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EXPERIMENTAL ARTICLES

Diversity of Effector Genes in Plant Pathogenic Bacteria of Genus *Xanthomonas*[1]

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Abstract—Gram-negative plant pathogenic bacteria are secreting into plant cell a special type of pathogenicity-related proteins called effectors. They are capable of suppressing plant innate immunity or stimulating synthesis and export of metabolites desired by the pathogen. We identified a number of effector-coding genes typical of xanthomonads analyzing 8 completely sequenced genomes of genus *Xanthomonas*. Using representative collection provided by Russian Research Institute of Phytopathology we identified genetic diversity of effector gene loci in population of *Xanthomonas* bacteria. Patterns of effector genes were identified for individual strains and statistic linkage between particular genes and race of the pathogen was established. For the first time several untypical effector genes were found in strains of *Xanthomonas campestris* pv. *campestris*.

Key words: bacteria, avirulence, pathogenesis, genetic diversity.

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Bacteria of genus *Xanthomonas* (xanthomonads) belong to gram-negative gamma-proteobacteria and can infect nearly every cultivated plant specie causing diseases with diverse symptoms. Xanthomonads include over 25 genomospecies identified by DNA:DNA hybridization, MLST analysis, and in some cases by phenotype [1], and other 150 pathovars differed by host plant range and symptoms of the diseases caused [2]. Many species and pathovars of xanthomonads are divided into numerous physiologic races recognized in scheme of gene-for-gene interaction by reaction of differential plants with specific resistance genes. The basic hypothesis of Flore for gene-for-gene interaction suggests that product of plant resistance gene directly or indirectly interacts with product of avirulence gene in pathogen [3]. Xanthomonads population infecting plants of different species and families have high genetic diversity including variable patterns of avirulence genes [4].

Proteins secreted via the Type 3 Secretion System (T3SS) into host cell are one of major pathogenicity mechanisms of gram-negative bacteria [5]. Proteins, delivered by the T3SS are called T3SS effectors. The majority of effector have unknown function yet. Some known effector proteins can block signals of innate immunity pathway [6]. Entering the host cell they prevent development of plant reaction on non-specific elicitors—MAMPs (microbe associated metabolic profiles) [7]. Effector proteins are essential for virulence and host specificity of all bacteria species with T3SS [8].

Over 20 effector genes were described for xanthomonads in previous research [9]. In a few cases they can determine race of the pathogen, in others—control the pathogen aggressiveness [10]. Homologous effectors were found in bacteria of different species and genera, and horizontal transfer of them was postulated [6]. Publication of several complete genomes of plant pathogenic bacteria accelerated identification of new effector genes by identification of conservative promoters, specific signals and domens [11]. Eight strains of xanthomonads were sequenced and annotated as well at the time of this article preparation. Generally, the effector gene content was diverse even between the strains of a single species.

The aim of current study was to identify content of effector genes in natural population of *Xanthomonas campestris* pv. *campestris*, crucifers pathogen, and compare the spectrum of the loci with previously studied physiologic and taxonomic features of the bacteria including race and pathovar.

MATERIALS AND METHODS

Bacteria and Cultivation

53 strains of several species, pathovars and races of World-wide origin were obtained through the collection of Russian Research Institute of Phytopathology. Identity of all bacteria was confirmed by PCR ampli-

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fication of specific fragments of genes *tonB, cytP450*, and *gyrB* [12, 13]. Previously sequenced strains of *X. campestris* NCPPB528^T, LMG8004 and reference strains of species *X. vesicatoria* and *X. phaseoli* were used as control. All the strains are described in the Table 1. The bacteria were stored at sterile water and grown at King's B medium [14] at 28°C.

Bacterial DNA Extraction, PCR Analysis and DNA Sequencing

Nineteen genes coding T3SS effectors and present at least in two complete sequenced genomes of xanthomonads were selected based on database of National Center of Biotechnological Information (NCBI). All specific primer pairs for every gene were designed for conservative DNA fragments.

Total bacterial DNA was extracted from suspension grown overnight in LB broth at 28°C by modified SDS-STAB protocol [15]. "Encyclo" PCR kit ("Evrogen", Russia) was used for PCR analysis as recommended. Profile of PCR was: initial denaturation 94°C—4 min; followed by 25 cycles: denaturation 94°C—20 s, annealing at temperature specific for each primer pair—30 s, elongation 72°C—45 s; final elongation 72°C—2 min, 4°C—5 min. All PCRs were duplicated at each experiment and each experiment was repeated at least twice (totally 4 repeats at least). The PCR fragments were detected by ethidium bromide staining after electrophoresis in 0.8–2% agarose gel depending on expected fragment size. Three parameters were evaluated: presence/absence of the fragment; the fragment size (band mobility); and the fragment amount (band intensivity). PCR products were purified by Wizard kit of "Promega Co." (United States) after electrophoresis in 0.8% low-melting agarose and sequenced as recommended [16] by automatic sequenator ABI 373 "Perkin Elmer", United States, as recommended. Each fragment was sequenced at least twice in both directions.

