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L.A. PASICHNYK, L.M. BUTSENKO*

Zabolotnyi Institute of Microbiology and Virology, NAS of Ukraine,
154 Academika Zabolotnoho Str., Kyiv, 03143, Ukraine

*Author for correspondence; e-mail: imv_phyto@ukr.net

MODERNIZATION OF THE PSEUDOMONAS SYRINGAE PATHOVARS SEROGROUPING SCHEME

In 1979, L.T. Pastushenko and I.D. Symonovych developed a scheme of serogrouping phytopathogenic bacteria of the Pseudomonas genus, which is still used now. However, today's using this serogrouping scheme is complicated by the lack of all data accumulated over the years of its application. Moreover, the scheme does not correspond to the modern taxonomy of phytopathogenic bacteria of the Pseudomonas genus. ***Aim.*** *On the basis of own experimental results and data of scientific literature, to carry out modernization of the serogrouping scheme of phytopathogenic bacteria of the Pseudomonas genus.* ***Methods.*** *The strains of Pseudomonas syringae pathovars such as atrofaciens, coronafaciens, tabaci, which were isolated from plants of wheat, rye, oats, tobacco, and various species of affected weeds in different regions of Ukraine have been studied in the work. Antigenic properties of bacterial strains were studied by agglutination and precipitation reactions (the Ouchterlony double immunodiffusion techniques) using antisera to P. syringae strains of nine serological groups (I, II, III, IV, V, VI, VII, VIII, IX). To carry out the precipitation reaction, O- and OH-antigens were obtained by a modified Grasse's method. The presence of the same number of precipitation lines of the studied antigens as the number of lines with homologous antiserum of the corresponding serogroup testified to their belonging to this serogroup according to the known serogrouping scheme of phytopathogenic bacteria developed in 1979 by L.T. Pastushenko and I.D. Symonovych.* ***Results.*** *It has been proved that strains of P. syringae pathovars isolated from different cereals (rye, wheat, oats) and segetal vegetation differ in antigenic composition. The antigenic composition of P. syringae strains depends on the host plant from which the pathogen was isolated. Strains of the causative agent of basal glume rot P. syringae pv. atrofaciens isolated from wheat belong to four serological groups (II, IV, V, VI), from rye — to five serological groups (I, II, IV, V, VI), as well as strains of this pathogen isolated from segetal vegetation of wheat agrophytocenosis. Strains of the halo bligth P. syringae pv. coronafaciens from affected oat plants belong to two serological groups (I, V). The serogrouping scheme has been supplemented by new data on the antigenic properties of P. syringae pv. tomato, the causative agent of the bacterial speck disease of tomatoes (Solanum lycopersicum), which is classified as serogroup IV. It has been found that P. syringae pv. tabaci strains, which cause wildfire of tobacco, are part of three serogroups — VII, VIII, IX, and not of two ones, as was presented in the known scheme (1979).* ***Conclusions.*** *Therefore, on the basis of our*

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own research and literature data, the serogrouping scheme of phytopathogenic bacteria of the Pseudomonas genus developed by L.T. Pastushenko and I.D. Symonovych has been modernized. In the renovated scheme, current species' names of phytopathogens are present, this scheme introduces new information about the serogroups of P. syringae pv. atrofaciens isolated from rye seeds and plants, weeds, P. syringae pv. coronafaciens — from oat plants, P. syringae pv. tabaci — from tobacco, and P. syringae pv. tomato — from affected tomato plants.

Keywords: *Pseudomonas syringae, pathovars, agglutination, precipitation, serogrouping scheme, serogroups.*

Phytopathogenic bacteria of *Pseudomonas syringae* pathovars cause diseases of most species of cultivated plants and many wild species. The taxonomic status of pathovars of this species of microorganisms has been debated over the past fifty years and has not yet been definitively established. Depending on the ability to infect a particular plant species, *P. syringae* bacteria were divided into 40 pathovars [1, 2]. The use of new research methods has established the existence of the so-called "*P. syringae* complex", which includes up to ten species of *Pseudomonas* and 60 pathovars of *P. syringae* [3]. Based on the results of ribotyping and DNA-DNA hybridization of strains of each pathovar of *P. syringae*, nine genotypes were identified [4]. However, for a larger number of bacterial strains, no phenotypic or biochemical characteristics were identified on the basis of which they could be attributed to a particular pathovar or genotype. Immunospecificity-based methods are used to address the taxonomic status and identify strains of phytopathogenic bacteria. The use of such methods is based on knowledge of the serological specificity of bacteria. According to serological properties, strains of *P. syringae* pathogens are heterogeneous, and strains of one pathogen can belong to several serological groups [5—7]. Therefore, the identification of *P. syringae* strains must take into account their serological heterogeneity.

