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RESEARCH PAPERS

Defense Response Mechanisms of Ginseng Callus Cultures Induced by *Yersinia pseudotuberculosis*, a Human Pathogen

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Abstract—The effect of *Yersinia pseudotuberculosis*, the bacterial pathogen affecting humans and animals, on growth of ginseng (*Panax ginseng* C.A. Mey) cell cultures was studied. The bacteria strongly induced the expression of phenylalanine ammonia-lyase and β-1,3-glucanase, the proteins encoded by the defense-related genes of ginseng and inhibited the normal ginseng callus growth but did not affect the resistant cell cultures. The thermostable and thermolabile protein toxins of these bacteria are lethal to mice when induced parentherally, and they also induced the expression of the defense-related genes in ginseng callus cultures. At the same time, the ginseng cells completely suppressed the bacterial cell growth. These data suggest that the ginseng cells recognized the yersinia and developed the immune response to this pathogen. The interaction between the ginseng cells and *Y. pseudotuberculosis* is similar to the hypersensitive response of plants to plant pathogens.

Key words: Yersinia pseudotuberculosis - Panax ginseng - phenylalanine ammonia-lyase - β-1,3-glucanase - pathogenesis - plant–microbe interactions

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INRODUCTION

Yersinia pseudotuberculosis are ubiquitous bacteria affecting humans and animals. Plants were found to play a significant role in ecology and epidemiology of *Y. pseudotuberculosis* [1–3]. It is also known that humans do not get directly infected with the pseudotuberculosis by other humans or animals. They are normally infected through consumption of food contaminated by these bacteria, mainly of vegetables that have been stored at low temperature where *Yersinia pseudotuberculosis* actively multiply and become highly virulent [4].

Evidence exists that yersinia can also reside in trees and herbs. Under laboratory conditions, they can penetrate into the seedlings of lettuce, cabbage, oat, peas, potato, and *Elodea canadensis*, an aquatic plant [7—9].

The understanding of the fact that many bacterial pathogens affecting plants and animals have common ancient ancestors and function similarly is growing [10–12]. Recognition of the phytopathogens by plants at the molecular level occurs through the interaction of protein products of *R-Avr* genes, where *R* is a plant disease-resistance gene while *Avr* (stands for avirulence) is a gene of a pathogenic microbe [13]. Many phytopathogenic microorganisms can inhabit the plant surface without causing a disease. Under certain circumstances, bacteria penetrate into plants through stomata or wounds, multiply in the apoplast, and thus establish the initial contact with the plant cells. During this contact, the *Hrp*, a hypersensitive response and pathogenicity locus of the bacteria that includes a cluster of genes encoding a type III protein secretion system in bacteria is induced [12]. The activity of most *Avr* genes depends on the activity of the *Hrp* locus. The *Hrp* locus of phytopathogens is similar to corresponding loci encoding a type III protein secretion system in human pathogens, including the *Yersinia, Shigella,* and *Salmonella* [14]. The common mechanism of pathogenesis was initially demonstrated for *Pseudomonas aeruginosa*, when the samples of this clinically isolated human pathogen were shown to be able to affect plants, nematodes, insects, and mice [11]. The complete necrosis of arabidopsis leaves was observed on the 4th to 5th day after plant treatment with the bacterial suspension.

Abbreviations: Avr—avirulence;CFU—colony-forming unit; HR—hypersensitive response; *hrp*—hypersensitive response and pathogenicity genes; PAL—phenylalanine ammonia-lyase; Pg-glu1—*Panax ginseng* β-1,3-glucanase; PgPAL—*P. ginseng* PAL; TLTYp—thermolabile toxin of *Yersinia pseudotuberculosis*; TSTYp—thermostable toxine of *Y. pseudotuberculosis*; Yop—*Yersinia* outer protein.

The *dsbA* gene along with its homologuous human pathogens (including *Shigella flexneri* and *Vibrio cholera*) and plant pathogens (*P. aeruginosa* and *Erwinia chrysanthemi*) as well as *hrpM*, *gacS*, and *gacA* genes are common evolutionally conserved determinants of the pathogenicity. The functions of the *Hrp* gene family have already been mentioned above; the *gacS* gene encodes a two-component sensor kinase, its activity being regulated by the *gacA* gene.

Another important pathogenicity factors are the YopJ family proteins initially isolated from *Yersinia*; their homologues were subsequently found in phytopathogens, including *Erwinia, Pseudomonas*, and *Xanthomonas* and even in *Rhizobium*, a symbiotic bacterium [12]. A common function of these proteins, acting as cysteine proteases, is a destruction of signaling systems of host organisms, protecting them against pathogens [15]. These genes developed together in the ancient Gram-negative organisms as environmental adaptors. They help the modern bacteria to infect a wide range of host organisms. It should be also noted that many pathogenicity factors of plants and animals are different [12].

