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EXPERIMENTAL ARTICLES

The Serological Heterogeneity of *Pseudomonas syringae* pv. *atrofaciens* Strains and Their Ecological Niches

L. A. Pasichnik*,[1], L. M. Yakovleva*, R. I. Gvozdyak*, and V. I. Vassilev**

**Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, ul. Zabolotnogo 154, Kiev, 03143 Ukraine*

***Malkov Institute for Plant Genetic Resources, 4122 Sadovo, Plovdiv, Bulgaria*

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Abstract—The paper deals with a comparative analysis of the serological and ecological properties of *Pseudomonas syringae* pv. *atrofaciens* strains from the collections of microbial cultures at the Malkov Institute for Plant Genetic Resources and Zabolotny Institute of Microbiology and Virology. All of the strains from the Bulgarian collection, except for one, fall into five serogroups (II through VI) of the classification system of Pastushenko and Simonovich. The *P. syringae* pv. *atrofaciens* strains isolated from Bulgarian and Ukrainian wheats belong mainly to serogroups II and IV, respectively. The strains that were isolated from rye plants belong to serogroup I. The strains isolated from sorghum and Sudan grass belong to serogroups II, IV, and VI. Serogroup III includes the *P. syringae* pv. *atrofaciens* strains that were isolated from cereals in the United Kingdom but not in Ukraine.

Key words: *Pseudomonas syringae* pv. *atrofaciens*, serogrouping, cereals, ecological niches.

Pseudomonas syringae pv. *atrofaciens* (McCulloch 1920) Young *et al.* 1978 is the causal agent of basal glume rot of cereals, which can affect all parts of the plant and grain, causing spot blotch and empty ears and thus reducing the crop yield. The disease is widespread in Central and Eastern Europe [1–4]. In Bulgaria, *P. syringae* pv. *atrofaciens* often occurs in associations with fungi of the genera *Alternaria, Fusarium*, and *Drechslera*. In Ukraine, *P. syringae* pv. *atrofaciens* parasitizes on wheat, rye, and barley. The strains isolated from affected and host plants are similar in physiological and biochemical characteristics but differ serologically. According to the presence of specific thermostable antigenic complexes, the *P. syringae* pv. *atrofaciens* strains isolated from affected wheat tissue were divided by Pastushenko *et al.* [5] into four groups, which correspond to serogroups II, IV, V, and VI of the classification system of Pastushenko and Simonovich [6]. The *P. syringae* pv. *atrofaciens* strains isolated from Ukrainian ryes fall into five (I, II, IV, V, and VI) serogroups [7]. No relationship was revealed between the serological properties of *P. syringae* pv. *atrofaciens* strains and the degree of their virulence, the plant organ from which they were isolated, and the region of their occurrence (steppe, forest steppe, and Polesie) in Ukraine.

This work was undertaken with the aim to establish a relationship between the serological and ecological properties of *Pseudomonas syringae* pv. *atrofaciens* strains from the Bulgarian and Ukrainian collections of microbial cultures.

[1] Corresponding author. E-mail: phyto_pas@mail.ru

MATERIALS AND METHODS

The organisms studied in this work were 16 strains (Table 1) of *P. syringae* pv. *atrofaciens* (McCulloch 1920) Young *et al.* 1978 from the collection of microbial cultures at the Malkov Institute for Plant Genetic Resources, including 14 strains isolated in Bulgaria, 1 strain isolated in the United Kingdom, and 1 strain isolated in the United States. The strains were isolated from the affected plants of the wheats *Triticum aestivum* and *Triticum monococcum*, 6 strains being isolated from black glumes and grains, 6 strains from necrotic leaves, 1 strain from an ungerminated pink grain, 1 strain from the root rot zone, and 2 strains from zones with unknown lesion types.

The strains were tested for pathogenicity by injecting them into wheat plants in the booting stage. The serological properties of the strains were studied by the agglutination and the Ouchterlony double immunodiffusion techniques [8] using the O and OH antigens prepared by a modified method of Grasse [10]. Immune antisera were prepared by the conventional method [9] to the representatives of nine serogroups [6] obtained from the collection of microbial cultures at the Zabolotny Institute of Microbiology and Virology: *P. syringae* pv. *syringae* 8511 (PDDCC 281, UKM B-1027) of serogroup I; *P. syringae* pv. *atrofaciens* K-1025 and 7864 of serogroup II; *P. syringae* pv. *syringae* P-55 of serogroup III; *P. syringae* pv. *atrofaciens* ICMP 4394 (UKM B-1011) of serogroup IV; *P. syringae* pv. *atrofaciens* 948 of serogroup V; *P. syringae* pv. *atrofaciens* 7194 (UKM B-1115) and *P. syringae* pv. *syringae* 8299 of serogroup VI; *P. syringae* pv. *tabaci* 223 of serogroup VII; *P. syringae*