Statistic comparison of the effector genes content, frequency and virulence/avirulence of the strains was made by Statistica 6.0 package "StatSoft", United States, using Discriminant Analysis and Student's test.

RESULTS AND DISCUSSION

Genomes of eight completely sequenced at the time of this study strains of xanthomonads (*X. axonopodis* pv. *citri 306, X. campestris* pv. *campestris* LMG8004, *X. campestris* pv. *campestris* ATCC33913 (=NCPPB528^T), *X. campestris* pv. *campestris* B-100, *X. euvesicatoria* (*campestris* pv. *vesicatoria*) 85-10, *X. oryzae* pv. *oryzae* KACC10331, *X. oryzae* pv. *oryzae* MAFF 311018, and *X. oryzae* pv. *oryzae* PXO99A) were analyzed to compare the list of annotated T3SS effector genes occurred at least in two strains. Nineteen genes were selected: *avrBs3, avrXacE1, avrXacE2, HolPsyAE, avrXccA2, avrXccC, avrBs1, avrBs1.1, avrXccE1, avrXccB, AvrRxv, AvrRxo1, HopPtoH, xopB, xopC, xopD, xopJ, XopF2*, and *xopO*, including both known and unknown function ones. Using the BLAST program from Web-site of NCBI, the gene homologues with minimal amino acid similarity over 80% were searched in the complete genomes. Obtained genes are shown at the Tables 2 and 3. It was clear, that none of the genes was present in all genomes but some ones were found in more than one genome.

Conservative regions of each homologous gene group were identified and primers corresponding to such DNA fragments were designed (Table 3). PCR conditions were optimized for each primer pair using type strains of the bacteria with sequenced complete genomes and known set of T3SS effector genes. Identity of obtained PCR fragments was confirmed by direct sequencing of each fragment in several randomly chosen analyzed strains (data are not shown).

Average frequencies for the analyzed genes among 53 strains are shown at the Fig. 1. Occurrences of the genes in all strains, in *X. campestris*, and in strains of other species are given by three different lines. Based of the obtained results, the genes were divided into three groups: (1) present in all the studied strains; (2) present in all strains of *X. campestris*; (3) present in particular strains of *X. campestris* and probably associated with specific virulence or symptoms caused by the bacteria.

It is possible to see on the Fig. 1 that *xopD* was the most frequent gene (85% of strains) in both *X. campestris* pv. *campestris* and other species, probably indicating a high importance of this gene for pathogenesis. Several genes including *avrBs1.1, avrBs1*, and *avrXccC* were present in over 50% of strains. We found that gene *avrXccC* was present in strains of *X. campestris* pv. *campestris* only and can be a marker for races 1–4 of this pathogen. Each of the genes *avrXccA2*; *avrXccE1, avrXccB,* and *HopPtoH* were found in approximately 40% of strains. Genes *XacE1* and *XacE2* previously found in plant quarantine bacteria *X. citri* 306 were present in a few strains of *X. campestris* pv. *campestris* as well.

It is remarkable that some 30% of strains in both *X. campestris* pv. *campestris* and other species carried genes homologous to *avrBs3* group. Previously, this group of genes was not found in *X. campestris* pv. *campestris*, and only recently was discovered in *X. campestris* pv. *armoraciae* [17]. Proteins of this gene are eukaryotic transcription factors and cause changes in host cell metabolism and development. Among the studied strains presence of homologues of *avrBs3* in strains of *X. campestris* pv. *armoraciae* Xa373, Xa 5rei and *X. campestris* pv. *campestris* BT-1, FB 1020, HRI 5181, Eruka 1924, 33436, HRI 5186, Xcc B-36, and Thlaspi1 could be associated with ability of the strains to cause leaf spot symptoms besides black rot.