Due to the aforementioned facts, the hypersensitivity and other defense responses similar to the responses to plant pathogens should be expected in plant cells treated with *Y. pseudotuberculosis.* Earlier we studied the effects of *Y. pseudotuberculosis* and its toxins on growth of callus cultures of cabbage (*Brassica oleracea*), *Panax ginseng*, *Aristolochia manchuriensis*, and *Lithospermum erytrorhizon* [16, 17].The bacteria intensively grew in cabbage callus cultures; their numbers increased from 2 to 8 lgCFU/g of raw mass, starting from the third day of culturing and remained unchanged for up to 60 days, affecting the cabbage cells. This type of interaction suggests that the plant cells did not recognize the pathogen, which agreed with the known fact that cabbage is a major natural host of *Y. pseudotuberculosis* and with the role of cabbage in transmission of the infection to humans. In *Lithospermum erytrorhizon* calli (strain BK-39), where large amounts of antibacterial phytoalexins are synthesized [18], the yersinia only started to grow on the 6th day of culturing; their number grew from 4 to 7 lgCFU/g of raw mass and then slightly declined on the 50th day as the culture started to age. No visible interaction between the bacteria and plants was noted, and a decline in the bacterial mass was related to the effect of phytoalexins. The *Aristolochia manchuriensis* calli showed a more interesting type of interaction: the bacteria grew fast, and their mass increased from 4 to 9 lgCFU/g of raw mass. However, starting from the 11th day, their mass permanently decreased and no new colonies appeared after the 39th day. The ginseng calli responded violently: the plant cells died to destroy the bacteria infecting them. The initial growth of bacteria from 4 to 6.5 lgCFU/g of raw mass ended on the 3rd day of co-cultivation; after that their mass quickly declined and no new colonies were detected on the 11th day. This suggested a hypersensitive type of response (a so-called hr-syndrome) in plants against the *Xanthomonas campestris, P. aeruginosa*, and *E. chrysanthemi* due to the *R-Avr* interaction taking place [10, 12, 15, 19].

The objective of this study was to investigate and characterize the defense responses of ginseng cells against *Y. pseudotuberculosis.* Three types of ginseng cell cultures were used, of which one was a control and the remaining two were transgenic lines with the low and high *rolC* gene expression level, respectively. The *rolC* gene expression makes the plant cells highly resistant due to the high rate of biosynthesis of secondary metabolites [20] and the constitutive expression of PR-2, the pathogenesis-related proteins [21].

MATERIALS AND METHODS

Bacteria and toxins. *Yersinia pseudotuberculosis* (Enterobacteriaceae family) str. 512 I from the Institute of Epidemiology and Microbiology live culture collection was used for the study. Yersinia were cultured on the agar-solidified medium at 18 to 20°C for 24 h. The bacterial samples were serially tenfold diluted with distilled water according to the *Tarasevich* cloudiness scale [16]. TSTYp, the thermostable protein toxin of *Y. pseudotuberculosis* str.512 I carrying a virulence plasmid pYV,; and TLTYP, a thermolabile protein toxin of *Y. pseudotuberculosis* str.512 I lacking the above plasmid, were also used. The toxins were isolated from bacterial lysates, using the ion exchange and gel filtration techniques [22]. The amount of protein in toxin samples was measured according to Bradford [23].

Cell cultures. GV, 2c2, and 2cR2 *Panax ginseng* C.A. Mey. calli were cultivated on agar-solidified W_{4CPA} medium [20, 24]. The production protocols, as well as the molecular and biological characteristics of these cultures have been described earlier [25]. GV is a control culture obtained by transformation of strain 1c calli (the collection code @BCKK-BP@ 41) with pPCV002, an empty binary vector. 2c2 and 2cR2 cultures obtained by transformation of the same strain with pPCV002-CaMVC vector are characterized by low and high *rolC* gene expression, respectively. The level of *rolC* transcripts in 2cR2 was approximately ten times higher that in 2c2 [21].

Coculturing. 10 to 20 μl of TLTYP (60 μg/ml), TSTYp (2.5 mg/ml), or the bacterial suspension (10^5 bacteria/ml) was applied to the callus surface under aseptic conditions during the exponential growth phase of ginseng cells (on the 15th day of culturing). The calli–bacteria and calli–toxin systems were incubated at 24°C and 70% RH in the dark. Sterile distilled water was applied to the control ginseng calli. For measuring the amount of viable yersinia in calli, the samples were dynamically transferred to the differential diagnosis medium [4], incubated at 37°C for 36 to 48 h; the number of resulting colonies was counted, and the

lgCFU of bacteria per g of raw mass of callus was determined.

Differential gene expression analysis. On the first and third days of experiment, the total RNA was isolated from cell cultures using the Yellow Solve kit (Silex, Russia) for differential analysis of PAL and Pg-glu1 gene expression in ginseng calli treated with bacteria and toxins. cDNA was obtained using the reverse transcription kit with an M-MLV reverse transcriptase according to the manufacturer's instructions with minor modifications [26]. cDNA synthesis was carried out using the BioRad amplifier (United States) programmed for 37°C for 40 min, 70°C for 10 min, and cooling at 4°C. 0.2 to 2.0 μl of cDNA were used for amplification of actin, *PAL* and *Pg-glu1* genes as described earlier [21, 26].