Researchers have long been involved in finding species- or strain-specific bacterial antigens. L.T. Pastushenko and I.D. Symonovych found a difference in the set of OH- antigens of phytopathogenic pseudomonas, using cross-agglutination reactions of OH- sera and bacterial cells [8]. Based on the identified group-specific antigens in the gel agar precipitation reaction using O- and OH-antisera and thermolabile and thermostable antigens obtained by the Grasse method, the serogrouping scheme of phytopathogenic bacteria of the *Pseudomonas* genus was developed [9]. The scheme includes nine groups. It has been successfully used by employees of the Department of Phytopathogenic Bacteria for a long time. It should be noted that many scientists have devoted their research to the development of serogrouping schemes for phytopathogenic bacteria of the genus *Pseudomonas* [6, 7, 10, 11]. However, to date, there has been no serotyping scheme for these bacteria, which could be generally accepted [12, 13]. Therefore, we continue to use the serogrouping scheme developed by L.T. Pastushenko and I.D. Symonovych, which over time has been supplemented with new data on the serogrouping of *P. syringae* pathovars, obtained by the employees of the Department of Phytopathogenic Bacteria. As a result, today the application of this scheme is complicated by the lack of all data accumulated over the years of its application. Besides, it does not correspond to the modern taxonomy of phytopathogenic bacteria of the genus *Pseudomonas*. After all, over the past twenty years, the taxonomy of phytopathogenic bacteria of the genus *Pseudomonas* has undergone significant changes: some species have been transferred to the rank of *Pseudomonas syringae* pathovars or even taken out of the boundaries of the species.

Therefore, the **purpose** of our work was to modernize the serogrouping scheme of phytopathogenic bacteria of the *Pseudomonas* genus developed by L.T. Pastushenko and I.D. Symonovych on the basis of our own experimental results and data from the scientific literature.

Materials and methods. Strains of phytopathogenic bacteria *Pseudomonas syringae* pathovars of *atrofaciens*, *coronafaciens*, and *tabaci*, which were isolated in different regions of Ukraine from plants of wheat, rye, oats, tobacco, and various species of affected weeds, have been studied. The antigenic properties of bacterial strains were studied using agglutination and precipitation reactions [14, 15]. We used antisera to *P. syringae* strains of nine serological groups as follows: *P. syringae* pv. *syringae* 8511 (UKM B-1027, NCPPB 281), *P. syringae* pv. *atrofaciens* 8281 (UKM B-1013) — serogroup I; *P. syringae* pv. *atrofaciens* K-1025 — serogroup II; *P. syringae* pv. *syringae* P-55 — serogroup III: *P. syringae* pv. *atrofaciens* 4394 (PDDCC 4394, UKM B-1011) — serogroup IV; *P. syringae* pv. *atrofaciens* 948 — serogroup V; *P. syringae* pv. *atrofaciens* 7194 (UKM B-1115) — serogroup VI; *P. syringae* pv. *tabaci* 223 — serogroup VII; *P. syringae* pv. *tabaci* 225 — serogroup VIII; *P. syringae* pv. *lachrymans* 7591 — serogroup IX.

The specific precipitation reaction (the Ouchterlony double immunodiffusion technique) was used to determine the affiliation of strains to specific serological groups [14]. To carry out the reaction, O- and OH- antigens were obtained by the modified Grasse method [16]. To determine the affiliation of the studied phytopathogenic strains to the serological group, the known serogrouping scheme of phytopathogenic bacteria was used [9].

In order to renovate the serogrouping scheme of *P. syringae* pathovars, the scientific literature was analyzed and changes were made regarding the taxonomic status of phytopathogenic bacteria.

Results. On the basis of the results obtained, the serogrouping scheme developed by L.T. Pastushenko and I.D. Symonovych [9] was added with information on the serological properties of *P. syringae* pv. *atrofaciens* isolated from different cereals. It has been found that strains of *P. syringae* pathovars, isolated from different cereals (rye, wheat, oats) and segetal vegetation, differ in antigenic composition, which depends on the host plant the pathogen was isolated from.