The last group of genes including *xopC, Xop F2, xopB, HolPsyAE, xopO, xopJ, avrRxo1*, and *AvrRxv*

Table 1. Strains of xanthomonads used in this study

Species, pathovar, race	Strain	Original name	Source
X. campestris pv. *incanae*	FB 1310	ATCC13462	1
X. campestris pv. *armoraciae*	Xca 347	NCPPB347^T	2
X. campestris pv. *armoraciae*	Xa 5 rei	–	2
X. campestris pv. *armoraciae*	Xa373	–	2
X. campestris pv. *campestris*,			
Race 1	PHW231	–	2
Race 4	D2	–	3
Race 3	LMG8004	–	4
Race 1	Xcc231	PHW231	4
Race 4	FB 1011	–	1
Race 4	FB 1020	CFBP 2350	1
Race 4	FB 1207	B-24	1
Race 3	FB 1223^T	NCPPB 528 ^{T}	1
Race 3	FB 1240	FB 1223^T	1
Race 4	FB 1257	R1-4-11 X. sp	1
Race 1	Xcc B-1	–	1
Race 1	Xcc B-30	–	1
Race 1	Xcc B-36	–	1
Race 1	Xcc B-65	–	1
Race 1	Xcc B-65 rei	–	1
Race 4	Xcc B-89	–	1
Race 4	Xcc B-94	–	1
Race 4	Xcc BT-1	–	1
Race 1	Xcc 401	–	1
Race 1	Xcc 403	–	1
Race 1	Xcc 422	–	1
Race 1	Xcc 430	–	1
Race 1	Xcc 460	–	1
Race 3	Xc Er 1	–	5
Race 3	Xc Er 6	–	5
Race 1	33435	–	1
Race 5	Xcc 58-10	–	1
Race 1	Eruka1	–	5
Race 5	Thlaspi1	–	5
Race 6	Bnigra 1	–	5
Race 4	Xn 19a	–	5
Race 4	HRI 1279a	–	2
Race 1	HRI 5181	–	2
Race 1	HRI 5184	–	2
Race 1	HRI 5186	–	2
X. perforance	938 (C)	–	6
X. gardneri	GA_2(D)	–	6
X. euvesicatoria	Xv153(A)	–	6
X. vesicatoria	5235 (B)	–	2
X. vesicatoria	56 (B)	–	6
X.vitians	FB 1317	NCPPB 976	1
X.vitians	FB 1402	X22	1
X.vitians	FB 1403	X23	1
X. arboricola	1392	–	7
X. arboricola	1393	–	7
X. arboricola	1394	–	7
X. arboricola	1395	–	7
X. arboricola	1396	–	7
X. arboricola	1397	–	7

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Table 2. Content of T3SS effector-coding genes in complete sequence genomes of eight strains of xanthomonads

Sequenced strain	T3SS effector gene
X. citri 306 (NC_003919)	*avrBs3, avrXacE1, avrXacE2, avrXccE1*
X. campestris pv. *campestris* 8004 (NC_007086)	*avrBs1, avrXccA2, avrXccB, avrXccC, avrXccE1, xopD*
X. campestris pv. *campestris* ATCC 33913 (NC_003902)	*avrBs1, avrBs1.1, avrXccA2, avrXccB, avrXccC, avrXccE1, xopD*
X. campestris pv. *campestris* B-100 (NC_010688)	*avrXccA2, avrXccC, avrBs1, avrBs1.1, HopPtoH, xopD*
X. vesicatoria 85-10 (NC_007508)	*avrBs1, avrBs1.1, avrRxo1, avrRxv, avrXacE1, avrXccE1, HolPsyAE, HopPtoH, xopB, xopC, xopD, xopF2, xopJ, xopO, avrXacE1(XopE1); avrBs3* (on a plasmid)
X. oryzae pv. *oryzae* KACC10331 (NC_006834)	*avrBs3, HolPsyAE, HopPtoH*
X. oryzae pv. *oryzae* MAFF 311018 (NC_007705)	*avrBs3, HolPsyAE, HopPtoH*
X. oryzae pv. *oryzae* PXO99A (NC_010717)	*avrBs3, HolPsyAE, HopPtoH*

Table 3. Sequence of consensus DNA primers for PCR amplification of the studied T3SS effector genes, annealing temperature (Ta), and expected PCR fragment size