***PAL* gene expression analysis.** Based on the known nucleotide sequences of *PAL* genes of *A. thaliana, Lactuca sativa, Rubus idaeus, L. erythrorhizon, Glycine max, Pisum sativum, Cicer arietinum*, and *Phaseolus vulgaris* (NM_129260, NM_115186, AF411134, AF299330, AF237955, X52953, Q01861, Q04593, Q9SMK9, and P19143, respectively), the conservative area primers 5'-ARGCYGCYGCYATYATGGA and 5'-GGRGTGCCYTGRAARTT were designed. The annealing temperature was 53°C, which enabled us to obtain a signal within the band required (266 bp) without using the additional signal markers; the elongation time was 25 s. The detailed technique for measuring the *Pg-glu1* gene expression level was described earlier in [21].

The *PAL* and *Pg-glu1* gene expression were normalized to the actin gene expression level [21]. The amplification products were extracted from gel using the Glass Milk kit (Silex) and cloned into the pTZ57R/T vector (Fermentas, Lithuania) according to manufacturer's instructions.

RT-PCR product sequencing. The RT-PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, United States) and following the manufacturer's instructions. The Biology and Soil Institute based ABI 310 Genetic Analyzer (Applied Biosystems) was used for sequencing. The detailed sequencing protocol was described earlier in [21]. The nucleotide sequences of ginseng *PAL* and *Pg-glu1* genes obtained were aligned with known sequences of corresponding genes of other organisms using the BioEdit 7.0.8 software and the NCBI BLAST resource. The *PAL* genes are known to make a large family in the plant genome. 20 to 30 clones were sequenced in each cell culture during the experiment. All sequences were numbered in the order they were analyzed.

Based on the qualitative analysis of representation of different *PAL* genes in the samples and the total *PAL* gene expression using the degenerate primers;, the expression of each subfamily of *PAL* genes was determined and expressed in relative units. The individual *PAL* gene expression level was normalized to the actin gene expression and the weighted average *PAL* gene expression level was calculated for each clone studied [27]. As was shown earlier [27], this approach is as efficient for gene expression analysis as a commonly used real time-PCR method. Moreover, it enables to determine the expression of each individual gene within the family.

Measurement results were processed with STATISTICA 8.0 software package. Values were expressed as the means and their standard deviations. The significance of the differences was statistically evaluated using a Student's *t*-test. In all experiments, significance was determined at the $P < 0.05$ levels.

RESULTS

Growth of Ginseng Cell Cultures in the Presence of Yersinia

In the first set of experiments, the growth profiles of ginseng cell cultures and *Y. pseudotuberculosis* bacteria during cocultivation were obtained. The GV cells grew fast, and on the 40th day of culturing, their weight was 15 times the initial weight of the inoculum (Fig. 1a, curve *1*). *Y. pseudotuberculosis* almost completely depressed the GV culture growth (Fig. 1a, curve *2*) as compared to growth of the mock-treated control cells.

2Ò2 cell cultures also grew fast in control medium, and on the 40th day of culturing, their weight increased by a factor of 15 (Fig. 1b, curve *1*). In the presence of *Y. pseudotuberculosis*, the cells continued to grow (Fig. 1b, curve *2*) and their growth rate did not significantly differ from that of the mock-treated control cells. However, 2cR2 cell cultures grew slowly under control conditions, their weight on the 40th day of culturing was just three times the initial weight (Fig. 1c, curve *1*). After addition of *Y. pseudotuberculosis*, the 2ÒR2 cells continued to grow (Fig. 1c, curve *2*), and their growth rate did not significantly differ from that of the mock-treated control cells.

The analysis of viability of *Y. pseudotuberculosis* in the infected calli showed that the number of bacteria in all experimental samples considerably declined on the third day of coculturing, and, on the seventh day of all experiments, the presence of bacteria was not detected (Fig. 2).

PAL and Pg-glu1 Gene Expression in Ginseng Cell Cultures

In the second set of experiments, the expression of *PAL* and *Pg-glu1* genes in *P. ginseng* GV calli in presence of *Y. pseudotuberculosis* and its toxins was studied. The plant *PAL* gene family consists of about 30 to 40 genes [28]; however, no *PAL* gene sequences of ginseng are available. This is why the degenerate primers had to be constructed to match all the potential *PAL* genes of ginseng. Sequencing of the PCR products amplified from ginseng cDNA samples indicated the

