

RESEARCH PAPERS

Effect of *Bacillus* Bacteria on Hydrogen Peroxide Content and Gene Expression of Hydrolase Inhibitors in Potato Plants Infected with *Phytophthora infestans* (Mont.) de Bary

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Received March 2, 2021; revised March 17, 2021; accepted March 17, 2021

Abstract—Late blight of potato (*Solanum tuberosum* L.) caused by the oomycete *Phytophthora infestans* (Mont.) de Bary was studied in respect to the disease resistance induced by *Bacillus subtilis* Cohn (strains 26D and 11VM) and *B. thuringiensis* Berliner (strains B-5351 and B-6066) bacteria. The content of hydrogen peroxide, activities of antioxidant (catalases and peroxidases) and hydrolytic (proteases and amylases) enzymes, together with the gene expression of inhibitors of hydrolases, as the parameters related to the resistance, were assessed. The 15-day-old plants of a cv. Rannyaya Rosa susceptible to late blight were derived from microtubers. The plants were sprayed with bacterial suspension (10^8 cells/mL) of one of the mentioned strains of *B. subtilis* or *B. thuringiensis*. After 5 days, part of the plants was inoculated with *P. infestans* zoospores (10^5 spores/mL). After 6, 24, or 48 h postinoculation, the plants were fixed for biochemical analyses. It was found that either *B. subtilis* or *B. thuringiensis* reduced the late blight severity on the potato leaves; the effect depended on the particular bacterial strain applied. This was apparently a consequence of H_2O_2 accumulation and increased expression of the genes encoding protease and amylase inhibitors. The transcriptional activity of the genes of hydrolase inhibitors was stimulated by *B. subtilis* and *B. thuringiensis* to different extents. This suggests the existence of different strain-dependent pathways of establishment of bacteria-induced potato resistance to *P. infestans*.

Keywords: *Solanum tuberosum*, *Phytophthora infestans*, *Bacillus subtilis*, *Bacillus thuringiensis*, antioxidant enzymes, hydrolases, hydrolase inhibitors, hydrogen peroxide, gene expression, induced resistance

DOI: 10.1134/S1021443721060194

INTRODUCTION

Late blight is the most devastating disease of potato crop affecting almost any part of the plant: leaves, stems, tubers, flowers, and berries. The infection entails the losses, which are expressed in decrease in mass and quality of tubers, death of the infected vegetating shoots during tuber formation, and massive rot of the stored tubers. The causal agent of the disease is the oomycete *Phytophthora infestans* (Mont.) de Bary, which is a hemibiotrophic pathogen. It starts to behave as a biotroph at early stages of the disease and later turns to the necrotrophic manner of nutrition. The host-plant is inoculated with zoospores that penetrate through the lenticels, stomata, and wounds of the tissues. The zoosporangia can germinate to form the

hyphae immediately infecting the plant or produce numerous zoospores capable of germination [1].

Making crops more robust against pathogens is an urgent task of contemporary plant growing. In this regard, the most prospective strategy includes microbiological approaches exploiting potentials of plants and soil-born microbes. The ecologically safe formulations, protecting plants from biotic and abiotic stresses, are often based on Plant Growth-Promoting Bacteria (PGPB) [2]. Such bacteria of the *Bacillus* genus appear to be especially promising because of their high efficiency, long-lasting vital capacity, and widespread natural antagonism to diverse phytopathogenic fungi. Many *Bacillus*-based bioformulations have been designed to protect plants from phytopathogens and are regarded as prospective agents controlling plant diseases and pests [3].

The microorganism-based bioformulations are peculiar in that they nonspecifically activate plant defense systems. Some representatives of *Bacillus* bac-

Abbreviations: CAT—catalase; ISR—induced systemic resistance; PB—phosphate buffer; PGPB—plant growth-promoting bacteria; PI—proteinase inhibitors; PO—peroxidase; PR—proteins—pathogenesis-related proteins; SAR—systemic acquired resistance.