Gene	Sequences or forward and reverse primers, 5'–3'	Ta, °C	Fragment, bp
avrBs3	GCATTYGATGANGCCATGAC CCCTGATGCCTGGAGGATACG	62	149
avrXacE1	GGTCCGGCGGTTCTACTCA GGCGAGATGGGCTGTGCGT	65	360
avrXacE2	CAATGACCGCCCTGGACGAA TGCACAAGGCGAACAACACGA	62	221
HolPsyAE	CGCTGATCTATGCCGTACCCA AGAAGGTGAGCTGCCGATCCA	65	318
avrXccA2	AACGCTATGTGCCGCTCAA CGCCCTGAATACTAACCTCAT	62	405
avrXccC	TGGTCTCAGCCCGTATGGAA GCCGCCTCAAAGAAGTAACGA	57	418
avrBs1	TTG CTG TCA AGT GCG GTT TGT CAG CCG CCG TAG TGA ATC AG	56	488
avrBs1.1	ATGATATCATCGAACGGCAACGTAAA TGCTTGGCGTACTCGTAGAATAGAGG	57	113
avrXccE1	ACC CTA CAG CCG CAC GAA GTT CGC TCC GTC CGT TGC TRA G	59	197
avrXccB	GTCATCGTTATCCGCCATC CGTGGCAAGGTGTCCGTCT	65	192
AvrRxv	CATCTGGTCCGCTCCTACGA CGCCTCAATCACAGCAAACGA	57	321
AvrRxo1	GACACATCAACAGGCTACTCAG CGAACTCTTGAATACATCTGG	56	346
HopPtoH	TAGAATGGAGCCCGCATAG CGGCTTCGGATGTTTCGTC	59	180
xopB	GCCGACTTGTTTCTGCCGACC CGCCGTCTCAACCGTGAAT	65	179
xopC	GAGGTTATCGGCACATTAGGTT CCGTTCCTCAATCCAAGCGTC	57	852
xopD	CCGACTCGCAGGTAGTGACCAT TCGCCGCAGGAATAACCGTC	57	316
xopJ	ATCCAGCACTTTGACTCGCCTAT CGCTTGCTTCGCCATTAGGT	56	154
Xop F2	TGAACGCCTGTCTCGCTAA CGGACGCTGCCTACGAA	56	825
xopO	CGGTGGAGGCAAGCGAGTT CCCAGCAAAGAACGCAAATCA	57	228

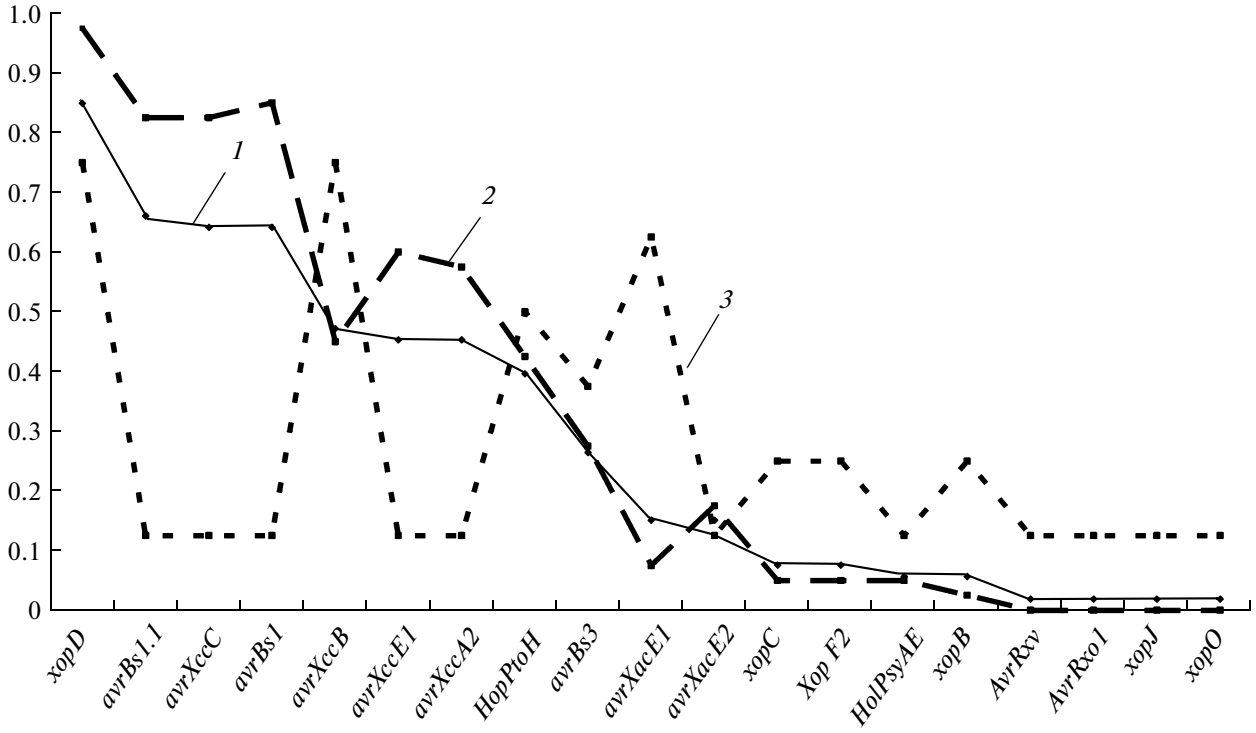

Fig. 1. Frequency of PCR fragments specific for the T3SS effector genes in the whole population of analyzed strains (*1*), in *X. campestris* pv. *campestris* (*2*), and in strains of other species (*3*).

