of widespread use of microbial exopolysaccharide xampan in many industries was shown. It has been found that xampan displays detoxification and radioprotective properties by activating functions of protective antioxidant, which helps to normalize the microflora of the gastrointestinal tract, normalizes phagocytic and enzymatic functions and shows the antimutagenic activity. Therefore xampan is recommended as a functionally active low-calorie dietary additive and therapeutic feeding. The biological gel (preparation EPAA) has been created on the basis of xampan and acrylamide; its promising use in woodworking, textile, microbiological industry and agriculture has been shown [15, 18].

But beginning from 2006 the Department started studying the role of phytopathogenic bacteria in modern farming systems. Intensification of agricultural production has led to significant alterations in the properties of phytopathogenic bacteria and, accordingly, to the interactions in the system soil – plant – phytopathogenic bacteria.

Scientists of the Department have realized monitoring of wheat bacterial diseases, taking into account different doses of fertilizers and crop precursor when studying the ecological role of phytopathogenic bacteria in formation of balanced agrocommunity; bacteria-agents were isolated and characterized [14]. It was shown that the symptom of development of the main wheat disease caused by *P. syringae* pv. *atrofaciens* varied depending on farming techniques, stages of plant growth and environmental factors. Adding of different doses of nitrogen, phosphate and potash fertilizers, particularly elevated, increases the probability of wheat damage by *P. syringae* pv. *atrofaciens* (the agent of basal bacteriosis) and *Xanthomonas translucens* (agent of black bacteriosis). *P. syringae* pv. *atrofaciens* strains isolated from infected wheat plants in the experiment affected the following weeds: sow-thistles, field horsetail and couch grass [14].

The main agents of bacterial diseases in the surveyed research and in industrial crops of soybeans are *P. savastanoi* pv. *glycinea* (angular leaf spot of soybean) and *Xanthomonas axonopodis* pv. *glycines* (pustule bacteriosis). Secondary pathogens are *P. syringae* pv. *tabaci* and rusty spot pathogen of soybean *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* [5, 9]. Fatty acid composition of cellular lipids proved to be effective chemotaxonomic technique for identification of the agent of both generic and species level.

Monitoring of the agents of bacterial diseases of weeds in the wheat and soybeans agrophytocenoses was performed for the first time. Symptoms of the bacterial diseases of perennial weeds: field bindweed, dandelion, thistle field, couch grass and horsetail, were described; the agents were identified as *P. syringae*, *P. carotovorum* subsp. *carotovorum*, *P. agglomerans and C. flaccumfaciens* [19, 20]. The yeasts pathogenic to plants were isolated for the first time. It was found that the surface of healthy weeds which grow in cereal crops was colonized by phytopathogenic bacteria of genus *Pseudomonas* [6].

Polyphage *P. syringae* pv. *syringae*, basal bacteriosis agent of wheat *P. syringae* pv. *atrofaciens* and pathogen of soybean *C. flaccumfaciens* demonstrate high aggressiveness under artificial inoculation of some weed species. Therefore perennial weeds serve as one of ecological niches for survival of the agents of bacterioses under the adverse or extreme environmental conditions.

It has been found that the main cause of tomato bacterial wilt is *Clavibacter michiganensis* subsp. *michiganensis*, the agent of tomato bacterial cancer. It is especially dangerous in greenhouses, causing massive damage of plants during fruiting period [5]. It has been revealed that the aggressiveness and phenotype characteristics of *C. michiganensis* subsp. *michiganensis* strains isolated from different ecological niches in Ukraine differ from those obtained in other regions of the world by quantity of the assimilated carbon compound. These high aggressive strains lose partially their aggressiveness during storage in the collection.

Fatty acid composition of cellular lipids *C. michiganensis* subsp. *michiganensis* under different conditions of cultivation is in a narrow range of C_{14} -C_{18} and belongs to izoanteizo type.