teria are known to carry out an endophytic lifestyle within plant tissues. They suppress growth and development of causal agents of plant diseases by means of secreted hydrolytic enzymes. In addition, their discharge of low-molecular-weight lipopeptides triggers the generation of hydrogen peroxide and activates oxylipin signaling defense system regulating activity of inhibitors of proteinases [4]. The crop-protecting action of the formulations based on *Bacillus* spp. may be mediated by H₂O₂, which is involved in the increased gene expression of PR-proteins. Presumably, *Bacillus* bacteria promote ROS generation that initiates transduction of the signals initiating, in turn, different defense responses. It was reported that plant treatment with *Bacillus subtilis* forwards an establishment of an induced systemic resistance (ISR), which is mediated by jasmonic acid [5]. Meanwhile, the bacteria of this genus can promote alternative systemic acquired resistance (SAR), which functions through the salicylate signaling pathway [6]. Despite a good deal of evidence on suppression of phytopathogens by *Bacillus* bacteria, the actual mechanism of the corresponding plant resistance remains unclear. It was suggested that the specificity of defense responses caused by *Bacillus* bacteria might depend on the nature of the particular producer organism [3].

The purpose of this work was to examine the effects of different strains of *B. subtilis* and *B. thuringiensis* on the content of hydrogen peroxide, the activities of antioxidative and hydrolytic enzymes, and the transcriptional activity of the genes of hydrolase inhibitors in potato plants in relation to the resistance of the plants to the late blight caused by the oomycete *P. infestans*.

MATERIALS AND METHODS

Objects. The plants of potato (*Solanum tuberosum* L.) were derived from microtubers of the cv. Rannyaya Rosa susceptible to late blight. The microtubers were planted into a container with TerraVita soil (Nord Palp, Russia) to a depth of 3–4 cm. The soil contained high-moor peat decomposed to different extents, purified bank sand, perlite, complex mineral fertilizer, and biohumus (pH 6.0–6.5). The plants were grown for 15 days in a growth chamber before application of bacteria.

The bacterial cultures of *Bacillus thuringiensis* Berliner (B-5351 and B-6066 strains) were obtained from the All-Russia Collection of Industrial Microorganisms. The *B. subtilis* Cohn bacteria of the 26D strain were isolated from the commercial bioformulation Phytosporin-M (Bashinkom, Russia) and those of the 11VM strain were purchased from the collection of the Skryabin Institute of Biochemistry and Physiology of Microorganisms of Russian Academy of Sciences. The bacteria were maintained on a liquid Luria-Bertani medium for 72 h followed by dilution of the suspension with distilled water to a desired concentration.

The culture of the late blight causal oomycete (*Phytophthora infestans* (Mont.) de Bary, Bashkirskii

isolate) was taken from the collection of the Institute of Biochemistry and Genetics.

Treatment of plants with bacteria, inoculation with pathogen, and assessment of disease symptoms. The plants were treated with a suspension culture of one bacterial strain (final titer of 10⁸ cells/mL, 5 mL/plant). After 5 days, part of the plants was inoculated with a *P. infestans* spore suspension (10⁶ spores/mL, 5 mL/plant).

The disease symptoms were quantitatively scored in 5 days postinoculation as the size of affected area (percentage of a total area of the lamina). The leaves were photographed, and their digital photo images were analyzed with the ImageJ program (National Institutes of Health, Bethesda, United States).

In 6, 24, and 48 h after the late blight inoculation, the control and infected plants were fixed in liquid nitrogen to be stored at –80°C until biochemical analyses.

Assay for hydrogen peroxide. The leaves were disrupted in 25 mM Na-phosphate buffer (PB), pH 6.2, at a 1 : 3 ratio (w/v), and the homogenate was centrifuged at 10000g for 20 min in a 5415R centrifuge (Eppendorf, Germany). Content of H₂O₂ was assayed in the supernatant using xylenol orange dye [7]. The reagent contained 0.074% of Mohr's salt in 5.81% sulfuric acid and 0.009% xylenol orange in 1.82% sorbitol at a 1 : 100 ratio. The optical density of the reaction products was measured at 560 nm at a Biospek-Mini spectrophotometer (Shimadzu, Japan).