was found in strains of so-called *"X. axonopodis"* (*X. citri, X. phaseoli, X. euvesicatoria*). This group had the largest number of the studied genes.

Among the *X. campestris* pv. *campestris*, 7 of 19 genes in average were detected in strains of races 5, 6 and pathovar *X. campestris* pv. *armoraciae*. The smallest numbers of genes (5 per strain) was in strains of race 4.

It is important, that some effector genes present in population of *X. campestris* pv. *campestris* are typical of bacteria of other genera like *Pseudomonas, Ralstonia* (*avrBs3, avrXccE1, avrXccB*), or other species of xanthomonads (*avrXacE1, avrXacE2, xopC, HopPtoH, HolPsyAE*). Those genes are absent in completely sequenced *X. campestris* pv. *campestris* strains, and it is highly possible that they were laterally transferred to some other strains.

Analysis of the PCR fragments for genes *avrXccA2, avrXacE1, avrXacE2, avrBs3, xopC*, and *xopF2* showed differences in size, but direct sequencing of such alleles showed single nucleotide changes only. It is possible, that several variants of such genes were present in the strains.

Six analyzed strains of *X. arboricola* from brassicas were completely free of the studied effectors. These bacteria can attack host plant of different families including *Rosaceae, Brassicaceae, Compositacea*, and *Poaceae* at higher temperature, and probably, less need suppression of host non-specific immunity.

There was no complete correspondence between content of T3SS effector genes among the studied strains (Fig. 2) and their previously studied virulence to differential plants [18]. Such result was rather expectable considering limited information about the gene presence/absence obtained by PCR, and possible influence of allelic variation or differences in gene expression *in planta*.

So as, we cannot see the full matching between presence/absence of the genes and differential plant reaction, we evaluated statistic correlation between individual genes and plant response. The differential plants for *X. campestris* races are represented by 3 different genomes with specific loci of resistance: *Rxcc1* for genome B (*Brassica nigra, B. juncea, B. carinata*); *Rxcc3* for genome C (*B. oleracea* Miracle F_1); and *Rxcc4* for genome A (*B. rapa, B. napus*). To test such hypothesis we made statistic analysis of linkage between particular effector genes and reaction of plants with those three resistance genes (Table 4). For such analysis all strains were divided in two groups (virulent/avirulent) according to reaction of plants with particular resistance type, and correspondence between the group and presence/absence of each of 19 genes was tested by Discriminant Analysis and Student's test.

Significant correlation was found for avirulence to plants with gene *Rxcc1* and presence of *avrXccC, avrXacE1* or absence of gene *xopB* (by Discriminate Analysis). Analysis by Student's test showed correla-