Antagonistic activity of eight strains of the *Bacillus* genus against the agent of tomato bacterioses was investigated. Six stable highly active antagonists with a wide spectrum of activity against phytopathogens of tomato, non-toxic, with a stimulating effect for tomato seedlings can be promising for biopesticide creation on their basis to protect tomatoes against bacterial pathogens. It was found that *Bacillus subtilis* IMV-7023 and *B. pumilus* 3 take influence on development of the pathological process that is caused by *C. michiganensis* subsp. *michiganensis* on tomatoes and reduce the disease symptoms [17].

It was shown on several phenotype properties that the agent of wet watery rot of lupine – *Pseudomonas xanthochlora* has great affinity for the representatives of species *P. marginalis*. An analysis of the nucleotide sequence of 16S rRNA gene showed high homology (98-99%) of the investigated strains of *P. xanthochlora* with both species *P. fluorescens* and *P. marginalis*. Based on the analysis of phenotype and genotype signs the investigated strains were assigned to the species *P. marginalis* [7, 8].

Bacterial diseases of rape, which agents are bacteria of genera *Pectobacterium*, *Xanthomonas* and *Pseudomonas* were revealed and described [10].

Culture liquids and lipopolyssacharides (LPS) of *P. syringae* pv. *atrofaciens* 9400 and 9417, as well as neopathotype strains of *P. syringae* pv. *syringae* UKM B-1027 and *P. syringae* pv. *atrofaciens* UKM B-1011 do not cause mutations in the Ames test. LPS of *P. syringae* pv. *atrofaciens* strains show protective activity against mutagenic effect of potassium dichromate and N-methyl-N'-nitro-N'-nitrozo huanidine on the test strains of *Salmonella typhimurium* TA98 and TA100 [3].

It was first found for phytopathogenic bacteria that *P. syringae* can produce exometabolites that affect the genetic apparatus of plants. It was determined that LPS of phytopathogenic bacteria cause degradation of plant chromosomes. Quantity of chromosomal aberrations increases in the apical meristem cells of *Allum cepa* roots under the influence of LPS solutions of *P. syringae* pv. *atrofaciens (*strains 9400 and 9417) and culture fluid of *P. syringae* pv. *atrofaciens* (avirulent strain 9417). It was revealed that LPS preparations of *P. syringae* pv. *atrofaciens* 9400 and 9417 strains alter the physiological condition of *A. cepa* seedlings, as evidenced by the increase of peroxidase activity and content of malonic aldehyde in plants [2, 3].

The ability of LPS of *P. syringae* pv. *atrofaciens* 9400 and 9417 strains at concentrations of 1.0 and 0.1 mg/ml to prevent tumor formation induced by the test strains of *Agrobacterium tumefaciens* on potato explants was established.

Thus we have found that wheat lesions caused by the basal bacteriosis agent *P. syringae* pv. *atrofaciens* enhances the increase of the amounts of nitrogen, phosphate and potash fertilizers.

It was established that soybean may be affected by the agent of angular spot *P. savastanoi* pv. *glycinea* and pustule bacteriosis *X. axonopodis* pv. *glycines*, as well as by *P. syringae* pv. *tabaci* and the rusty spot soybean pathogen *C. flaccumfaciens* pv. *flaccumfaciens* which is new for Ukraine. The distribution of soybean agent bacterioses depends on the type of plants, agrocultivation technologies, quantitative load of pesticides and use of biological control.

It was shown that perennial weeds were affected by bacteriosis agents of agricultural crops.

Today and tomorrow the natural (organic) agricultural production is the only among a wide range of management practices on the Earth that does no harm to the environment. The systems of organic production are based on specific and precise requirements (standards) to the production process to support optimal ecosystem condition at the social, environmental and economic levels. The recycling of nutrients and strengthening of natural processes help to maintain soil fertility and ensure profitable production. The level of pests and plant diseases is controlled naturally and by preventive, biological and other modern scientific methods [1].

However, the data on monitoring and genetic diversity of phytopathogenic bacteria in the organic farming system are very insignificant or nonexistent. Therefore, in recent years the scientists of the Department focused their attention on the above mentioned problems.

