DIVERSITY OF PHYTOPATHOGENIC BACTERIA OF GENUS XANTHOMONAS ISOLATED FROM POACEAE PLANTS IN RUSSIA

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We reviewed the published data on a composition of plant pathogenic bacteria of the genus Xanthomonas damaging plant of family Poaceae (cereals) in Russia together with our new data obtained from surveys of cereal seeds from different regions of Russia. We assayed the xanthomonads on cereal seeds from different regions and have identified for the first time pathogens similar to species *X. hortorum*, *X. campestris*, *X. cynarae*, *X. pisi*, and *X. gardeneri*, which have not been reported yet on the cereal crops.

Key words: Cereals, bacterial leaf streak, bacterial leaf blight of rice, black chaff, Xanthomonas translucens, Xanthomonas oryzae, Xanthomonas arboricola.

Cereals are main crops of agricultural production in Russia. Therefore, stable yields are a critical for national food security of Russia. However, the main grain crops (wheat, barley, rye, rice, oats) are affected by a number of pathogens that can significantly reduce the yield. Some authors described rapid spreading of bacterial diseases of cereals in recent years [1; 2]. They have underlined several main reasons of the increasing of bacterial diseases severity including:

— Emergence of new aggressive species and biotypes (races) of phytopathogenic bacteria;

— Climate changes;

— Inappropriate agrotechnology of growing, harvesting and storage of seeds;

— Lack of accurate diagnosis of pathogens [1].

Plant diseases caused by phytopathogenic bacteria of the genus Xanthomonas are among the most common and associated with serious economic losses at different regions of Russia, since they affect the most important cereals, cruciferous, solanaceous, legumes, and other crops. Bacterial diseases caused by xanthomonads on the cereal crops are among the most harmful diseases transmitted by seeds [3]. They include bacterial leaf streak (BLS) (causing agent -Xanthomonas translucens) [4]. Lesion on the plant ear is known as black chaff (BCH) is caused by the same pathogen and affects crops mostly in countries of warm and humid climate [11].

Several specialized forms of the pathogen were described based on differentiation by the preferred host plants: X. translucens pv. undulosa (wheat pathogen), X. translucens pv. secalis (rye pathogen), X. translucens pv. hordei (barley pathogen), and some others, rare in Russia.

The pathogen was first isolated and identified from barley [4], and later from wheat [5], rye [6], perennial grasses [7] and, finally, from triticale [8].

Some early reports showing wide-spread distribution of BCH were found later contradictory and unreliable, since the initial symptoms of the disease were often mixed with symptoms of abiotic stress [9; 10]. The information about spreading of the pathogen in Argentina, Brazil, China, Mexico, Pakistan, the USA, Turkey, and Ethiopia at 1980s was more reliable [11]. BLS and BCH are causing lesion on leaves, stems, stalks, grain, and the most commonly seen on the ears. Depending on the growing area and local weather favorable for development of the pathogen, this disease can reduce the potential wheat yield by 5—90%. It was proved that 50% flag leaf damage takes away 13—34% of potential yield depending on the cultivar susceptibility and climatic conditions [12].

The most harmful for cereals species — Xanthomonas oryzae, which include two pathovars: X. oryzae pv. oryzae (causing agent of bacterial leaf blight — BLB) and X. oryzae pv. oryzicola (bacterial leaf streak — BLS) [13]. Rice is the principal host plant for both pathovars. Rice crop is cultivated in Russian Federation at Northern Caucasus, Krasnodar and Far East regions on the area > 200,000 ha. The bacterial diseases are among the most dangerous for rice across the tropical and subtropical climate areas and have high phytosanitary risk for the EPPO region. Both BLS and BLB are absent in Russian Federation and Europe.

For the first time, rice leaf blight (BLB) found in Fukuoka Prefecture, Japan, in 1884 [14]. BLB is causing huge economic losses worldwide [15]. The disease is common in all rice-growing countries in Asia, Africa, Latin America, Australia (Northern Territory, Queensland), and the territory of the Caribbean region [16]. Bacterial leaf streak (BLS) was first discovered in Philippines at 1918 [14]. BLS is widespread in tropical and subtropical Asia: China, Thailand, Malaysia, India, Vietnam, Philippines and Indonesia, but has not been found yet in temperate regions, such as Japan and Korea [17]. Recently, the disease has become a serious problem in West Africa, and has reached epidemic stage in China (J.L. Notteghem, personal communication). Currently, there was no evidence of dissemination of this disease in the United States.