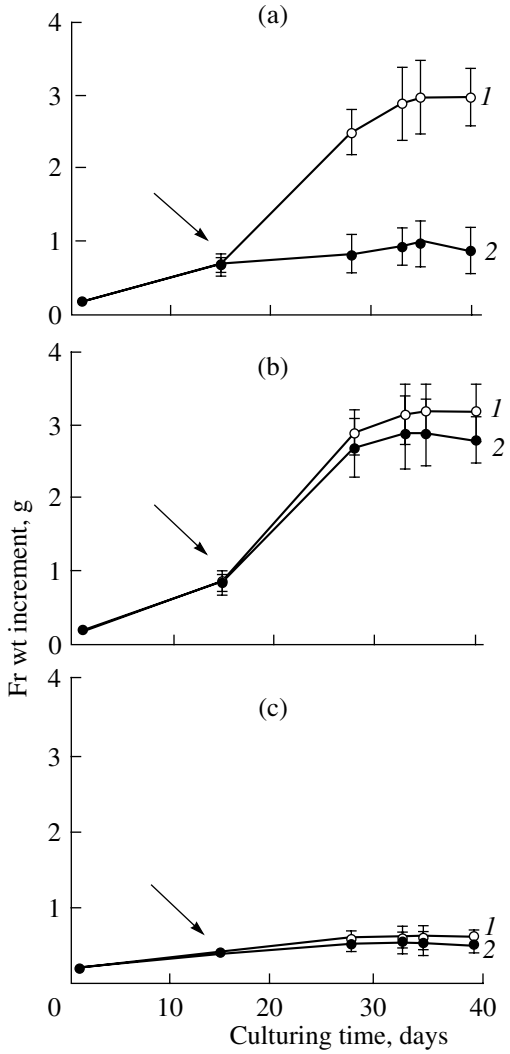

Fig. 1. Ginseng cell growth profile under normal conditions and in association with *Y. pseudotuberculosis.*
(a) Strain GV; (b) strain 2c2; (c) strain 2cR2.
(*1*) Bacteria-free medium; (*2*) coculturing with bacteria. The arrow shows time of application of yersinia to calli.

presence of about 30 *PAL* genes that could be grouped into three subfamilies on the basis of their nucleotide sequences. These subfamilies were called PgPAL1, PgPAL2, and PgPAL3 (in the order they were sequenced). The deduced amino acid sequences of all corresponding proteins of ginseng were found to be close to the earlier described PAL proteins of other plants, showing 98 to 99% identity. The genes of plant β-1,3-glucanases are also a large gene family; thus, the degenerate primers had to be designed on the basis of known sequences of these genes. Sequencing of the ginseng cDNA amplification products showed that the *Pg-glu1* gene is mainly expressed in ginseng cultures. Other glucanase genes were also detected (*Pg-glu2, Pg-glu3*); however, in all experiments their expression was below 10% of the total gene family expression.

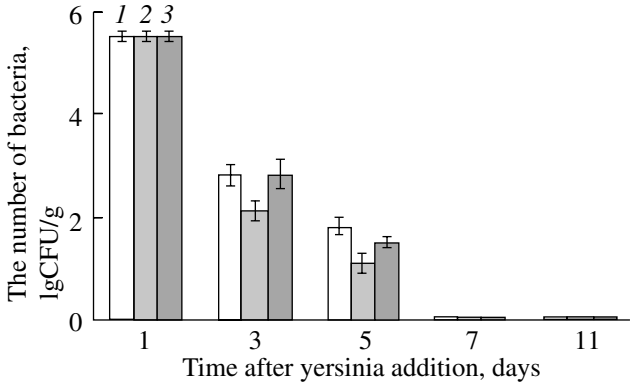

Fig. 2. The number of viable *Y. pseudotuberculosis* cells in ginseng cell cultures.
(*1*) Strain GV; (*2*) strain 2c2; (*3*) strain 2cR2.

The lowest level of *PAL* and *Pg-glu1* gene expression was detected in GV cell cultures (Fig. 3), whereas the highest level was observed in the 2cR2 cells. The expression level of the *PAL* gene in 2cR2 cells was three times higher that in GV cells (Fig. 3a). The expression level of the *Pg-glu1* gene in 2cR2 cells was 27 times higher that in GV cells (Fig. 3b). The expression level of *PAL* and *Pg-glu1* genes in 2c2 cultures was between the corresponding levels for those in GV and 2cR2.

The expression of *PAL* in ginseng GV cultures was found to be induced by *Y. pseudotuberculosis* and TLTYp, making 2 to 4 times the level in control plants on the very first day of culturing (Fig. 4). The increased expression of *PAL* genes was still detected on the 3rd day of coculturing with yersinia, although their total level of expression in samples declined. The induction of *PgPAL1* subfamily genes was the strongest (Fig. 5). The expression of *Pg-glu1*, a glucanase gene, significantly grew (3 to 5 times than that in control samples) during the first day of experiment; its high level was also detected on the third day of culturing with yersinia (Fig. 6). The earlier described ginseng cell death dynamics [16, 17] suggests that, on the third day of coculturing of ginseng calli with yersinia, more than 50% of cells died. A decline of the immune response by the third day of coculturing is obviously explained by massive cell death. By the 5th day, dead cells made 65%, by the 7th day, 73%, and by the 18th day, 96% of the total number of cells [16, 17]. These results were obtained for R-1 strain of ginseng calli. The GV cell growth profile provided in Fig. 1 largely agrees with these data.