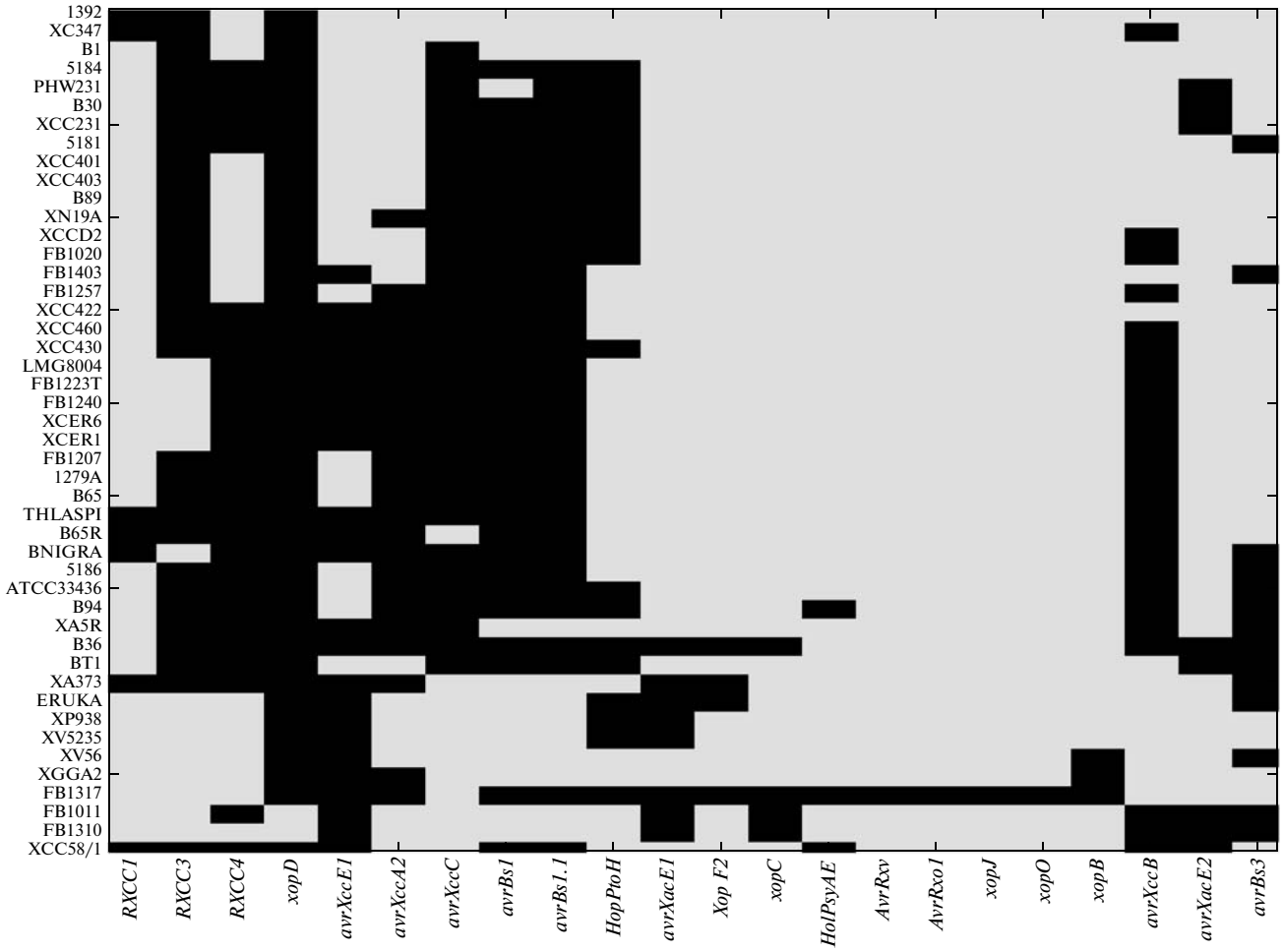

Fig. 2. Interaction of 46 studied strains of xanthomonads (except *X. arboricola*) with plants carrying three major resistance loci of brassicas (*Rxcc1*, *Rxcc3*, and *Rxcc4*) and occurrence of PCR fragments specific for 19 T3SS effector genes. Dark field—positive (susceptible reaction or DNA fragment presence), light field—negative (resistant reaction or DNA fragment absence).

tion of such trait with presence of genes *avrXccC* and *HopPtoH*. Significant linkages between virulence to plants with gene *Rxcc3* and presence of *avrXccC* and absence of *avrXccE1, avrXacE1* and *xopB* (Student's test), *avrXocE1* and *xopB* (Discriminant Analysis) were found as well. It was proved before that *avrXccC* controls avirulence to plants with gene *Rxcc1* [19], and present in races 1, 3, and 4, but occurrence of such gene in strains of virulent to *Rxcc1* genotype probably explained by its inactivation.

Reliable correlation between virulence to plants with *Rxcc3* and absence of *avrXccE1* in bacteria confirms opinion of Yong-Qiang He et al. [20], that protein AvrXccE1 provides avirulence of *X. campestris* pv. *campestris* to Chinese cabbage (*B. rapa*) Zhongbai-83, with resistance gene *R3*. Avirulence of bacteria to plants with gene *Rxcc4* correlated to presence of genes *avrBs1.1, avrXccB, avrXccA2* (Student's test) and genes *avrXccA2, avrXacE2, xopC* (Discriminant Analysis).

The lack of clear correspondence between T3SS effector presence/absence and race of the strain can be partly explained by the hypothesis described by Ignatov et al. [15] about three multi-allelic loci of resistance genes in genomes of the differential plants from 3 major species *B. oleracea, B. rapa* and *B. nigra*, and possible participation of multi-allelic variants of effector genes from the pathogen side.