As it has been found, strains of the causative agent of basal bacteriosis *P. syringae* pv. *atrofaciens* isolated from wheat grown in different farming systems belong to four serological groups, namely II, IV, V, VI [17] (Table 1).

Representatives of *P. syringae* pv. *atrofaciens* isolated from affected seeds and rye plants belong to five serological groups — I, II, IV, V, VI [18], as well as strains of this pathogen isolated from segetal vegetation of wheat agrophytocenosis (Table 1).

Strains of the halo blight pathogen *P. syringae* pv. *coronafaciens* from affected oat plants belong to two serological groups (I and V) (Table 1).

Strains of *P. syringae* pv. *atrofaciens* isolated from cereals with homologous antisera formed 2—3 lines of precipitation, on the basis of which they are assigned to the appropriate serogroups. Strains of serogroup V isolated from wheat did not react with antisera to representatives of other serogroups (Table 2). Representatives of *P. syringae* pv. *atrofaciens* serogroup IV in addition to 2—3 lines of precipitation with homologous antiserum, formed one line of precipitation with antisera to strains of this species of serogroups II and VI. Strains of serogroup VI also reacted with antisera to representatives of serogroups II and IV. This indicates that they have common antigens with representatives of other serogroups (II, IV), in addition to antigens characteristic for serogroup VI.

Strains of *P. syringae* pv. *atrofaciens* isolated from weeds give a precipitation reaction (2—3 lines) with homologous sera only.

Table 1. **Serological groups of bacteria isolated from cereals and weeds**

Bacteria	Host plant	Distribution of strains into serogroups,%				
		I	II	IV	V	VI
P. syringae pv. *atrofaciens*	Wheat	0	31	51	5	13
	Rye	50	26	11	4	9
	Weeds	3	22	53	3	13
P. syringae pv. *coronafaciens*	Oat	14	0	0	86	0

Agglutination and precipitation reactions are used to determine the antigenic properties of bacteria [14, 15]. To establish the affiliation of the experimental strains of *P. syringae* pathovars to a particular serogroup, we used the double immunodiffusion techniques for O- and OH- antigens with antisera of those serological groups with which the studied antigens in the agglutination reaction react in high titer — 6400-12800-25600. The presence of the same number of precipitation lines of the studied antigens as the number of lines with homologous antiserum of the corresponding serogroup indicated their belonging to this serogroup.

Table 2. **Interpretation of the results of the precipitation reaction in establishing the serological affiliation of *P. syringae* pv. *atrofaciens***

P. syringae pv. *atrofaciens* strains	Number of precipitation lines with antisera to *P. syringae* strains, serogroups				
	I	II	IV	V	VI
Strains of serogroup I	2—3	0	0	0	0
Strains of serogroup II	0	2—3	0	0	0—1
Strains of serogroup IV	0	1	2—3	0	1
Strains of serogroup V	0	0	0	2—3	0
Strains of serogroup VI	0	1	1	0	2—3

The serogrouping scheme [9] is supplemented with new data on the antigenic properties of the agent *P. syringae* pv. *tomato*, not represented in it. Strains C-9, C-13, C-28, and C-46 obtained from affected tomatoes showed a serological affinity with antiserum to a typical strain of *P. syringae* pv. *tomato* R140.

The high titer in the agglutination reaction of the studied strains with antiserum to representatives of serogroup IV and the lack of positive reaction with antisera to representatives of other serogroups indicate their belonging to serogroup IV similar to the serogroup of phytopathogenic bacteria of *P. syringae* [19].