At 2010 we reported for the first time isolation of new bacterial pathogen of cereals and other crops — Xanthomonas arboricola [1, 3], causing loss of wheat, rye, oats, barley, sunflower, and cruciferous crops. The symptoms caused by this pathogen did not differ from diseases caused by other types of bacteria of the genus Xanthomonas, specializing on the same host plants.

Bacterial spot, blight, wilt, cancer, caused by the bacterium, can be considered as the most harmful bacterial disease for plants of families Rosaceae and Euphorbiaceae, walnut, birch, willow and banana. Bacterial diseases of plants caused by X. arboricola pv. pruni, X. arboricola pv. corylina, X. arboricola pv. juglandis, X. arboricola pv. populi were described in Russia [18], but the pathogens have not been properly studied.

Recently, there was an evidence of new aggressive strains of phytopathogenic bacteria that infect a wide range of crops that has strengthened severity of bacterial diseases and caused considerable economic losses [1]. To study the bacterial species infecting plants of cereal crops we examined almost 190 seed lots of seeds of rice, wheat, rye, and barley harvested at 2006—2012 at different regions of Russia for presence of bacterial pathogens (tabl. 1, 2). During the examination of the seed lot, we selected healthy-looking seeds and seeds with visual symptoms of disease: shriveled, underdeveloped, and gray with a yellowish exudate withered. Bacteria were cultured on medium YDC [19] at 27 °C for 48 hours. Grown single colonies of bacteria were subcultured and stored in 15% glycerol at –70 °C.

Table 1

Occurrence of *Xanthomonas* species in rice seeds of domestic origin

Region of origin	Tested seed lots number	Frequency of occurrence, % (real number)			
		X. oryzae	*X. campestris*	*X. vesicatoria*	*X. arboricola*
Kuban (provided by VNIIF)	12	0 (0)	33 (4)	0 (0)	25 (3)
Dagestan, Kizlyar	68	0 (0)	8,8 (6)	7,35 (5)	4,4 (3)
Dagestan, Kazbek reg.	20	0 (0)	0 (0)	0 (0)	5 (2)
Dagestan, Gunib reg.	10	0 (0)	20 (2)	0 (0)	0 (0)
Primorie region, Anuchin	16	0 (0)	12,5 (2)	0 (0)	6,25 (1)
Average	—	0	11,1	5,8	7,1
Total lots number*	126	0	14	5	9

Table 2

Occurrence of bacteria of the genus Xanthomonas on seeds of cereal crops

Host plant	Anaysed lots number	Frequency of occurrence, % (real number)						
		X. campestris	*X. arboricola*	*X. cynarae*	*X. hortorum*	*X. pisi*	*X. gardeneri*	*X. translucens*
Wheat	30	8,3 (2)	20 (5)	20 (5)	30 (7)	8,3(2)	8,3 (2)	0 (0)
Barley	20	0 (0)	55 (11)	0 (0)	10 (2)	0 (0)	0 (0)	5 (1)
Rye	11	18 (2)	0 (0)	36 (4)	36 (4)	0 (0)	9 (1)	0 (0)
Average %		7,27	29,1	16,3	23,6	2,6	5,4	1,8
Total	55	4	16	9	13	2	3	1

All the bacteria were tested for hypersensitive reactions (HR) on leaves of tobacco, plectrantus, and geranium, and positive were selected for pathogenicity tests. The tests of pathogenicity for all HR-positive strains were conducted on plants of families Poaceae (wheat, rye, barley, oats, rice), Solanaceae (tomato), Brassicaceae (kale, turnip, mustard), and Compositae (sunflower). The seed were provided by companies "Segris", "Our Garden", others, and checked for viability and pathogens contamination. All the seeds were surface-sterilized before sowing by 10% solution of commercial bleach. Plants were grown in 15 cm pots to the stage of 3—5 true leaf and inoculated by spraying the edge of the sheet and topping with bacterial suspension (concentration 107 CFU / ml). The suspension was a obtained from 2-days culture of the bacteria grown on YDC agar at 28 °C. Plants of each sample were inoculated into two three experiments for each strain, and incubated at constant temperature of 24 °C and 28 °C. Symptom development was counting in 20 days after inoculation using 2-point scale: 0 — no lesions, 1 — local necrosis, or vascular lesion. Virulent strains were used for further analysis.

Identification of the isolated strains was performed by PCR using specific primers [3; 13] and MultiLocus Sequencing Analysis (MLST) as described elsewhere [3; 19; 20].