Assay for catalase (CAT) activity. Plant tissue was homogenized in 50 mM PB, pH 7.8, at a 1 : 10 ratio (w/v). The homogenate was centrifuged at 12000g for 10 min followed by measurements of the enzymatic activity [8]. The reaction was initiated by mixing 0.1 mL supernatant (water in the control) with 0.2 mL of 0.03% H₂O₂. After 10 min of the reaction, it was ceased by addition of 1 mL of 4% ammonium molybdate with a subsequent measurement of A₄₁₀. The activity of catalase (EC 1.11.1.6) was calculated by the following formula:

$$E = (A_{\text{wat}} - A_{\text{sup}}) / (K \times V \times t),$$

where E is catalase activity (mol H₂O₂/L); A_{wat} and A_{sup} are optical densities of the samples containing water or supernatant, respectively; V is volume of the added sample (0.1 mL); t is incubation time (600 s); and K is molar extinction coefficient of the complex of H₂O₂ with ammonium molybdate ($22.2 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$).

Peroxidase (PO) activity was assayed in the cytoplasmic fraction. Leaf pieces were disrupted in 10 mM PB, pH 6.2, at a 1 : 3 ratio (w/v), and the homogenate was centrifuged at 12000g for 25 min. The activity of peroxidase (EC 1.11.1.7) was determined in the supernatant by a micromethod [9] based on an oxidation of the PO substrate *o*-phenylenediamine. Optical density was read at a Benchmark Microplate Reader spectrophotometer (BioRad, United States) at 490 nm. The

change in the optical density after 1 min of the reaction was taken as one unit of the enzymatic activity.

Estimating transcriptional activity of the genes of potato inhibitors of protease and amylase. Total RNA was isolated from the plants with TRIzol according to the protocol of the manufacturer (Molecular Research Center, United States). To prepare cDNA based on the m-RNA of the tested samples, the reverse transcription reaction was carried on using M-MuLV reverse transcriptase according to the manufacturer's protocol (Sintol, Russia). The accumulation of transcripts of the genes of amylase inhibitor (GenBank number XM006351484) and those of amylase inhibitor (number JX683427) (Supplementary Materials, Table 1) were analyzed by a classic real-time PCR at an iCycler iQ5 Real-Time PCR Detection System (Bio-Rad, United States). The intercalating SYBR Green I dye (Sintol) was used. Changes in the transcriptional activity (number of mRNA copies of each gene) were derived from a level of normalized expression using the software of an iCycler iQ5 Real-Time Detection System (Bio-Rad). The histograms report the rates of gene expression as compared with the initial point representing the untreated control.

Activities of amylases and proteases were assayed by hydrolysis of starch or BSA, respectively [10]. These substrates, taken at 1% final concentration, were immobilized in 4% PAAG. The samples possessing the enzymatic amylolytic and proteolytic activities were applied onto PAAG and were incubated at 37°C for 20 min followed by staining with Lugol's iodine or Coomassie G-250 solution, respectively. The enzymatic activities were determined densitometrically by calibration curves drawn using standard preparations of amylase from *Aspergillus niger* or bovine trypsin (Sigma, United States). The activities were expressed as $\mu\text{mol substrate}/(\text{g protein min})$. The protein content in the samples was estimated by Bradford.

Statistics. The experiments included five biological replications. The biochemical parameters and transcriptional activities were measured at least three times. The histograms represent sample means with their 95% confidence intervals. To estimate significance of sample means' differences, the analysis of variance followed by the Duncan's multiple range test (at 95% reliability level) were carried out with the Statistica 13 program. The significantly differing means are indicated with different letters on the histograms.

RESULTS AND DISCUSSION

Effect of Different Strains of B. subtilis and B. thuringiensis on Resistance of Potato Plants to P. infestans Infection

On the infected plants of the susceptible cv. Rannaya Rosa, a difference was observed in a late blight severity between the untreated control and the plants pretreated with *B. subtilis* or *B. thuringiensis* bacteria