DISCUSSION

Our study showed that the expression of *PgPAL1, PgPAL2, PgPAL3*, the phenylalanine ammonia-lyase, *Pg-glu1*, and β-1,3-glucanase encoding genes in ginseng GV calli increased already on the first day of coculturing with *Y. pseudotuberculosis*. This is an evidence of fast recognition of the pathogen and the induction of

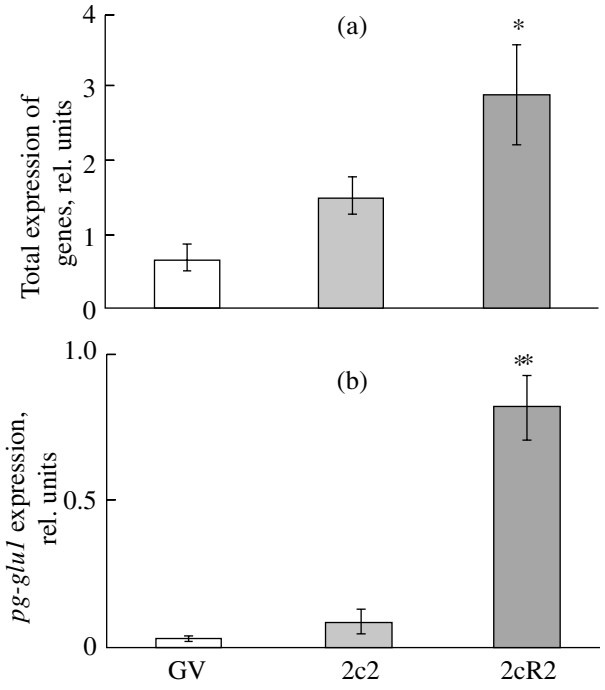

Fig. 3. The expression profiles of the *PAL* and *pg-glu1* genes in ginseng cell cultures.
The ginseng actin gene expression level was taken as 1. $*P < 0.05$.

the defense response in ginseng cells. The general trend in interaction of bacterial and plant cells was the absence of viable bacterial cells and the termination of the growth of ginseng cell by the seventh day of the coculturing. This looks like a typical hypersensitive response that happens after the introduction of a normal pathogen into the plant cells (like, for example, *P. aeruginosa*). These data clearly indicate that the *Y. pseudotuberculosis* bacteria are recognized by ginseng cells, which therefore develop the immune response against this pathogen. It is not possible however to clearly identify the type of the immune response at this stage, because *R*, the resistance gene of ginseng and *Avr*, the pathogenicity factor of yersinia need to be identified and their interaction needs to be proven. As was noted in the Introduction section, no barrier for such interaction exists in principle because the pathogenicity factors of *Erwinia, Pseudomonas*, and *Xanthomonas*, the plant pathogens and *Yersinia*, the human pathogen, are similar. *Y. pseudotuberculosis* is obviously the second bacterial species after *P. aeruginosa* capable of induction of the immune response in both animals and plants.

The resistant ginseng callus cells with systemic immunity induced by the high levels of the *rolC* gene expression developed normally after application of yersinia while the bacterial cells died. This indicates that the induced immune system response manifested by the expression of *PAL* and β-1,3-glucanases genes is sufficient for the protection of ginseng cells against this

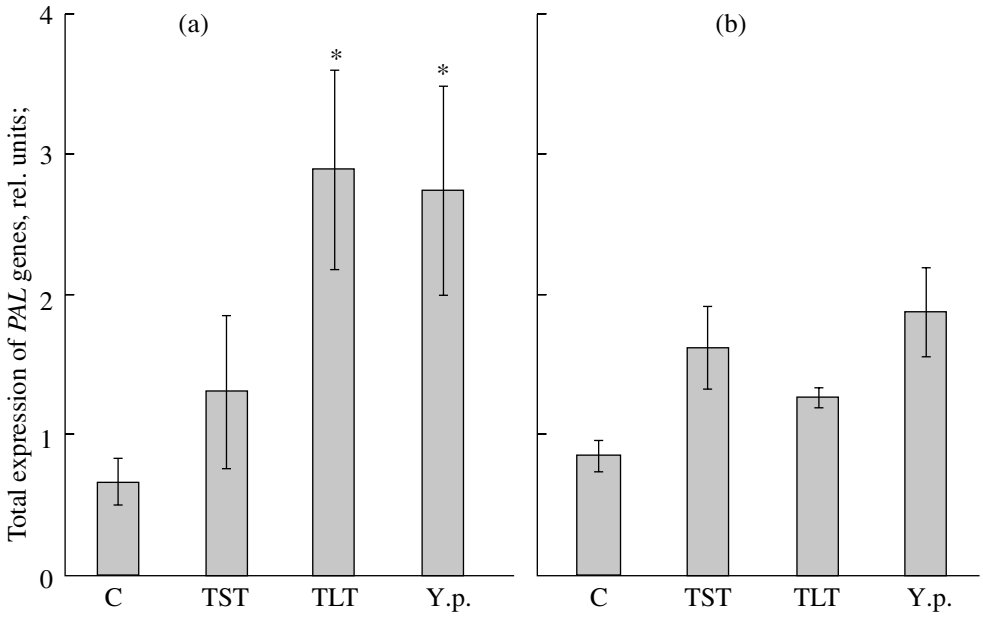

Fig. 4. The changing level of total *PAL* gene expression in ginseng GV cell cultures after application of *Y. pseudotuberculosis* and their toxins.
The actin gene expression level was taken as 1. $*P < 0.05$.
(*a*) On the 1st day of experiment; (*b*) on the 3rd day of experiment.
C—control cells; TST—TSTYp added; TLT—TLTYp added; Y.p.—bacteria added.