The activity of heat-stable somatic antigens of strains *P. syring*ae pv. *tabaci* with the antisera

Table 3. **Antigenic characteristics of *P. syringae* pv. *tabaci* strains**

Antigens of strains	Quantity of lines in ODD with serogroup antisera									Serogroup
	I	II	III	IV	V	VI	VII	VIII	IX	
Homologous strain system	2	3	2	2	2	2	2	2	2-3	
P-27	0	0	0	1 w	0	0	nd	2	1 w	VIII
P-28	0	0	0	1 w	0	0	nd	2	3	IX
P-29	0	0	0	0	0	0	2	0	1 w	VII
P-30	0	0	0	0	0	0	2	0	1 w	VII
P-31	0	0	0	0	0	0	2	0	1 w	VII
P-32	0	0	0	1 w	0	0	0	2	1 w	VIII
P-33	0	0	0	1 w	0	0	0	2	1 w	VIII
P-34	0	0	0	1 w	0	0	0	2	1 w	VIII
P-35	0	0	0	1 w	0	0	0	2	1 w	VIII
P-36	0	0	0	1 w	0	0	0	2	1 w	VIII
P-37	0	0	0	0	0	0	0		2	IX
P-38	0	0	0	1 w	0	0	0	2	1 w	VIII
P-39	0	0	0	1 w	0	0	0	2	1 w	VIII

w — weak line, nd — not determined

of nine *Pseudomonas syringae* serogroups of L.T. Pastushenko and I.D. Symonovych [9] was used for serological classification of *P. syringae* pv. *tabaci*. As a result, most of the strains showed high serological activity to serogroup VIII. Minor antigens gave a reaction with antisera to strains of serogroups IV and IX (Table 3).

Several strains of *P. syringae* pv. *tabaci* formed two lines of precipitation with antiserum to serogroup VII. They had low antigenic relationships with representatives of serogroup IX, forming one weak line precipitation with antiserum to serogroup IX strains. *P. syringae* pv. *tabaci* P-37 revealed antigenic complexes only with serogroup IX [20].

Based on these findings, strains of the causal agent of wildfire of tobacco *P. syringae* pv. *tabaci* are assigned to three serological groups (VII, VIII, IX) according to the scheme [9].

Thus, the serogrouping scheme of L.T. Pastushenko and I.D. Symonovych [9] is expanded by the number of serological groups of *P. syringae* pv. *tabaci*.

Given that phytopathogenic species of bacteria of the genus *Pseudomonas* were transferred to the rank of *Pseudomonas syringae* pathovars and new results obtained in the study of serological properties of *P. syringae* strains, the updated serogrouping scheme of bacteria is as follows (Table 4).

Table 4. **Serological groups of phytopathogenic bacteria of *Pseudomonas syringae* pathovars**

Bacteria	Host plant	Serological group								
		I	II	III	IV	V	VI	VII	VIII	IX
P. syringae (P. cerasi)*	Fruit trees, poplars		+							
P. syringae (P. holci)*	Sorghum, Sudan grass	+	+		+		+			
P. syringae (P. lupini)*	Lupine	+	+	+	+					
P. syringae (*P. wieringae*)*	Beet				+					
P. syringae (P. syringae f. populi)*	Fodder beans		+							
P. syringae (P. syringae f. populi)*	Fruit trees		+	+						
P. syringae (P. vignae)*	Bean				+					
P. syringae pv. *aptata*	Beet				+					
P. syringae pv. *atrofaciens*	Wheat		+		+	+	+			
P. syringae pv. *atrofaciens*	Rye	+	+		+	+	+			
P. syringae pv. *atrofaciens*	Weeds	+	+		+	+	+			
P. syringae pv. *coronafaciens*	Oat	+				+				
P. savastanoi pv. *glycinea (P. syringae* pv. *glycinea**)*	Soya				+					
P. syringae pv. *lachrymans*	Cucumbers, pumpkins									+
P. syringae pv. *maculicola*	Cabbage	+								
P. syringae pv. *morsprunorum*	Fruit trees		+							
P. savastanoi pv. *phaseolicola (P. syringae* pv. *phaseolicola**)*	Bean						+			
P. syringae pv. *pisi*	Pisi				+					
P. syringae pv. *syringae*	Fruit trees, poplars	+	+	+						
P. syringae pv. *tabaci*	Tobacco							+	+	+
P. syringae pv. *tomato*	Tomato				+					

Note: * — the pathovar status was not assigned [1]; ** — synonym (another scientific name)