Results. In many of the tested lots of cereals bacteria of the genus Xanthomonas were identified. The Table 1 shows that bacteria of the genus Xanthomonas: X. campestris, X. vesicatoria and X. arboricola were isolated from seeds of domestically grown rice in 21 lots of 126. No X. oryzae strains were found in domestically produced rice seeds.

Among the analyzed 30 seed lots of wheat, 12 lots of rye, and 20 seed lots of barley (harvest 2011) from 7 regions of Russia: Altai Krai, Voronezh region, Kursk region, Ryazan region, Tambov region, Amur Oblast, Omsk region, provided by VNIIF and Seed Testing Laboratory (Institute of General Genetics by N.I. Vavilov) we showed the presence of xanthomonads of several species in 48 of 67 seed lots, but X. translucens was found in only one lot of barley seeds (tabl. 2). Other common types of other bacteria on wheat, rye, oats, and barley seeds were: Curtobacterium spp, Pantoea sp., Pseudomonas sp., Microbacterium sp.

The Table 2 shows that X. arboricola was the most frequently found pathogenic species on the seeds of cereal crops — 22 seed lots of 190 were infected by this pathogen. This species was detected in the samples of rice, wheat and barley.

The appearance of X. arboricola in Russia on new plant species [3] at the same time increasing severity of this species in areas of traditional damage by the pathogen — Turkey, Caucasus, Balkans, Central Asia, China, the USA, Australia and Africa, suggesting an extension of the pathogen population to European part of the Russian Federation and further increase of the number of host plant species.

X. hortorum was the second most common pathogen found on cereals seeds. Bacteria of X. hortorum cause plant diseases of willow (genus Hederae), Pelargonium (Pelargonii), dandelion (Taraxaci), salad (Vitians). This type was found in the seed samples of wheat, barley and rye.

X. campestris was isolated from seed lots of rice (with highest frequency), wheat and rye. It was believed that X. campestris affects brassicas, Solanaceae and Cucurbitacea plants (X. campestris pv. raphani) only. Although, A. Ignatov has identified bacteria from rice and cabbage plants grown at rice paddy field at winter season after summer rice crop as predecessor, and the isolates were identified by us as X. campestris pv. campestris (data not shown).

It was unexpected, that only one seed lot of barley carried infection of X. translucens. But, previous evaluation of some 100 xanthomonads collected at 1995—2001 from cereals and deposited to Russian State Collection of Plant Pathogenic Bacteria at VNIIF also showed very rare occurrence of this species on cereal crops in Russia (summary of final report for project ISTC 1771p, www.istc.ru).

In this work we have identified pathogens X. campestris, X. cynarae, X. pisi, and X. gardeneri for the first time on cereals seeds and confirmed their virulence to corresponding host plants. Normally, these species attack plants from seeds. Asteraceae (Compositaceae), Legumes, Solanaceae. In available literature there is no data on infection by X. hortorum, X. campestris X. cynarae, X. pisi, and X. gardeneri of plants within the family Poaceae that suggests that these species are moving towards parasitism on new host plants, like X. arboricola. The emergence of new bacterial pathogens moved from culture to culture, makes difficult traditional control measures and reduces the efficiency of monitoring of seeds and planting material purity [1].

In all the analyzed samples of rice, neither X. oryzae pv. oryzae (BLB) nor X. oryzae pv. oryzicola (BLS) have been identified by classic PCR and Real Time PCR for with specific primers for Xanthomonas oryzae. We can conclude that this pathogen is still absent in Russian Federation.

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ВИДОВОЕ РАЗНООБРАЗИЕ ФИТОПАТОГЕННЫХ БАКТЕРИЙ РОДА XANTHOMONAS, ПОРАЖАЮЩИХ РАСТЕНИЯ СЕМЕЙСТВА МЯТЛИКОВЫЕ В РОССИИ

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В работе были приведены литературные данные о видовом составе фитопатогенных бактерий рода Xanthomonas, поражающих растения семейства Мятликовые. А также собственные данные, полученные в результате обследований семян зерновых культур из различных регионов РФ на наличии патогена. При проведении исследований впервые были идентифицированы возбудители *X. hortorum*, *X. campestris*, *X. cynarae*, *X. pisi*, и *X. gardeneri*, которые ранее на злаковых культурах не выявлялись.

Ключевые слова: злаковые культуры, бактериальная штриховатость листьев, бактериальный ожог листьев риса, черный бактериоз, *Xanthomonas translucens*, *Xanthomonas oryzae*, *Xanthomonas arboricola*.

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