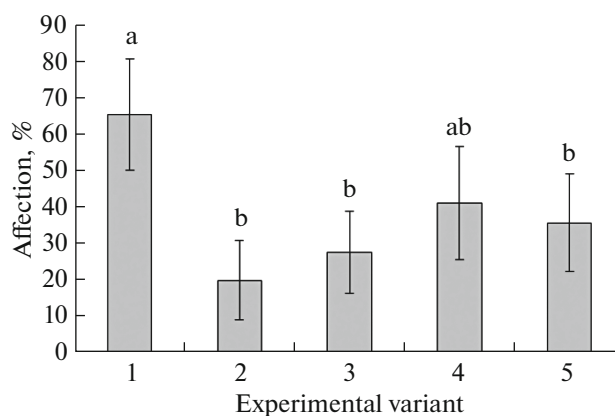


Fig. 1. Late blight severity on potato leaves pretreated with different strains of *Bacillus subtilis* or *Bacillus thuringiensis* and inoculated with *Phytophthora infestans*. (1) Untreated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066. Different letters indicate significantly differing means.

(Fig. 1). The control leaves were affected by $65 \pm 15\%$. This index was significantly lower in the bacteria-treated counterparts: down to 20 ± 11 and $28 \pm 11\%$, respectively, for the *B. subtilis* 26D and 11VM strains. For the *B. thuringiensis*, B-6066 and B-5351 strains, the corresponding values were 36 ± 13 and $41 \pm 15\%$. In general, the resistance of potato to late blight was promoted by all the tested strains but to different extent. *B. subtilis* 26D was the most efficient in agreement with the results obtained on tubers [11] and in vitro culture [12] of *S. tuberosum*.

Effect of Different Strains of B. subtilis and B. thuringiensis on Hydrogen Peroxide Content in Potato Plants Infected with P. infestans

Some mechanisms of the *Bacillus*-induced potato resistance to late blight may be related to changes in the hydrogen peroxide level in plant tissues. We actually found that the H_2O_2 concentration progressively increased in the plants that were treated with *B. subtilis* but not inoculated with *P. infestans*. The plants treated with the *B. subtilis* 11VM and *B. thuringiensis* B-6066 strains demonstrated the largest difference from the control plants after 48 h postinoculation with the pathogen (Fig. 2).

The effect might be due to antistress properties of metabolites of *B. subtilis*. Endophytic bacteria of the *Bacillus* genus are known to activate antioxidant enzymes of the plant [13]. Meanwhile, we revealed that the H_2O_2 level significantly increased in the potato leaves at the early stage of infection (6 h postinoculation) if the leaves had been pretreated with bacteria, especially *B. subtilis* 26D and *B. thuringiensis* B-6066 (Fig. 2). However, in 48 h postinoculation, this parameter was more or less uniform in the plants

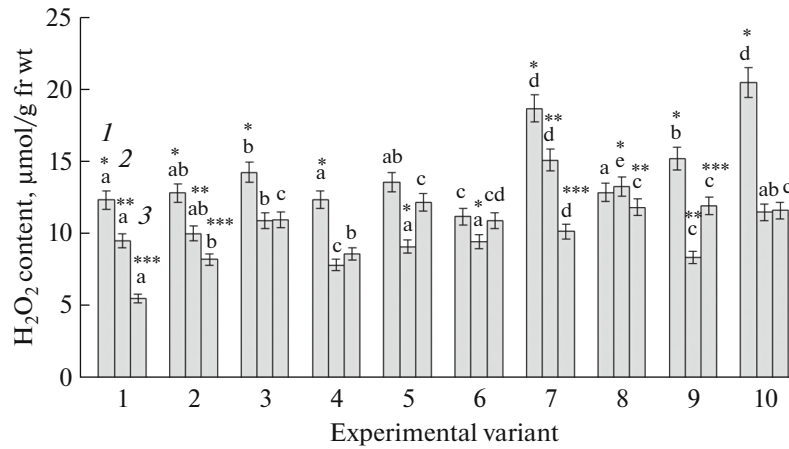


Fig. 2. Content of hydrogen peroxide in potato plants pretreated with different strains of *B. subtilis* or *B. thuringiensis* and/or inoculated with *P. infestans*. (1) Untreated uninoculated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066; (6) *P. infestans*; (7) *B. subtilis* 26D + *P. infestans*; (8) *B. subtilis* 11VM + *P. infestans*; (9) *B. thuringiensis* B-5351 + *P. infestans*; (10) *B. thuringiensis* B-6066 + *P. infestans*. Time after inoculation: (1) 6 h; (2) 24 h; and (3) 48 h. Different letters indicate significantly differing means corresponding to equal postinoculation time. Asterisks indicate significantly differing means corresponding to a counterpart of treatment but different postinoculation times.

treated with different bacterial strains and did not differ from the untreated infected control.