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Table 1. Characteristics of the strains under study

Strain	Country, author, year	Host species and cultivar	Lesions
P1	Bulgaria, V. Vassilev, 1978	*Triticum aestivum*, Sadovo-1	Typical lesions of basal glume rot with black glumes
P2	Bulgaria, V. Vassilev, 1979	*T. aestivum*, 28	Typical lesions of basal glume rot with black glumes
P3	Bulgaria, V. Vassilev, 1979	*T. aestivum*, Sadovo S	Brown spot at the leaf base
P4	Bulgaria, V. Vassilev, 1980	*T. aestivum*, Trakiya	Small necrotic spots with yellow aureoles on the leaf surface
P5	Bulgaria, V. Vassilev, 198	*T. aestivum*, Kavkaz	Dry rot of boot leaves
P6	Bulgaria, V. Vassilev, 1981	*T. aestivum*, Skorospelka 35	Typical lesions of basal glume rot with black glumes
P7	Bulgaria, V. Vassilev, 1981	*T. monococcum*	Small necrotic spots with yellow aureoles on the leaf surface
P8	Bulgaria, V. Vassilev, 1983	*T. aestivum*, Charodeika	Root rot, yellowing and stunting of plants
P9	Bulgaria, V. Vassilev, 1982	*T. aestivum*, Rubezh	Ungerminated pink grain
P10	Bulgaria, V. Vassilev, 1982	*T. aestivum*, 301	Typical lesions of basal glume rot with black glumes
P11	Bulgaria, V. Vassilev, 1983	*T. aestivum*, Rusalka	Leaf blotch
P12	Bulgaria, V. Vassilev, 1986	*T. aestivum*, 03348K	Yellow leaf with black strokes
P13	Bulgaria, V. Vassilev, 1986	*T. aestivum*, Kiten	Black strokes on glume
P14	Bulgaria, V. Vassilev, 1986	*T. aestivum*, Montojoe	Typical lesions of basal glume rot with black glumes
P18	United Kingdom, NCPPB117	*T. aestivum*	
P21	USA, J.D. Otta, 1972	*T. aestivum*	

Note: Empty cells mean that no data was available. NCPPB, National Collection of Phytopathogenic Bacteria, the United Kingdom.

pv. *tabaci* 225 of serogroup VIII; and *P. syringae* pv. *lachrymans* 7595 (UKM B-1039) of serogroup IX. The strains under study were classified into serogroups according to the classification scheme of Pastushenko and Simonovich [6].

RESULTS AND DISCUSSION

Experiments showed that 14 of the 16 *P. syringae* pv. *atrofaciens* strains obtained from the Bulgarian collection of microbial cultures were pathogenic to wheat, whereas the remaining 2 strains (P8 and P14) were avirulent. The virulence of the strains did not depend on the time of their storage in the collection.

Agglutination reactions with the OH antisera to the nine serogroups (Table 2) showed that the 16 Bulgarian *P. syringae* pv. *atrofaciens* strains were serologically heterogeneous, some species-specific agglutinogens of these strains being common to several of the *P. syringae* pathovars (*atrofaciens*, *syringae*, *tabaci*, and *lachrymans*).

The investigation of the *P. syringae* pv. *atrofaciens* strains by the more specific serological reaction of double immunodiffusion (which is commonly used for the serogrouping of phytopathogenic bacteria *P. syringae* [6, 7, 11–13]) showed that strains P1–P7 and P9–P11 produced two to four precipitin lines with the antisera to the *P. syringae* pv. *atrofaciens* K-1025 and 7864 strains of serogroup II (Table 3). Correspondingly, the ten strains (P1 through P7 and P9 through P11) were referred to this serogroup. The *P. syringae* pv. *atrofaciens* strains from serogroup II were heterogeneous with respect to their thermostable antigens and the structure of O-specific polysaccharides [14].

Strains P8 and P13 produced three precipitin lines with the antisera to the *P. syringae* pv. *atrofaciens* of serogroup VI, whereas strain P12 produced three lines with the antiserum to strain 4394 from serogroup IV. The strain *P. syringae* pv. *atrofaciens* P18 isolated from affected wheat plants in the United Kingdom produced three precipitin lines with the serogroup III antiserum and one faint line with the antisera to the members of serogroups II and VI. Strain P21 reacted only with the serogroup V antiserum. Strain P14 produced a faint precipitin line with the antiserum to *P. syringae* pv. *atrofaciens* 4394 (serogroup IV), which did not allow this strain to be assigned to any of the serogroups. None of the strains under study produced precipitin lines with the antisera to serogroups VII, VIII, and IX. For this reason, the respective data are not presented in Table 3.