Table

Investigation of plants bacterial diseases in Ukraine

Plants	Researchers (years of study)	*Isolated pathogens*
Cereals (wheat, rye, barley, oat, millet, rice)	I.L. Serbinov (1925-1928); Ye. Ye.Fomin (1928); N.V. Broyakovskiy (1929); G.F. Trunov (1939); K.A. Bogovyk (1958); F.Ye. Nemliyenko (1948-1957); K.G. Beltyukova (1944-1949); I.B. Korolyeva (1975-1989); S.S. Sydorenko, N.P. Pravoshynska (1975--1977); V.F. Peresypkin, M.D. Minko (1978-1989); R.I. Gvozdyak (1982-2010), L.A. Pasichnyk (1982-2012), S.F. Khodos (1990-2011)	*Acidovorax avenae* subsp. *avenae* (*B. avenae*), *Bacillus subtilis*, *Pantoea agglomerans* (*Erwinia herbicola*), *Pectobacterium carotovorum* (*E. carotovora*), *Pseudomonas syringae* pv. *atrofaciens*, *P. syringae* pv. *coronafaciens*, *P. syringae* pv. *syringae* (*P. oryzicola*), *Pseudomonas fluorescens, Xanthomonas oryzae* pv. *oryzae*
Maize, sorghum, Sudan grass	I.L. Serbinov (1925); F.Ye. Nemliyenko (1936); K.G. Beltyukova, E.A. Balinska (1950); L.T. Pastushenko (1961-1964)	*P. carotovorum* (*E. carotovora*), *P. syringae* (*P. holci*), *P. syringae* pv. *syringae*, *X. vasicola* pv. *holcicola* (*X. holcicola*)
Legumes (bean, pea, soybean, lupine, lentil, alfalfa)	M.S. Zayanchkovska (1934-1938); K.G. Beltyukova (1949-1969); I.T. Zhavoronkova (1930-1933); S.Z. Aizenberg (1962-1969); I.B. Korolyeva (1966-1974); V.O. Muras (1962-1985), H.V. Zhitkevych (1984-2012); R.I. Gvozdyak and L.A. Dankevych (2003-2012); T.T. Gnatyuk (2011-2012)	*Clavibacter insidiosum*, *Curtobacterium flaccumfaciens*, *P. agglomerans* (*E. herbicola*), *P. carotovorum* (*E. carotovora*), *P. marginalis* (*P. xanthochlora*), *P. savastanoi* pv. *glycinea* (*P. syringae* pv. *glycinea*), *P. savastanoi* pv. *phaseolicola*, *P. syringae* pv. *syringae* (*P. vignae*), *P. syringae* pv. *pisi*, *P. syringae* pv. *tabaci*, *P. syringae* (*P. lupini*), *X. axonopodis* pv. *glycines*, *X. axonopodis* pv. *phaseoli*
Potato	K.G. Beltyukova (1934-1935); O.D. Belova (1930-1940); A.A. Koryshkov (1932); V.M. Polozhenets (1980-2010); L.V. Nemerytska (2002-2005); H.V. Zhitkevych, L.M. Ukrayinets (2005-2008); I.V. Demchuk, M.I. Demchynska, S.M. Moroz (2004-2005)	*B. subtilis*, *Clavibacter michiganensis* ssp. *sepedonicus* (*B. sepedonicum*), *Pectobacterium atrosepticum* (*E. phytophthora*), *P. carotovorum* (*B. carotovorum*), *P. fluorescens*, *P. marginalis* (*P. xanthochlora*), Ralstonia – similar bacteria
Carrot	R.I. Gvozdyak, S.F. Khodos (1985-1989); V.O. Muras , Dao Kim Oan (1984-1985)	*P. carotovorum* (*E. carotovora*), *P. fluorescens*
Tomato	I.L. Serbinov (1921-1922); K.G. Beltyukova (1950); S.S. Sydorenko, S.Z. Aizenberg (1975); L.V. Kabashna, M.A. Shaban, G.O. Bykova (1989-1991); R.I. Gvozdyak, S.M. Moroz, L.M. Yakovleva, Ye.P. Chernenko (2005-2009)	*C. michiganensis* subsp. *michiganensis*, *Erwinia rhapontici*, *P. agglomerans* (*E. herbicola*), *P. carotovorum* (*E. carotovora*), *P. corrugata**, *P. fluorescens*, *P. marginalis* pv. *marginalis* (*P. marginalis*), *P. syringae* pv. *tomato*, *Ralstonia solanacearum*, *X. vesicatoria*
Sweet pepper	O.P. Korobko, Debes Bakri (1985-1990)	*P. fluorescens*, *P. viridiflava*
Cucumber, melon, watermelon	A.O. Potebnya (1915); O.P. Korobko (1968-1998); Yu. Salex (1975-1977); R.I. Gvozdyak (1998)	*Erwinia toxica***, *Pseudomonas burgeri***, *P. carotovorum* (*E. carotovora*), *P. syringae* pv. *lachrymans*, *Xanthomonas cucurbitae*
Cabbage	F.I. Gordienko (1940); S.S. Sydorenko (1964); A.B. Marchenko (2000-2005)	*P. agglomerans* (*E. herbicola*), *P. carotovorum* (*E. carotovora*), *P. syringae* pv. *maculicola* (*P. maculicola*), *X. campestris* pv. *campestris*
Onion	L.V. Kabashna, R.I. Gvozdyak, S.S. Sydorenko, L.M. Yakovleva (1963-1988)	*Burkholderia gladioli* pv. *alliicola* (*P. alliicola*), *P. agglomerans* (*E. herbicola*), *P. carotovorum* (*E. carotovora*), *Pseudomonas aeruginosa*
Cotton	S.M. Moskovets (1936-1960); P.T. Estifeyev (1937); O.P. Lebedeva (1939), S.E. Gomolyako, K.A. Votolkina (1940); K.G. Beltyukova (1940-1950); A.K. Vasylkova (1940)	*Xanthomonas citri* subsp. *malvacearum* (*B. malvacearum*), *X. necrosis***
Hemp	G.A. Demekhina (1949); G.F. Trunov, I.V. Bogovyk (1954-1955)	*Pseudomonas cannabinae*