The resistance of potato to *P. infestans* is determined to a large extent by a hypersensitive response. This implies changes in the hydrogen peroxide concentration in the plant tissues in response to the pathogen's ingress. In this regard, H₂O₂ can be considered as an essential signaling agent that is involved in transduction of intracellular signals controlling gene expression and activation of defense systems of the plant. In turn, accumulating H₂O₂ increases cytosolic concentration of calcium ions fulfilling important functions of signal transduction into the plant genome [14]. Presumably, *B. subtilis* and *B. thuringiensis* bacteria favor the early ROS generation and initiate transmission of the signals triggering different defense systems. Bacteria of *Bacillus* genus elevate plant resistance to pathogens not only due to secretion of antibiotics but also because of the accumulation of phenols together with pro- and antioxidant enzymes in the infection site [15]. Apparently, PGPB sensitize plant tissues to penetration of pathogens and prepare the plant defense system to perform early defense reactions subsequently.

Effect of Different Strains of B. subtilis and B. thuringiensis on Activities of Antioxidant Enzymes in Potato Plants Infected with P. infestans

During pathogenesis, many metabolic processes can modulate the level of hydrogen peroxide in the plant tissues. To a large extent, these changes are associated with the changes in activities of antioxidant enzymes. Catalase is one of the essential constituents of an enzymatic antioxidant system [16]. In our experiments, healthy plants exhibited an enhanced CAT activity only after their treatment with *B. subtilis* 26D (Fig. 3). In the untreated infected plants, the activity

decreased in the course of the infection process. In the plants pretreated with *B. thuringiensis* B-6066 or, especially, *B. subtilis* 11VM, the catalase activity significantly increased over the 24-h postinoculation period.

CAT activity can be considerably modified by signaling molecules. In this regard, hydrogen peroxide is both signal and substrate for this enzyme. The influence of H₂O₂ on the activity of plant catalases is ambiguous. For example, peroxide, depending on its concentration, either inhibits [17] or stimulates [18] the CAT activity in wheat seedlings. Salicylic acid is also capable of CAT inhibition. This effect is involved in the hypersensitive reaction and can further activate the CAT gene expression and enhance the enzyme synthesis [19].

Another enzyme dealing with hydrogen peroxide is peroxidase. It is involved in both generation and elimination of H₂O₂. The chief functions of PO include protection of a plant organism from a harmful action of ROS and direct participation in the differentiation processes of tissues and organs of higher plants.

In our experiments with healthy plants, only the ones pretreated with *B. subtilis* 11VM manifested an activation of PO in comparison with the untreated control (Fig. 4). Inoculation with *P. infestans* elevated the PO activity as against the healthy counterparts after 24 h postinoculation. In the infected plants, all the tested bacterial strains of *B. subtilis* and *B. thuringiensis* increased the PO activity in comparison with the control as the inoculation with the pathogen alone.

The important trait of peroxidase is the ability of switching to catalase activity finally preventing accumulation of surplus H₂O₂. This feature was reported for several apoplastic forms of PO [20]. In addition, the extensive multigenic family of typical class III peroxidases of the plants participates in the cell wall reinforcement due to oxidative reactions between proteins and