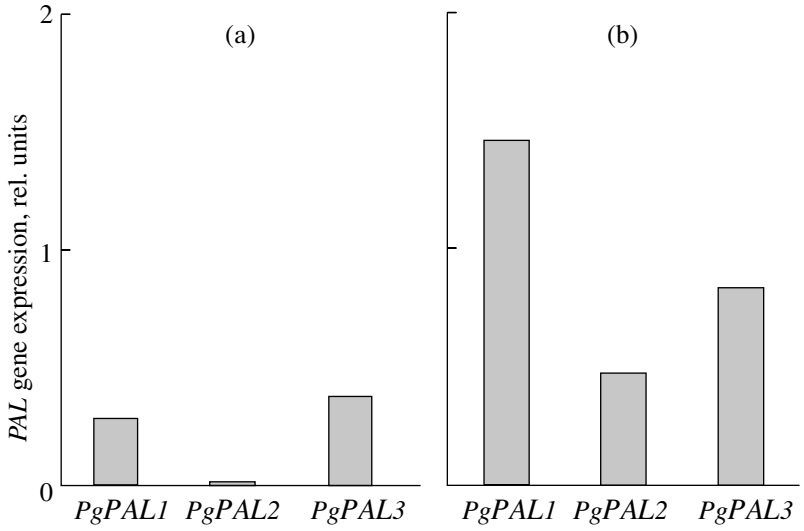

Fig. 5. *PAL* gene expression in ginseng GV cell cultures.
The ginseng actin gene expression level was taken as 1.
(*a*) Control cells cultured under normal conditions; (*b*) cells cocultured with *Y. pseudotuberculosis.*

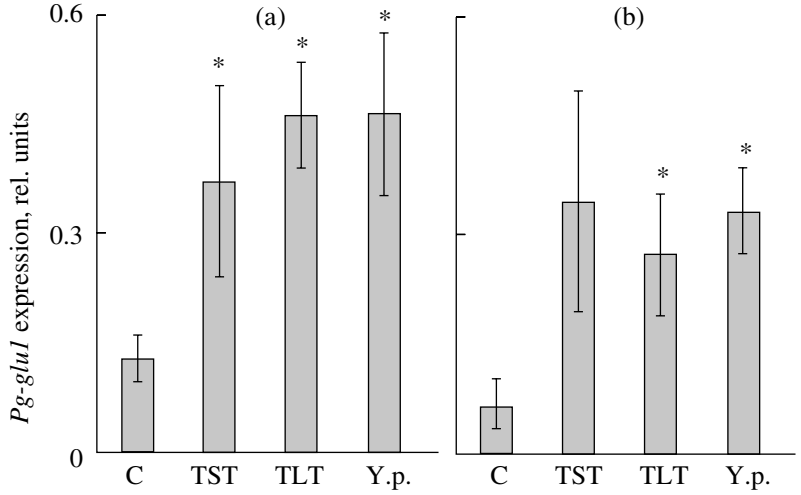

Fig. 6. The changing level of total *Pg-glu1* gene expression in ginseng GV cell cultures after adding the *Y. pseudotuberculosis* and their toxins.
The actin gene expression level was taken as 1. *P <0.05.
(*a*) On the 1st day of experiment; (*b*) on the 3rd day of experiment.
C—control cells; TST—TSTYp added; TLT—TLTYp added; Y.p.—bacteria added

pathogen. The exact pathway of ginseng GV callus cell death caused by yersinia (necrosis or apoptosis) is still unclear.

The immune response of the cells to the TSTYp and TLTYp toxins was similar to that caused by *Y. pseudotuberculosis.* This indicates that the interaction of yersinia with plant cells can be mediated by the secreted toxins. Unfortunately, the molecular structures of TSTYp and TLTYp toxins are unknown, and it is not possible so far to compare these toxins with the known toxins of *Yersinia* spp. Sequencing of genes encoding these toxins would help to identify the *Avr* gene(s) of *Y. pseudotuberculosis* interacting with plant cells. For example, seven homologues of YopT, an Avr protein of the type III protein secretion system of yersinia, are found in *P. syringae* and *Ralstonia*, the plant pathogens, and three homologues are found in *Rhizobium* and *Bradyrhizobium*, the plant symbiotic bacteria [29]. HopPtoN and HopPtoC, the Avr proteases that belong to the same Yopt protein family, were found in *P. syrin-*

gae pv. *tomato* DC3000 line [29]. YopJ/P, the protein of the type III protein secretion system of yersinia causing the apoptosis of macrophages, shows similarity with AvrRxv, the avirulence factor of *X. campestris*, a plant pathogen; YopE is a homologue of ExoS, a cytotoxin secreted by *P. aeruginosa*, and YopM was found in *Rhizobium sym*-plasmids [30].

The future studies may show that there is no clear boundary between the pathogens affecting animals and plants. *Agrobacterium tumefaciens*, the plant pathogen, was recently shown to mediate the transformation of human cell cultures [31, 32] and sea urchin embryos [33], using the pathogenicity factors of the type IV protein secretion system.