Here, we reported about screening of a limited number of effector genes known from analysis of completely sequenced bacteria in core collection of *X. campestris* pv. *campestris* and strains of several related species. High level of T3SS effectors content variability was shown for the analyzed species and its pathovars. For the first time we found in *X. campestris* pv. *campestris* the effector genes untypical for this pathogen, and showed possible association between the effector gene content and number with the type of disease symptoms and major race of the pathogen. Some the genes present in *X. campestris* pv. *campestris* are obviously obtained via lateral transfer from other

Table 4. Statistic analysis of correspondence between reaction of differential plants with resistance genes *Rxcc1*, *Rxcc3*, and *Rxcc4* (Vicente et al., 2001) and presence/absence of 19 T3SS effector genes. Only significant interactions are shown

(A) Discriminant analysis

Group, gene	Wilkinson's test	Partial correlation coeff.	F-test	*p*-level
RXCC1				
avrXccC	0.726136	0.489696	38.55709	0.000000
avrXacE1	0.495308	0.717908	14.53861	0.000504
xopB	0.604451	0.588279	25.89530	0.000011
RXCC3				
avrXccE1	0.556214	0.734261	14.11461	0.000562
xopB	0.492692	0.828928	8.04875	0.007187
RXCC4				
avrXccA2	0.546088	0.629952	22.32205	0.000031
avrXacE2	0.523100	0.657636	19.78274	0.000073
xopC	0.411010	0.836985	7.40106	0.009777

(B) Student's test

	Average for group 1*	Average for group 0	*t*-value	Freedom value	*p*-level
RXCC1					
avrXccC	0.285	0.800	–3.00	45	0.0042
HopPtoH	0.000	0.525	–2.72	45	0.0092
RXCC3					
avrXccC	0.848	0.428	3.188	45	0.0026
avrXccE1	0.303	1.000	–5.55	45	0.000001
avrXacE1	0.060	0.428	–3.35	45	0.0016
xopB	0.000	0.214	–2.93	45	0.0052
RXCC4					
avrBs1.1	0.526	0.892	–3.037	45	0.0039
avrXccB	0.263	0.714	–3.32	45	0.0017
avrXccA2	0.210	0.714	–3.81	45	0.0004

* Group 1—strains virulent to corresponding resistance gene, Group 0—avirulent strains.

species of xanthomonads and/or other genera of bacteria. The results of this study are important for planning further steps in investigation of the gene role in interaction between pathogens and plant.

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REFERENCES

1. Jones, J.B., Lacy, G.H., Bouzar, H., Stall, R.E., and Schaad, N.W., Reclassification of the Xanthomonads Associated with Bacterial Spot Disease of Tomato and Pepper, *Syst. Appl. Microbiol.*, 2004, vol. 27, no. 6, pp. 755–762.
2. Vauterin, L., Rademaker, J., and Swings, J., Synopsis on the Taxonomy of the Genus *Xanthomonas, Phytopathology*, 2000, vol. 90, no. 7, pp. 677–682.
3. Flor, H.H., Current Status of the Gene-For-Gene Concept, *Annu. Rev. Phytopathol.*, 1971, vol. 9, pp. 275–296.
4. Bouzar, H., Jones, J.B., Stall, R.E., Hodge, N.C., Minsavage, G.V., Benedict, A.A., and Alvarez, A.M., Physiological, Chemical, Serological, and Pathogenic Analyses of a Worldwide Collection of *Xanthomonas campestris* pv. *vesicatoria, Phytopathology*, 1994, vol. 84, pp. 663–671.
5. Buttner, D. and Bonas, U., Who Comes First? How Plant Pathogenic Bacteria Orchestrate Type III Secretion, *Curr. Opin. Microbiol.*, 2006, vol. 9, pp. 193–200.
6. Alfano, J.R. and Collmer, A., Type III Secretion System Effector Proteins: Double Agents in Bacterial Disease and Plant Defense, *Annu. Rev. Phytopathol.*, 2004, vol. 42, pp. 385–414.

7. Coaker, G., Falick, A., and Staskawicz, B., Activation of a Phytopathogenic Bacterial Effector Protein by a Eukaryotic Cyclophilin, *Science*, 2005, vol. 308, no. 5721, pp. 548–550.

8. Grant, S.R., Fisher, E.J., Chang, J.H., Mole, B.M., and Dangl, J.L., Subterfuge and Manipulation: Type III Effector Proteins of Phytopathogenic Bacteria, *Annu. Rev. Microbiol.,* 2006, vol. 60, pp. 425–449.