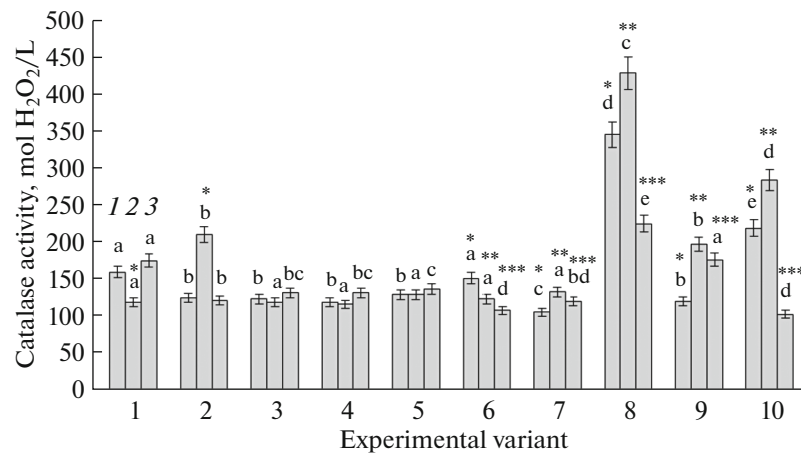


Fig. 3. Activity of catalase in potato plants pretreated with different strains of *B. subtilis* or *B. thuringiensis* and/or inoculated with *P. infestans*. (1) Untreated uninoculated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066; (6) *P. infestans*; (7) *B. subtilis* 26D + *P. infestans*; (8) *B. subtilis* 11VM + *P. infestans*; (9) *B. thuringiensis* B-5351 + *P. infestans*; (10) *B. thuringiensis* B-6066 + *P. infestans*. Time after inoculation: (1) 6 h, (2) 24 h, and (3) 48 h. Different letters indicate significantly differing means corresponding to equal postinoculation time. Asterisks indicate significantly differing means corresponding to a counterpart of treatment but different postinoculation times.

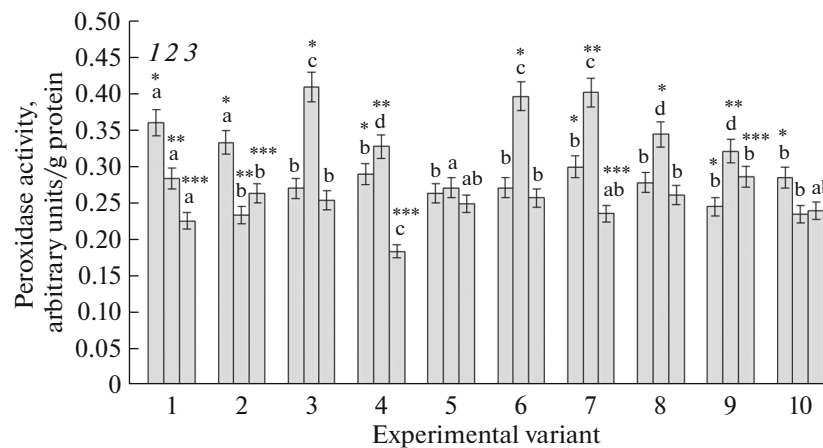


Fig. 4. Activity of peroxidase in potato plants pretreated with different strains of *B. subtilis* or *B. thuringiensis* and/or inoculated with *P. infestans*. (1) Untreated uninoculated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066; (6) *P. infestans*; (7) *B. subtilis* 26D + *P. infestans*; (8) *B. subtilis* 11VM + *P. infestans*; (9) *B. thuringiensis* B-5351 + *P. infestans*; (10) *B. thuringiensis* B-6066 + *P. infestans*. Time after inoculation: (1) 6 h, (2) 24 h, and (3) 48 h. Different letters indicate significantly differing means corresponding to equal postinoculation time. Asterisks indicate significantly differing means corresponding to a counterpart of treatment but different postinoculation times.

phenols. As a result, lignin sediments in the cell walls making them more resistant to pathogens' hydrolases. In the potato plants interacting with *Bacillus* bacteria, the H₂O₂ level might be controlled by decrease in the CAT activity or by modulation of the PO activity.

Effect of Different Strains of B. subtilis and B. thuringiensis on Transcriptional Activity of the Genes of Inhibitors of Protease and Amylase and on Activities of These Enzymes in Potato Plants Infected with P. infestans

The essential feature of plant–pathogen interactions, allowing of an assessment of pathogenic effects, is represented by hydrolytic enzymes of the pathogen.

They destroy the cell walls of the plants and promote its penetration into host's tissues [21]. The plant responds to this attack by synthesis of inhibitors of such enzymes [22].