In the character and degree of antigenic affinity, the *P. syringae* pv. *atrofaciens* strains from the Bulgarian collection of microbial cultures can be divided into five serogroups, II through VI (Table 4). Earlier, the classification system of Pastushenko and Simonovich served as the basis for the structural investigation of O-specific lipopolysaccharides and their relationship with the serological specificity of bacteria [15–18]. The avirulent strain P14 could not be assigned to any of the nine known serogroups and may represent a new serogroup within the classification system of Pastushenko and Simonovich. It should be noted that four of the five serogroups of *P. syringae* pv. *atrofaciens* that we identified correspond to sero-

Table 2. The agglutination reactions of the *P. syringae* pv. *atrofaciens* strains

Strains	Titers of agglutination reactions with antisera to members of nine serogroups								
	8511 I	K1025 II	P-55 III	4394 IV	948 V	8299 VI	223 VII	225 VIII	7595 IX
P1	3200	25600	1600	6400	3200	25600	400	3200	400
P2	3200	25600	1600	12800	800	12800	100	3200	800
P3	6400	12800	800	51200	1600	12800	100	3200	400
P4	0	51200	400	12800	51200	51200	51200	51200	200
P5	6400	12800	800	12800	1600	25600	100	1600	400
P6	12800	25600	1600	25600	800	25600	100	3200	400
P7	6400	12800	1600	12800	800	25600	100	1600	200
P8	3200	12800	12800	12800	800	25600	100	800	100
P9	3200	25600	3200	12800	3200	51200	200	51200	200
P10	3200	12800	1600	12800	800	51200	100	800	0
P11	6400	51200	800	25600	400	51200	0	3200	100
P12	3200	3200	200	12800	3200	51200	51200	25600	0
P13	800	6400	25600	100	3200	51200	100	800	200
P14	0	200	100	800	200	100	0	0	100
P18	6400	6400	51200	51200	12800	25600	100	51200	51200
P21	3200	1600	400	6400	6400	3200	0	800	100
8511	**25600**	3200	800	6400	6400	100	6400	400	100
K-1025	1600	**51200**	3200	25600	6400	51200	100	1600	100
P-55	100	25600	**51200**	25600	3200	25600	100	100	800
4394	800	6400	100	**6400**	1600	3200	0	3200	0
948	3200	1600	0	12800	**25600**	400	0	800	100
8299	800	12800	12800	200	6400	**51200**	0	800	200
7595	100	100	800	400	400	400	0	6400	**12800**
223	3200	100	200	12800	200	800	**6400**	0	100
225	0	0	0	3200	400	800	0	**12800**	1600

Note: Titers of homologous agglutination reactions are given in bold.

groups SYR1, MOP2, APTRIS, and PHA within the classification system of Saunier *et al.* [19] (Table 4). As can be seen from this table, ten *P. syringae* pv. *atrofaciens* strains belong to serogroup II and two strains (P8 and P13) belong to serogroup VI, whereas each of the other serogroups (III, IV, and V) includes only one strain. None of the *P. syringae* pv. *atrofaciens* strains was found to belong to serogroup I, which is the most abundant serogroup among the Ukrainian strains of *P. syringae* pv. *atrofaciens* pathogenic to rye. The data presented here are in agreement with the earlier observations that the *P. syringae* pv. *atrofaciens* strains of serogroup V are few [6, 7, 20].

Serogroup III includes only one *P. syringae* pv. *atrofaciens* strain, P18, which was isolated in the United Kingdom. One of the three *P. syringae* strains classified by Pastushenko and Simonovich into serogroup III was also isolated in the United Kingdom (from a fruit crop). These data suggest that the *P. syringae* strains of serogroup III are restricted to the United Kingdom. It is likely that the *P. syringae* strains of this serogroup are the least frequent in nature. For instance, among the 16 *P. syringae* pv. *atrofaciens* strains from Canada, New Zealand, Greece, Germany, the United States, and Bulgaria classified by Saunier *et al.* [19], only one strain (*P. syringae* pv. *atrofaciens* 2256 from Greece) was found to belong to serogroup MOP2, which corresponds to serogroup III within the classification system of Pastushenko and Simonovich.