Discussion. Based on the molecular genetic studies, it has been established that phytopathogenic species of bacteria of the genus *Pseudomonas* have been transferred to the rank of *Pseudomonas syringae* parhovars [1]. Therefore, these data are included in the known the serogroupings scheme of phytopathogenic bacteria (Table 4). Some pathogens are not assigned the rank of pathovars. These are bacteria *P. cerasi* isolated from fruit trees and poplar, *P. holci* — from sorghum and Sudan grass, *P. lupini* — from lupine, *P. wieringae* — from beets, *P. syringae* f. *populi* — from fodder beans and fruit trees, *P. vignae* — from beans. Therefore, these agents of bacterial diseases belong to the species *P. syringae*. Two species of bacteria *P. glycinea* and *P. phaseolicola* based on DNA-DNA hybridization are identified as *Pseudomonas savastanoi* pv. *glycinea* and *P. savastanoi* pv. *phaseolicola*, respectively [21]. But in the scientific literature, some researchers use other names for these bacteria — *Pseudomonas syringae* pv. *glycinea* and *P. syringae* pv. *phaseolicola* [4, 22]. Therefore, in the updated of serogrouping scheme of phytopathogenic bacteria both names of these bacteria are given (Table 4). Moreover, the strains of these species react with antisera to representatives of the *P. syringae* pathovars of the corresponding serogroups and belong to the serological groups of the scheme L.T. Pastushenko and I.D. Symonovych (IV and VI).

The results of studying the serological properties of *P. syringae* pv. *atrofaciens* strains isolated from rye seeds and plants and *P. syringae* pv. *coronafaciens* from oat were added to the before-developed serogrouping scheme. Strains of *P. syringae* pv. *atrofaciens* belong to five serological groups (I, II, IV, V, VI), whereas those of *P. syringae* pv. *coronafaciens* from oat — to two groups (I, V). On the affected wheat plants, the agent *P. syringae* pv. *atrofaciens* belonging to serogroup I was not detected, but it was revealed among strains of phytopathogenic bacteria isolated from weeds of wheat agrophytocenosis.

It has been found that strains of serogroup I bacteria are characteric for *P. syringae* pv. *atrofaciens* isolated from rye [17, 18] and for *P. syringae* pv. *coronafaciens* from oats. Bacterial strains belonging to serogroup I are found among other pathovars of *P. syringae*, namely *syringae* and *maculicola*.

Thus, serogroup I has been supplemented with representatives of two pathogens — *P. syringae* pv. *atrofaciens* and *P. syringae* pv. *coronafaciens*, and serogroup V — with *P. syringae* pv. *coronafaciens*.

The serogrouping scheme of phytopathogenic bacteria of the genus *Pseudomonas* includes information on the serological properties of *P. syringae* pv. *tomato*, which belongs to serogroup IV [19].

On a wider number of *P. syringae* pv. *tabaci* strains, it has been found that the bacteria of this pathovar are part of three serogroups — VII, VIII, and IX. Moreover, strains of *P. syringae* of other pathovars are not part of serogroups VII and VIII; to serogroup IX in addition to *P. syringae* pv. *tabaci* belong only the bacterium *P. syringae* pv. *lachrymans* (Table 4). Strains of *P. syringae* belonging to serogroups III and V are rare. As for serogroup III bacteria, they are strains of *P. syringae* isolated from affected fruit and tree crops [9]. Serogroup V includes only strains of *P. syringae* pv. *atrofaciens* and *P. syringae* pv. *coronafaciens*, which affect cereals and weeds of wheat agrocenosis, but in small amount [5, 13, 17]. Strains of *P. syringae* pv. *atrofaciens* of this serogroup isolated from cereals are not numerous not only in Ukraine but also in Russia and the USA, and in Bulgaria they were not found at all [5]. It should be noted that strains of *P. syringae* pv. *coronafaciens* belonging to two serogroups (I and V) dominate in serogroup V [13].

The presence of *P. syringae* pv. *atrofaciens* strains in serogroups I and V isolated from weeds of wheat agrophytocenosis indicates their greater ability to adapt and survive in changing environmental conditions compared to this phytopathogen isolated from wheat.

Strains of *P. syringae* pv. *atrofaciens* belonging to serogroups II and IV dominated among the strains isolated from not only cereals but also other pathovars of *P. syringae*, representatives of agrophytocenosis [13]. It should be noted that serogroup IV includes the largest number of *P. syringae* pathovars that affect different crops (Table 4).

The predominance of bacterial strains of certain serogroups can be determined by their way of life and the host plant, as well as by their greater adaptability and survival in different conditions. In favor of the confinement of certain serogroups of phytopathogens to the host plant, there is evidence that they are usually represented by a large number of bacterial strains.