We found that treatments of potato plants with any tested strains of *B. subtilis* or *B. thuringiensis* bacteria, as well as an infection with *P. infestans*, stimulated accumulation of the transcripts of the amylase inhibitor gene in comparison with the control counterpart. In the infected plants pretreated with *B. thuringiensis* B-5351 or B-6066 strains, the transcription level of the amylase inhibitor gene was significantly higher than in the noninfected plants. The transcription activity of the protease inhibitor gene also increased, and the maximal effect occurred upon the treatment with *B.*

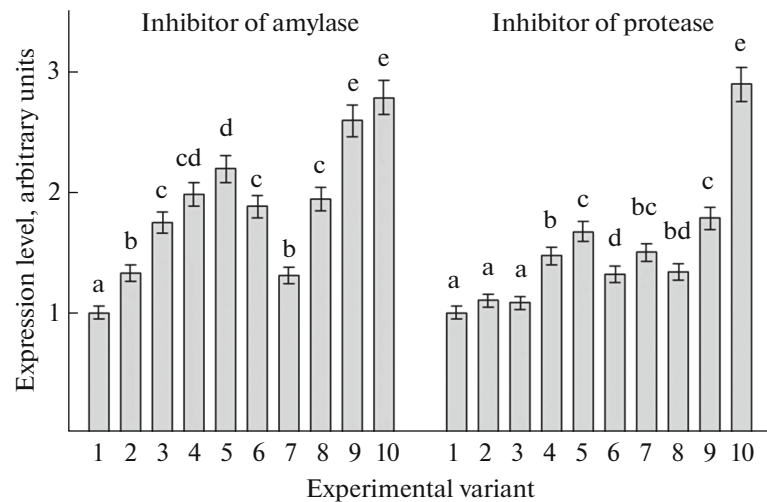


Fig. 5. Transcriptional activities of the inhibitor genes of amylase and protease in potato plants pretreated with different strains of *B. subtilis* or *B. thuringiensis* and/or inoculated with *P. infestans*. (1) Untreated uninoculated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066; (6) *P. infestans*; (7) *B. subtilis* 26D + *P. infestans*; (8) *B. subtilis* 11VM + *P. infestans*; (9) *B. thuringiensis* B-5351 + *P. infestans*; (10) *B. thuringiensis* B-6066 + *P. infestans*. Time after inoculation was 48 h. Different letters indicate significantly differing means corresponding to a particular gene.

thuringiensis B-6066 of either healthy or infected plants (Fig. 5). It seems that infection of potato with *P. infestans* may initiate de novo synthesis of the inhibitors capable of suppression of activities of amylase and proteinase enzymes. It is known that the content of hydrolase inhibitors rises in planta at the expense of synthesis of novel forms of inhibitors rather than accumulation of constitutive compounds [23]. The inhibitors of proteases and amylases are the most common in the plants [20].

Amylolytic activity is characteristic of most taxonomical groups of plant pathogens; these enzymes are almost always constitutive proteins. However, amylase is absent in oomycetes, including the *Phytophthora* genus. These pathogens use the corresponding enzymes of the host to decompose starch and activate their biosynthesis in the colonized plant tissues [24]. Presumably, the increased transcriptional activity of highly-specific amylase inhibitors, which is triggered by bacterial metabolites, hinders growth and development of *P. infestans* in the plant tissues.

Our studies revealed significantly lower activity of amylases in the healthy plants treated with *B. thuringiensis* B-5351 than in the control. Meanwhile, this parameter markedly increased after the potato treatment with *B. subtilis* 11VM (Fig. 6). In the infected plants, the protease activity significantly decreased if they had been pretreated with *B. thuringiensis* B-6066.

The high proteolytic activity in the infected tissues not only supplies amino acids for growth and development of the pathogenic microbe but can also neutralize protective proteins of potato, such as lectins inhibiting hydrolases. Thus, the extracellular metal-proteinase of phytopathogenic bacterium *Erwinia carotovora* (Jones) Waldee cleaves lectin, which is involved in

protection of potato [25]. Apparently, the increased gene transcriptional activity of the protease inhibitor aims at suppression of inhibitors of exogenous proteases and favors disease resistance in potato. The key role in initiation of formation of inhibitors of protective proteases belongs to the membrane receptor system—the protein of approximately 160 kDa molecular mass [26]. In response to wounding, it causes membrane depolarization accompanied by opening ionic channels and sharp increase in an intracellular content of calcium ions. These events lead to activation of MAP-kinase and phospholipase. Some associated reactions yield jasmonic acid, which possibly acts as an activator of gene transcription of defense proteins [27].