		Continue of Table
Rubber	V.O. Kalinenko (1933-1940); P.I. Savynskyi (1937-1950); Ye.I. Yezerska (1948); N.S. Novikova (1948-1951); Ye.Ye. Fomin (1949-1950); K.G. Beltyukova (1952)	*P. agglomerans* (*E. herbicola*), *P. fluorescens*
Beet sugar	N.V. Troyanovskyi (1925); I.N. Trzhebinskyi (1931); R.L. Babytska (1934); F.I. Gordiyenko (1934); V.P. Muravyuv (1939); L.V. Kucherenko (1960-1968)	*Erwinia betae*** (*E. bussei*), *P. carotovorum* (*E. carotovora*), *P. fluorescens*, *P. syringae* (*P. wieringae*), *P. syringae* pv. *aptata* (*P. aptata*), *X. axonopodis* (*X. beticola*), *X. axonopodis* pv. *vasculorum*
Rape	V.F. Peresypkin (1948-1952); L.A. Dankevich and O.M. Zakharova (2011-2012)	*X. campestris*, *P. fluorescens*
Tobacco, shag	A.A. Popova (1927-1955); K.G. Beltyukova (1930-1940); O.P. Lebedyeva (1930); Ye.K. Samotsvetova (1930-1940); N.S. Novikova (1934)	*P. carotovorum* (*B. carotovorum*), *P. syringae* pv. *tabaci* (*P. tabaci*)
Grape	P.N. Kostyuk (1940-1950); B.N. Milkus, O.Ye. Gaidai, L.O. Konup, N.V. Limanska (1990-2010)	*Agrobacterium tumefaciens*
Fruit trees	S.A. Mokrzhetskyi (1907); A.S. Korvatskyi (1909); A.A. Yachevskyi (1910); I.L. Serbinov (1922); M.N. Rodygin (1930); A.K. Vasulkova (1964-1974); K.G. Beltyukova, L.T. Pastushenko, L.V. Oskerko (1966-1968); I.G. Skrypal (1967-1969); R.I. Kalinichenko (1968-1975); A.K. Babych (1970-1974); N.I. Petrushova, G.F. Govorova (1975); Yu.P. Sadovskyi, S.I. Shevchenko (1989-1999); R.I. Gvozdyak, M.I. Lukach, V.V. Symochko, O.V. Barbakar (1997-2005); A.M. Sadlyak (1970-2010); O.Ya. Bokshan (2003); I.M. Krym (2005)	*A. tumefaciens*, *Bacterium nodoantrum***, *E. amylovora*, *Erwinia horticola***, *P. carotovorum* (*E. carotovora*), *P. fluorescens*, *P. syringae* (*P. cerasi*), *P. syringae* pv. *morsprunorum* (*P. morsprunorum*), *X. arboricola* pv. *pruni* (*X. pruni*), Rickettsiae similar organisms
Forest trees	R.I. Gvozdyak (1967-2010); L.M. Yakovleva (1968-2001); S.F. Khodos (1967-1976); N.F. Zuikova (1967-1977); A.F. Goichuk (1980-2012); O.P. Korobko (2001); V.V. Rozenfeld (2007-2012)	*Bacillus populi***, *B. subtilis*, *Clostridium butyricum*, *E. horticola***, *Erwinia nimipressuralis*, *E. rhapontici*, *P. syringae*, *P. syringae* (*P. cerasi*), *P. fluorescens*
Flower culture	K.G. Beltyukova, S.Z. Aizenberg (1975); R.I. Gvozdyak, L.V. Kabashna, L.Ye. Ogorodnik, L.M. Yakovleva (1970-1989)	*Bacillus* sp., *P. carotovorum* (*E. aroideae*, *E. carotovora*), *P. fluorescens*, *P. fluoro-violaceus*, *Pseudomonas iridis***
Ginseng	S.Z. Aizenberg (1988); R.I. Gvozdyak, V.O. Muras, H.V. Zhitkevych (1991-1993)	*Pseudomonas cichorii*
Weeds	R.I. Gvozdyak, Л.М. Яковлева, L.A. Pasichnyk, T.M. Scherbyna (2002-2012); В.П. Патика (2006-2012), M.I. Lukach (2000-2002); O.V. Barbakar (2005)	*P. agglomerans* (*E. herbicola*), *P. carotovorum* subsp. *carotovorum* (*E. carotovora* subsp. *carotovora*), *Pseudomonas* sp., *P. syringae*, *P. viridiflava*, yellow pigmented bacteria
Aquatic plants	R.I. Gvozdyak, L.Ye. Ogorodnyk, L.M. Yakovleva (2003-2009)	*Bacillus* sp., *Erwinia* sp., *P. carotovorum* (*E. carotovora*), *Pseudomonas* sp.