9. Thieme, F., Koebnik, R., Bekel, T., Berger, C., Boch, J., Büttner, D., Caldana, C., Gaigalat, L., Goesmann, A., Kay, S., Kirchner, O., Lanz, C., Linke, B., McHardy, A.C., Meyer, F., Mittenhuber, G., Nies, D.H., Niesbach-Klösgen, U., Patschkowski, T., Rückert, C., Rupp, O., Schneiker, S., Schuster, S.C., Vorhölter, F.J., Weber, E., Pühler, A., Bonas, U., Bartels, D., and Kaiser, O., Insights into Genome Plasticity and Pathogenicity of the Plant Pathogenic Bacterium *Xanthomonas campestris* pv. *vesicatoria* Revealed by the Complete Genome Sequence, *J. Bacteriol.*, 2005, vol. 187, no. 21, pp. 7254–7266.

10. Kjemtrup, S., Nimchuk, Z., and Dangl, J.L., Effector Proteins of Phytopathogenic Bacteria: Bifunctional Signals in Virulence and Host Recognition, *Curr. Opin. Microbiol.*, 2000, vol. 3, pp. 73–78.

11. Buttner, D., Noel, L., Thieme, F., and Bonas, U., Genomic Approaches in *Xanthomonas campestris* pv. *vesicatoria* Allow Fishing for Virulence Genes, *J. Biotechnol.*, 2003, vol. 106, pp. 203–214.

12. Punina, N.V., Zotov, V.S., Ignatov, A.N., Kuznetsov, B.B., and Shaad, N., Assessment of Genetic Diversity of the 16S–23S rRNA Intergenic Transcribed Region, *gyrB* Gene, and Development of PCR Diagnostics of Phytopathogenic Xanthomonads, *Vestnik MGOU*, 2008, no. 2, pp. 3–17.

13. Tsygankova, S.V., Ignatov, A.N., Boulygina, E.S., Kuznetsov, B.B., and Korotkov, E.V., Genetic Intraspecies Relationships in *Xanthomonas campestris* pv. *campestris* Revealed by Novel rep-PCR Primers, *European J. Plant Pathol.,* 2004, vol. 110, no. 8, pp. 845–853.

14. Atlas, R.M., *Handbook of Microbiological Media*, Parks, L.C., Ed., Boca Raton: CRC, 1993.

15. Ignatov, A.N., Sechler, A., Schuenzel, E.L., Agarkova, I., Oliver, B., Vidaver, A.K., and Schaad, N.W., Genetic Diversity in Populations of *Xanthomonas campestris* pv. *campestris* in Cruciferous Weeds in Central Coastal California, *Phytopathology*, 2007, vol. 97, pp. 803–812.

16. Sanger, F., Nicklen, S., and Coulson, A.R., DNA Sequencing with Chain-Terminating Inhibitors, *Proc. Natl. Acad. Sci. USA*, 1977, vol. 84, pp. 5463–5467.

17. Kay, S., Boch, J., and Bonas, U., Characterization of AvrBs3-Like Effectors from a *Brassicaceae* Pathogen Reveals Virulence and Avirulence Activities and a Protein with a Novel Repeat Architecture, *Mol. Plant Microbe Interact.*, 2005, vol. 18, no. 8, pp. 838–848.

18. Ignatov, A., Mazurin, E., Dzhalilov, F., Matveeva, E., and Schaad, N.W., An Improved Model for Races of *Xanthomonas campestris* pv. *campestris*, HIS Brassica-2008, Lillehammer, Norway, pp. 26–28.

19. Castaneda, A., Reddy, J.D., El-Yacoubi, B., and Gabriel, D.W., Mutagenesis of All Eight *avr* Genes in *Xanthomonas campestris* pv. *campestris* Had no Detected Effect on Pathogenicity, but One *avr* Gene Affected Race Specificity, *Mol. Plant-Microbe Interact.*, 2005, vol. 18, pp. 1306–1317.

20. Yong-Qiang He, Liang Zhang, Bo-Le Jiang, Zheng-Shun Zhang, Rong-Qi Xu, Dong-Jie Tang, Jing Qin, Wei Jiang, Xia Zhang, Jie Liao, Jin-Ru Cao, Sui-Sheng Zhang, Xiao-Xia Liang, Mei-Liang Wie, Guang-Tao Lu, Jia-Xun Feng, Baoshan Chen, Jing Cheng, and Ji-Liang Tang, Comparative and Functional Genomics Reveals Genetic Diversity and Determinants of Host Specificity among Reference Strains and a Large Collection of Chinese Isolates of the Phytopathogen *Xanthomonas campestris* pv. *campestris*, Genome Biology, 2007, vol. 8, no. 10, R218.