It is suggested that PGPB synthesize and secrete exometabolites that are specific to particular bacterial strain. These compounds comprise peptides with antibiotic properties and universal signaling molecules—ethylene, salicylic, and jasmonic acids [28]. For example, treatment of pepper plant with *B. cereus* BS107 confers them resistance to bacterial rot caused by *Xanthomonas axonopodis* pv. *vesicatoria*. The establishment of the resistance involves the genes of protective PR proteins, which are activated upon pathogenesis. Of them, PR-1 is inducible by salicylic acid, PR-4 and PR-10 by jasmonic acid and ethylene, while others are induced by H₂O₂ [29]. Treatment of tomato plants with *B. subtilis* BEB-DN stimulates the expression of some genes governing ISR. Among them, the genes of inhibitors suppressing proteases and enzymes of lignin synthesis are the most active [30].

Our results infer that the mobilization of defense systems afforded by several strains of *Bacillus* bacteria in potato plants depends on accumulation of hydrogen peroxide and activation of antioxidant enzymes and

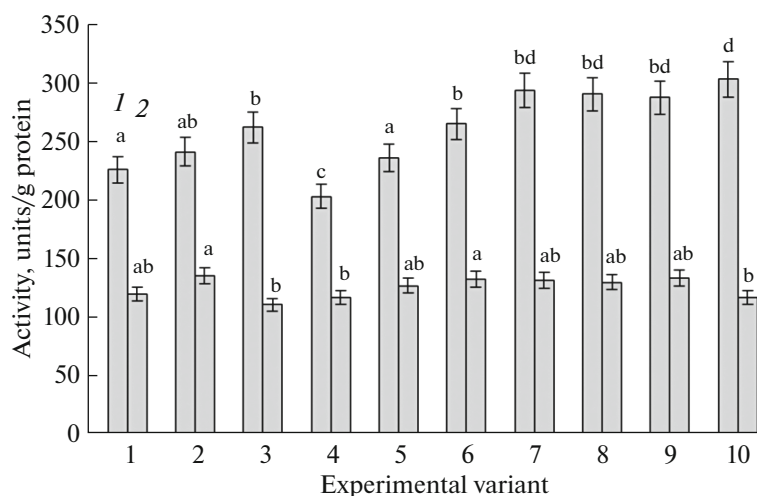


Fig. 6. Activities of (1) amylases and (2) proteases in potato plants pretreated with different strains of *B. subtilis* or *B. thuringiensis* and/or inoculated with *P. infestans*. (1) Untreated uninoculated control; (2) *B. subtilis* 26D; (3) *B. subtilis* 11VM; (4) *B. thuringiensis* B-5351; (5) *B. thuringiensis* B-6066; (6) *P. infestans*; (7) *B. subtilis* 26D + *P. infestans*; (8) *B. subtilis* 11VM + *P. infestans*; (9) *B. thuringiensis* B-5351 + *P. infestans*; (10) *B. thuringiensis* B-6066 + *P. infestans*. Time after inoculation was 48 h. Different letters indicate significantly differing means corresponding to one particular enzyme.

hydrolase inhibitors. The revealed difference in the rate of stimulation of transcriptional activities of the genes of hydrolase inhibitors caused by different strains of *B. subtilis* and *B. thuringiensis* suggests the existence of species- and strain-dependent pathways of induction of potato resistance to late blight.

ACKNOWLEDGMENTS

The equipment of Biomika Center of Collective Use (Department of Biochemical Research and Nanobiotechnology of the Agidel Regional Center of Collective Use) and that of the CODINK Unique Scientific Installation was used.

FUNDING

The work was partially carried out through State Task no. AAA-A21-121011990120-7 with support of the Russian Foundation for Basic Research and the Belarusian Republican Foundation for Fundamental Research (project no. 20-516-00005).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1134/S1021443721060194>.

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Translated by A. Aver'yanov