In brackets the name of the pathogen, during which it is described by the author. * Isolated by G.A. Bykova from tomatoes imported from Russia. ** The species name is not mentioned in the Bergey's Manual of Systematic Bacteriology

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ФІТОПАТОГЕННІ БАКТЕРІЇ В СИСТЕМІ СУЧАСНОГО ЗЕМЛЕРОБСТВА

Резюме

Охарактеризовано етапи вивчення бактеріальних хвороб сільськогосподарських культур і бур'янів за різних систем землеробства, досліджено біологічні властивості та ідентифіковано збудники хвороб традиційними і сучасними молекулярно-генетичними методами досліджень.

Ключові слова: фітопатогенні бактерії, бактеріальні хвороби рослин, сільськогосподарські культури, бур'яни, ліпополісахариди.

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ФИТОПАТОГЕННЫЕ БАКТЕРИИ В СИСТЕМЕ СОВРЕМЕННОГО ЗЕМЛЕДЕЛИЯ

Р е з ю м е

Охарактеризованы этапы изучения бактериальных болезней сельскохозяйственных культур и сорняков при различных системах земледелия, исследованы биологические свойства и идентифицированы возбудители болезней традиционными и современными молекулярно-генетическими методами исследований.

К л ю ч е в ы е с л о в а**:** фитопатогенные бактерии, бактериальные болезни растений, сельскохозяйственные культуры, сорняки, липополисахариды.

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