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EXPERIMENTAL PAPERS

Producing Cry-Toxins Endophytic Bacteria as an Alternative to Transgenic Plants to Protect Potato Plants against Colorado Potato Beetle

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Abstract—*Leptinotarsa decemlineata* (*Ld*) can develop resistance to chemical and biological insecticides. It is important to search for microbial symbionts of plants that increase their adaptive potential. It was shown that the endophytic cells of *Bacillus thuringiensis* B-5351 increases *Ld* larvae mortality and activates the transcription of genes of proteinase inhibitors and their protein products in potato plants after pest damage, in contrast to the "low endophytic" *B. thuringiensis* B-5659, which produces highly effective insectotoxins.

Keywords: *Leptinotarsa decemlineata*, *Bacillus thuringiensis*, proteinase inhibitors

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INTRODUCTION

Colorado potato beetle *Leptinotarsa decemlineata* Say (*Ld*) is the most important potato pest [1]. The main problem in protecting potato from this pest is the occurence of highly resistant to insecticides *Ld* populations [2]. 90% of all commercial bioinsecticides are based on strains of *Bacillus thuringiensis* [1]. Often *B. thuringiensis* and Cry toxins are considered along the same lines as chemical pesticides. *B. thuringiensis* strains have been reported that can exist endophytically, i.e., in the internal tissues of plants, where they are protected from external influences [3], and, apparently, are able to induce plant defense reactions against phytophages [5].

The aim is to study the effect of *B. thuringiensis* B-5351 and *B. thuringiensis* B-5658 strains on the transcriptional activity of the proteinase inhibitor gene and the activity of its product in potato plants and the mortality of Colorado potato beetle larvae after eating plants containing endophytic bacteria.

MATERIALS AND METHODS

We used strains *B. thuringiensis* B-5351 and *B. thuringiensis* B-5658, test-tube potato plants of the variety Early Rose, III instar larvae of *Ld* (collected in 2020, Chishmy, Bashkortostan). Their endophytic properties were assessed by the number of CFU of bacteria per g of wet weight of plants on the 7 day after treatment [5]. At the same time, the larvae were fed once with plants with endophytes or leaves, immersed in suspension of bacteria grown on LB liquid medium (10^8 cells/mL), as described in [5], on the 10th day after which mortality was calculated. In each variant, at least 30 larvae were examined. The transcriptional activity of trypsin inhibitor gene AY089962 was studied using Real-Time PCR; the activity of its protein product was studied using the BAPNA substrate [6] 1 h after plant damage by *Ld* larvae. Intact plants treated with water were used as control ones. The measurements were carried out in 3 biological replicates, in two independent experiments. Statistica 2.0 (Russia) was used for statistical processing, the significance of differences was detected by Tukey's *t*-test.

RESULTS AND DISCUSSION

The internal tissues of plants contain by one order more *B. thuringiensis* B-5351 than *B. thuringiensis* B-5689 (Table 1). At the same time, the suspension of the lat-

Table 1. Endophyticity of *B. thuringiensis* strains and their effect on *Ld* larval mortality (10 days after feeding)

		Control	B-5689	B-5351
CFU × 10^3/g [CFU/ × 10^3/g]		0	3.0 ± 0.11a*	70.0 ± 12.3b
Mortality, %	Suspension	4.2 ± 0.5a	64.6 ± 8.3b	28.7 ± 7.2c
	Endophytes	6.7 ± 0.5a	19.1 ± 4.8b	55.6 ± 5.8c

* Statistically significant differences in the corresponding parameter according to Tukey's *t*-test.

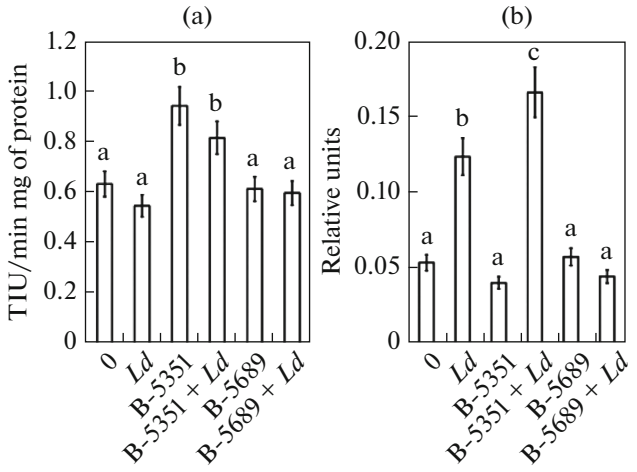

Fig. 1. Influence of *B. thuringiensis* on the activity of the trypsin inhibitor (a) and the number of transcripts of the *AY089962* gene encoding it (b) on 1 hour after plant damage *Ld* (different letters denote statistically significant differences in the corresponding parameters according to Tukey's *t*-test).

ter causes a significant increase in the mortality of larvae. Feeding plants containing endophytic cells of *B. thuringiensis* B-5689 results in much less larval mortality, while eating plants with endophytic *B. thuringiensis* B-5351 results in the death of more than 50% of larvae.

It was shown that *B. thuringiensis* B-5351 increases the trypsin inhibitor activity in both intact and *Ld*-damaged potato plants (Fig. 1a). At the same time, the content of AY089962 gene transcripts in damaged plants treated with water increased by 2.5 times compared to the control ones, and in plants treated with *B. thuringiensis* B-5351, by 3.4 times. *B. thuringiensis* B-5689 had no effect on both parameters (Fig. 1b).

We have shown that the strain *B. thuringiensis* B-5351 penetrates into the internal tissues of wheat and potato plants, and its insecticidal effect against *Ld* larvae is due to both the synthesis of Cry toxins [7], stimulation of transcription of proteinase inhibitor gene and activity of its protein product in host plants. Proteinase inhibitors are important protective compounds that reduce the activity of enzymes in the digestive tract of insects, and, accordingly, the food attractiveness of plants. Limiting the development of insect resistance to insecticides is possible with a multifactorial effect on the organism of pests [2], as we described in the case of plants treated with *B. thuringiensis* B-5351.

CONCLUSIONS

Mortality of larvae after feeding with a suspension of *B. thuringiensis*-5689 was the highest, which indicated the effectiveness of the toxins produced by the strain, however, its insecticidal effect as an endophyte was significantly lower than that of *B. thuringiensis* B-5351. We assume, it is due to its low ability to penetrate into the internal tissues of plants and influence the defense reactions of plants against phytophages.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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