To conclude, our study showed that *Y. pseudotuberculosis* causes a rapid induction of expression of protective genes in ginseng, inhibits the normal cell growth but does not affect the cell cultures of the resistant genotype.

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REFERENCES

1. Rozhkova, L.P. and Sharapova, T.A., Further Inquiry of Far-East Scarlet-Like Fever, *Dal'nevostochnaya skarlatinopodobnaya likhoradka (psevdotuberkulez cheloveka)* (Far-East Scarlet-Like Fever(Human Pseudotuberculosis)), Somov, G.P., Ed., Leningrad: Poligraficheskii Kombinat "Rotaprint", 1978, pp. 13–24.
2. Somov, G.P., Varvashevich, T.N., and Timchenko, N.F., *Psikhrofil'nost' patogennykh bakterii* (Psychrophilic Pathogenic Bacteria), Moscow: Nauka, Sibirskoe Otdelenie, 1991.
3. Kuznetsov, V.G. and Bagryantsev, V.N., Ecology of *Yersinia* and Yersiniosis, *Okruzhayushchaya sreda i zdorov'e naseleniya Vladivostoka* (Environment and the Health of Vladivostok Inhabitants), Kosolapov, A.B. and Preobrazhenskii, B.V., Eds., Vladivostok: Dal'nauka, 1998, pp. 154–164.
4. Somov, G.P., *Dal'nevostochnaya skarlatinopodobnaya likhoradka* (Far-East Scarlet-Like Fever), Moscow: Meditsina, 1979.
5. Gordeiko, V.A. and Shustrova, N.M., *Yersinia* in Plants, *Zh. Mikrobiol., Epidemiol. Immun.*, 1990, no. 11, pp. 16–18.
6. Markova, Yu.A., Romanenko, A.S., Igumnova, E.K., and Salyaev, R.K., Plants as Possible Reservoirs for Bacterial Pathogens of Human and Animals, *Dokl. Akad. Nauk*, 2002, vol. 386, pp. 277–279.
7. Shustrova, N.M., Misurenko, E.N., and Litvin, V.Yu., Possible Transfer of *Yersinia pseudotuberculosis* by Chain Soil–Plant–Animal, *Zh. Mikrobiol., Epidemiol. Immun.*, 1992, no. 4, pp. 10–12.
8. Litvin, V.Yu., Gintsburg, A.L., Pushkareva, V.I., Romanova, Yu.M., and Boev, B.V., *Epidemiologicheskie aspekty ekologii bakterii* (Epidemiological Aspects of Bacterial Ecology), Moscow: Farmus-print, 1998.
9. Markova, Yu.A., Romanenko, A.S., Klimov, V.T., and Chesnokova, M.V., Relationships of *Yersinia pseudotuberculosis* and Potato Plants *In Vitro*, *Zh. Stress. Fiziol. Biokh.,* 2006, vol. 2, no. 1, pp. 22–27.
10. Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S.P., Signaling in Plant–Microbe Interactions, *Science*, 1997, vol. 276, pp. 726–733.
11. Rahme, L.G., Ausubel, F.M., Cao, H., Drenkard, E., Goumnerov, B.C., Lau, G.W., Mahajan-Miklos, S., Plotnikova, J., Tan, M., Tsongalis, J., Walendziewicz, C.L., and Tompkins, R.G., Plants and Animals Share Functionally Common Bacterial Virulence Factors, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 8815–8821.
12. Staskawicz, B.J., Mudgett, M.B., Dangl, J.L., and Galan, J.E., Common and Contrasting Themes of Plant and Animal Disease, *Science*, 2001, vol. 292, pp. 2285–2289.
13. Hammond-Kosack, K.E. and Jones, J.D.G., Resistance Gene-Dependent Plant Defense Responses, *Plant Cell*, 1996, vol. 8, pp. 1773–1791.
14. Scofield, S.R., Tobias, Ch.M., Rathjen, J.P., Chang, J.H., Lavelle, D.T., Michelmore, R.W., and Staskawicz, B.J., Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato, *Science*, 1996, vol. 274, pp. 2063–2065.
15. Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., Mangel, W.F., Staskawicz, B.J., and Dixon, J.E., Disruption of Signaling by *Yersinia* Effector YopJ, a Ubiquitin-Like Protein Protease, *Science*, 2000, vol. 290, pp. 1594–1597.
16. Timchenko, N.F., Bulgakov, V.P., Bulakh, E.V., Yasnetskaya, E.G., and Zhuravlev, Yu.N., Relationships between *Yersinia, Listeria*, and *Salmonella* and Plant Cells, *Zh. Mikrobiol., Epidemiol. Immun.*, 2000, no. 1, pp. 6–10.
17. Timchenko, N., Eliseikina, M., Bulgakov, V., Bulakh, E., Yasnetskaya, E., Nedashkovskaya, E., and Zhuravlev, Yu., *Yersinia pseudotuberculosis*, Its Toxins and Plant Cells, *Adv. Exp. Med. Biol.*, 2003, vol. 529, pp. 169–171.
18. Bulgakov, V.P., Kozyrenko, M.M., Fedoreyev, S.A., Mishenko, N.P., Denisenko, V.A., Zvereva, L.V., Pokushalova, T.V., and Zhuravlev, Yu.N., Shikonin Production by p-Fluorophenylalanine-Resistant Cells of *Lithospermum erythrorhizon, Fitoterapia*, 2001, vol. 72, pp. 394–401.
19. Alfano, J.R. and Collmer, A., Bacterial Pathogens in Plants: Life up against the Wall, *Plant Cell*, 1996, vol. 8, pp. 1683–1698.
20. Bulgakov, V.P., Khodakovskaya, M.V., Labetskaya, N.V., Chernoded, G.K., and Zhuravlev, Yu.N., The Impact of Plant *rolC* Oncogene on Ginsenoside Production by Ginseng Hairy Root Cultures, *Phytochemistry*, 1998, vol. 49, pp. 1929–1934.
21. Kiselev, K.V., Kusaykin, M.I., Dubrovina, A.S., Bezverbny, D.A., Zvyagintseva, T.N., and Bulgakov, V.P.,