In general, the *P. syringae* pv. *atrofaciens* strains from different countries fall into six serogroups within the classification system of Pastushenko and Simonovich and into seven serogroups within the classification system of Saunier *et al.* (Table 5). It should be noted that Otta described six serogroups of the *P. syringae* strains isolated from winter wheat grain collected in the United States and Canada [12]. Unfortunately, none of the strains described by Otta was studied by Saunier *et al.* or by us. According to the data of Saunier *et al.*, the most abundant serogroup of the *P. syringae* pv. *atrofaciens* strains is SYR1, whereas APTRIS is the second-most-abundant serogroup (recall that SYR1 and APTRIS correspond to serogroups II and IV of the classification

Table 3. The antigenic properties of the *P. syringae* pv. *atrofaciens* strains in the double immunodiffusion test

Strains	The number of precipitin lines in reaction with antisera to members of various serogroups							
	8511 I	K-1025 II	7864 II	P-55 III	4394 IV	948 V	7194 VI	8299 VI
P1, P9, P10	0	**3**	**2**	0	1 (faint)	0	1	1
P11	0	**3**	**3**	0	1 (faint)	0	1	1
P2, P3, P4, P5, P6, P7	0	**4**	**2**	0	1 (faint)	0	1	1
P8	1	1 (faint)	1	0	1 (faint)	0	**3**	**3**
P12	0	1	0	0	**3**	0	1	0
P13	0	0	1	0	0	0	**3**	**3**
P14	0	0	0	0	1 (faint)	0	0	0
P18	0	0	1	**3**	0	0	0	1
P21	0	0	0	0	0	**2**	0	0
8511	**2**	0	0	0	0	ND	ND	ND
K-1025	0	**4**	**3**	0	1	0	1	1
7864	0	**4**	**3**	0	0	0	ND	0
4394	1 (faint)	1	0	0	**3**	0	0	1
948	0	ND	ND	0	ND	**2**	ND	ND
7194	0	ND	0	0	ND	ND	**3**	**3**
8299	0	0	ND	0	1	0	**3**	**3**

Note: ND stands for “not determined”.

Table 4. The serogrouping of the *P. syringae* pv. *atrofaciens* strains from the Bulgarian collection of microbial cultures

Strains	Percent of strains in the particular serogroup	Serogroup in the classification system of:	
		Pastushenko and Simonovich [6]	Saunier *et al.* [19]
P1, P2, P3, P4, P5, P6, P7, P9, P10, P11	62.5	II	SYR1
P18	6.25	III	MOP2
P12	6.25	IV	APTRIS
P21	6.25	V	
P8, P13	12.5	VI	PHA
P14	6.25	New serogroup	

Table 5. The distribution of the *P. syringae* pv. *atrofaciens* strains in different countries

Serogroup in the classification system of:		Countries	Ref.
Pastushenko and Simonovich [6]	Saunier *et al.* [19]		
I	PERSAVTOM1	Ukraine	[20]
	PERSAVTOM2		
II	SYR1	Bulgaria, Germany, Greece, Canada, New Zealand, USA, Ukraine	[6, 19] and this paper
III	MOP2	UK, Greece	[19] and this paper
IV	APTRIS	Bulgaria, Germany, New Zealand, Ukraine	[6, 19] and this paper
V		Russia, USA, Ukraine	[6] and this paper
VI	PHA	Bulgaria, Ukraine	[6] and this paper
	SYR2	Germany	[19]

system of Pastushenko and Simonovich [6]). The *P. syringae* pv. *atrofaciens* strains of these serogroups are encountered in various countries and on various continents. Serogroup I is typical of the *P. syringae* pv. *atrofaciens* strains isolated from rye [20]. The *P. syringae* pv. *atrofaciens* strains of serogroup III were isolated in the United Kingdom and Greece. The *P. syringae* pv. *atrofaciens* strains of serogroup V were isolated in the United States and Ukraine. At the same time, none of the strains of these two serogroups was found in Bulgaria.

The *P. syringae* pv. *atrofaciens* strains isolated in Bulgaria from necrotic wheat tissues were found to belong to serogroups II, IV, and VI (mainly to II). At the same time, the *P. syringae* pv. *atrofaciens* strains that were isolated in Ukraine from affected wheat plants belong to serogroup IV and those isolated from rye plants belong to serogroup I [20]. Almost all of the *P. syringae* (*P. holci*) strains isolated in Ukraine from sorghum and Sudan grass belong to serogroups II, IV, and VI, whereas only two of these strains belong to serogroup I [6]. Thus, there is some restriction of serogroups to particular host plants, which is in agreement with the observations of Otta [12]. For instance, the *P. syringae* pv. *atrofaciens* strains of serogroups II, IV, and VI were mainly isolated from affected wheat tissues. On the other hand, the pathovars *P. syringae* of serogroups VII, VIII, and IX do not occur on cereals, whereas the strains of serogroup I do occur but very rarely. Some serogroups (namely, II and IV) occur in different countries and even on different continents, which is likely due to their high adaptability to variable environmental conditions. The restriction of some serogroups to particular host plants is confirmed by the fact that such serogroups, as a rule, contain a great number of serologically related strains. For instance, most of the epiphytic *P. syringae* pv. *atrofaciens* strains isolated from rye belong to serogroup II [20]. The reason for this is as yet unknown.

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