Conclusions. Thus, based on our research and literature data the serogrouping scheme of phytopathogenic bacteria of the *Pseudomonas* genus, developed by L.T. Pastushenko and I.D. Symonovych [9], has been updated. It presents modern species names of phytopathogens and introduces new information about the serogrouping of *P. syringae* pv. *atrofaciens* strains isolated from rye seeds and plants, and weeds, *P. syringae* pv. *coronafaciens* — from oat plants, *P. syringae* pv. *tabaci* — from tobacco, and *P. syringae* pv. *tomato* — from affected tomato plants.

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Л.А. Пасічник, Л.М. Буценко

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143, Україна

МОДЕРНІЗАЦІЯ СХЕМИ СЕРОГРУПУВАННЯ ПАТОВАРІВ *PSEUDOMONAS SYRINGAE*

У 1979 році Л.Т. Пастушенко та І.Д. Симонович розробили схему серогрупування фітопатогенних бактерій роду *Pseudomonas*, яку спеціалісти використовують і сьогодні. Але застосування цієї схеми ускладнено відсутністю в ній усіх накопичених за роки її застосування даних і невідповідністю її сучасній таксономії фітопатогенних бактерій роду *Pseudomonas*. **Мета.** На основі власних експериментальних результатів і даних наукової літератури здійснити модернізацію схеми серогрупування фітопатогенних бактерій роду *Pseudomonas*. **Методи.** В роботі досліджено штами фітопатогенних бактерій *Pseudomonas syringae* патоварів *atrofaciens, coronafaciens, tabaci*, які ізольовані з рослин пшениці, жита, вівса, тютюну і різних бур'янів в різних регіонах України. Антигенні властивості штамів бактерій вивчали за реакціями аглютинації та преципітації (подвійної дифузії в агарі за Оухтерлоні) з використанням антисироватки до штамів *P. syringae* дев'яти серологічних груп (I, II, III, IV, V, VI, VII, VIII, IX). Для постановки цієї реакції отримували О- і ОН- антигени за модифікованим методом Грасе. Наявність однакової кількості ліній преципітації досліджуваних антигенів з кількістю ліній з гомологічною антисироваткою відповідної серогрупи свідчило про приналежність їх до цієї серогрупи за відомою схемою серогрупування фітопатогенних бактерій, розробленої в 1979 році Л.Т. Пастушенко та І.Д. Симонович. **Результати.** Доведено, що штами патоварів *P. syringae*, ізольовані з різних зернових культур (жита, пшениці, вівса) і сегетальної рослинності за антигенним складом різняться. Останній залежить від рослини-хазяїна, з якої вилучено патоген. Штами збудника базального бактеріозу *P. syringae* pv. *atrofaciens*, ізольовані з пшениці, належать до чотирьох серологічних груп (II, IV, V, VI), з жита — до п'яти серологічних груп (I, II, IV, V, VI), як і штами цього збудника, ізольовані з сегетальної рослинності агрофітоценозу пшениці. Штами збудника ореольного бактеріозу *P. syringae* pv. *coronafaciens* з уражених рослин вівса характеризуються належністю до двох серологічних груп (I, V). Схема серогрупування доповнена новими даними з антигенних властивостей *P. syringae* pv. *tomato*, збудника бактеріальної плямистості томатів (*Solanum lycopersicum*), який віднесено до серогрупи IV. На ширшому наборі штамів *P. syringae* pv. *tabaci*, які спричинюють дикий опік тютюну, встановлено, що бактерії цього патовару входять до складу трьох серогруп — VII, VIII і IX, а не до двох, як було представлено у відомій схемі 1979 року. **Висновки.** Отже, на основі власних досліджень та даних літератури модернізовано схему серогрупування фітопатогенних бактерій роду *Pseudomonas*, розроблену Л.Т. Пастушенко і І.Д. Симонович. У ній наведено сучасні видові назви фітопатогенів та внесено нову інформацію про серогрупування штамів *P. syringae* pv. *atrofaciens*, ізольованих із насіння та рослин жита, бур'янів, *P. syringae* pv. *coronafaciens* — з рослин вівса, *P. syringae* pv. *tabaci* — з тютюну, *P. syringae* pv. *tomato* — з уражених рослин томатів.

Ключові слова: *Pseudomonas syringae, патовари, аглютинація, преципітація, схема серогрупування, серогрупи.*