The *rolC* Gene Induces Expression of a Pathogenesis-Related β-1,3-Glucanase in Transformed Ginseng Cells, *Phytochemistry*, 2006, vol. 67, pp. 2225–2231.

22. Timchenko, N.F., Nedashkovskaya, E.P., Dolmatova, L.S., and Somova-Isachkova, L.M., *Toksiny Yersinia rseudotubersulosis* (*Yersinia pseudotuberculosis* Toxins), Vladivostok: Primorskii Poligrafkombinat, 2004.
23. Bradford, M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantites of Protein Utilizing the Principle of Protein–Dye Binding, *Anal. Biochem.*, 1976, vol. 86, pp. 248–254.
24. Yasnetskaya, E.G., Bulgakov, V.P., Gorbach, V.I., Shevchenko, N.M., Fedoreeva, L.I., Zhuravlev, Yu.N., and Kiselev, K.V., Ethephon- and Jasmonate-Elicited Pathogenesis-Related Ribonucleases in Cultured Ginseng Cells, *Russ. J. Plant Physiol.*, 2003, vol. 50, pp. 554–560.
25. Gorpenchenko, T.Y., Kiselev, K.V., Bulgakov, V.P., Tchernoded, G.K., Bragina, E.A., Khodakovskaya, M.V., Koren, O.G., Batygina, T.B., and Zhuravlev, Yu.N., The *Agrobacterium rhizogenes rolC*-Gene-Induced Somatic Embryogenesis and Shoot Organogenesis in *Panax ginseng* Transformed Calluses, *Planta*, 2006, vol. 223, pp. 457–467.
26. Bulgakov, V.P., Veselova, M.V., Tchernoded, G.K., Kiselev, K.V., Fedoreyev, S.A., and Zhuravlev, Yu.N., Inhibitory Effect of the *Agrobacterium rhizogenes rolC* Gene on Rabdosiin and Rosmarinic Acid Production in *Eritrichium sericeum* and *Lithospermum erythrorhizon* Transformed Cell Cultures, *Planta,* 2005, vol. 221, pp. 471–478.
27. Kiselev, K.V., Gorpenchenko, T.Y., Tchernoded, G.K., Dubrovina, A.S., Grishchenko, O.V., Bulgakov, V.P., and Zhuravlev, Yu.N., Calcium-Dependent Mechanism of Somatic Embryogenesis in *Panax ginseng* Cell Cultures Expressing the *rolC* Oncogene, *Mol. Biol.*, 2008, vol. 42, pp. 275–285.
28. Cramer, C.L., Edwards, K., Dron, M., Liang, X., Dildine, S.L., Bolwell, G.P., Dixon, R.A., Lamb, C.J., and Schuch, W., Phenylalanine Ammonia-Lyase Gene Organization and Structure, *Plant Mol. Biol.,* 1989, vol. 12, pp. 367–383.
29. Zhu, M., Shao, F., Innes, R.W., Dixon, J.E., and Xu, Z., The Crystal Structure of *Pseudomonas* Avirulence Protein *AvrPphB*: A Papain-Like Fold with a Distinct Substrate-Binding Site, *Proc. Natl. Acad. Sci. USA*, 2004, vol. 101, pp. 302–307.
30. Hueck, C.J., Type III Protein Secretion Systems in Bacterial Pathogens of Animals and Plants, *Microbiol. Mol. Biol. Rev.*, 1998, vol. 62, pp. 379–433.
31. Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., and Citovsky, V., Genetic Transformation of HeLa Cells by *Agrobacterium, Proc. Natl. Acad. Sci. USA*, 2001, vol. 98, pp. 1871–1876.
32. Lacroix, B., Tzfira, T., Vainstein, A., and Citovsky, V., A Case of Promiscuity: *Agrobacterium*'s Endless Hunt for New Partners, *Trends Genet.*, 2006, vol. 22, pp. 29–37.
33. Bulgakov, V.P., Kiselev, K.V., Yakovlev, K.V., Zhuravlev, Yu.N., Gontcharov, A.A., and Odintsova, N.A., *Agrobacterium*-Mediated Transformation of Sea Urchin Embryos, *Biotechnol. J.*, 2006, vol. 1, pp. 454–461.

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