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INSECTICIDAL AND POTATO GROWTH-STIMULATING ACTIVITY OF *BACILLUS THURINGIENSIS KURSTAKI* HD-1

Bacillus thuringiensis (Bt) produces Cry toxins against pest insects. Cry proteins are conformed by domains related to pore formation and recognition of protein receptors. Plant-induced systemic resistance (ISR) is triggered due to pest attack, it could be activated by Bacillus sp. Tecia solanivora (Ts) is a potato pest, susceptible to Cry1Ac and Cry1B proteins. This paper indicates the endorsement of Bt kurstaki HD-1 (BtkHD1) in relation to Ts control (Cry1Ac and Cry1B proteins), potato growth promotion, and plant ISR due to pests related to the BtkHD1-potato system. To ensure that ongoing quality control of BtkHD1 was maintained, crystal synthesis (microscopy), cry1 genes presence, and Cry protein production were checked. Bioassays Ts larvae and potato plantlets and an in silico *analysis of the hybrid Cry1Ac-Cry1Ba protein and potato ISR related to the BtkHD1 influence were performed. Bioassay on Ts larvae shows an* LC_{50} *of 536 ng/cm*^{2} *of diet. A potato growth promotion assay revealed the effect of BtkHD1 on the length and dry weight of stems. The prospective analysis took into account relevant factors affecting the biological function of the hybrid protein focused on domain II.* In silico *identification of 15 BtkHD1 proteins and 68 potato proteins related to plant ISR due to pests was completed. This project serves to validation of toxicity on Ts larvae and potato growth effect based on BtkHD1, including a forward analysis of the hybrid Cry1Ac1-Cry1Ba1, and proteins associated with this strain and potato for eliciting plant ISR due to pests.*

Keywords: *Bacillus thuringiensis strain ABTS-351, Cry protein, Tecia solanivora, Solanum tuberosum development promotion, induced systemic resistance.*

Crystal (Cry) proteins of *Bacillus thuringiensis* (Bt) strains exhibit insecticidal activity on moths, beetles, or mosquitos [1]. These proteins are an option in commercial agriculture, and this fact has led to the production of biological pesticides and genetically modified (GM) crops due to their specificity coupled with their safety for the environment and human health, and so

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today they replace the use of chemical insecticides [2]. Cry protoxins are solubilized by alkaline pH and proteolytically processed in insect midgut to the activated toxin form which undergoes specific binding to the cell membrane surface of cadherin (CADR) or other receptors (aminopeptidase N, alkaline phosphatase), and posterior insertion into the cell membrane to form lytic pores. It is accepted that Cry subunits are assembled into oligomers for insertion into the cell membrane. A second relevant described action mechanism is associated with the CADR receptor binding and activation of a signaling pathway with the interaction of G protein, adenylyl cyclase, cyclic AMP, and protein kinase A, resulting in the cytoskeleton disruption, channel formation, and cytolysis [3]. Activated Cry toxin comprises three structural domains: domain I has seven or eight amphipathic α-helices which disrupt the midgut epithelial cell membrane by forming ion pores; domains II and III (antiparallel β-sheets) are involved in specific folding and interaction with membrane receptors in the insect midgut. There are projects for *cry* gene-directed evolution to swap domain sequences that have been characterized to improve potency and/or to reach new specificities [3, 4]. Cry proteins are classified by amino acid sequence homology and structure [5]. This classification corresponds to specificity in some cases, e.g. Cry1 and Cry9 toxins have activity against Lepidoptera order, Cry2 toxins are active against Lepidoptera and Diptera orders, Cry3 and Cry7 are Coleopteran-specific toxins, and Cry4, Cry10, and Cry11 are Dipteran-specific toxins [2].

There are several lepidopteran models with a relevant Bt susceptibility for bioassays comparisons such as *Spodoptera frugiperda* and *Trichoplusia ni* [2]. *Tecia solanivora* (Ts) Povolny (Lepidoptera: Gelichiidae) is a potato pest in the Andes (America) causing about 20% of direct losses. Ts has susceptibility to Bt *kurstaki* HD-1 (BtkHD1)-carrying *cry1* genes, and Cry1Ac and Cry1B proteins among other Cry1 proteins, and GM potato lines expressing a *cry1Ac* gene have been generated [6, 7]. A bacterium developing in the root ecosystem of plants can increase adaptation to insect pests by induced systemic resistance (ISR) using hormones such as ethylene, jasmonic acid induced by bacterium elicitors as volatile organic compounds (VOCs) and lipopeptides [8]. It has been described that Bt has activity as a biofertilizer, however, scientists still know too little about the effects of inoculation of Bt during plant development or on the health of potato crops [9]. It has already been seen that Bt can be used to trigger tomato ISR against *Ralstonia solanacearum* [10], but there are no studies about the Bt's impact on plant response to insects. Nevertheless, studies on *Bacillus sp*. triggering ISR as a response to insects are advanced [11-13]. This paper presents results obtained through applying bioassays, both to assess existing Ts larvae mortality and to promote potato growth with the use of BtkHD1. This was accompanied by a theoretical analysis that takes into account the processes of protein evolution and relevant Cry proteins (Cry1Ac and Cry1B) with activity to Ts, as well as determination of BtkHD1 and potato proteins associated with the bacterium-plant interaction and ISR that collectively improve response to insect aggression.

Materials and Methods. ***Quality control.*** BtkHD1 (formerly ABTS-351) was obtained from a commercial formulation named Dipel™, which is commercialized by Valent BioSciences LLC (a subsidiary of Tokyo-based Sumitomo Chemical Co., Ltd.) in the United States. Dipel™ was registered by Abbott (1970). Schering marketed it from 1972 in Australia, also Bayer marketed Dipel™ Forte 2X under Abbott's permission from 1994 until 1997. Abbott has been marketing Dipel™ SC and DF since 1997. By 2000, Sumitomo had purchased Abbott agrochemicals, including Dipel™. The product was serially diluted and plated on nutrient agar at 30 ± 2°C an orbital shaker set with 150 rpm for 24 h. Afterwards, the bacterium was picked to ob-

serve the colony morphology and verify the culture purity. BtkHD1 was subsequently stored in glycerol at –80 °C and filter paper after 25 days of sporulation. BtkHD1 was cultured on nutrient agar. A wet mount slide was achieved by transferring one loopful of biomass onto a microscopic slide. The culture was spread in the thin film and then covered with a cover slip. The slide was examined for the presence of spores and parasporal crystals under a phase contrast microscope. Plasmid DNA from BtkHD1 was extracted according to He et al. [14]. DNA, $MgCl_2$, Taq DNA polymerase concentration, and hybridization temperature of primers are relevant components for PCR. Effects and interactions of these four components were assessed using three levels (DNA (50, 100, and 200 ng), $MgCl_2$ (1.5, 2.0, and 2.5 mM), Taq DNA polymerase (1, 2 and 3 U), and hybridization temperature of primers (50, 55 and 60 °C) with the Taguchi method [15] using E = 2k + 1 (E is the number of experiments, k is the number of factors), so, this applies, in this case, to obtaining a minimum necessary of nine experiments: E = 2 (4) + 1 = 9. DNA was then subjected to a *cry1* gene screen using PCR with a set of primers (forward 5'-CTGGATTTACAGGTGGGGATAT-3', reverse 5'-TGAGTCGCTTCGCATATTTGACT-3') [16] in 25 µl reaction that contained 1X Buffer (100 mM Tris-HCl pH 9.0, 500 mM KCl, 1.0% Triton X-100), 0.2 mM concentrations of each dNTP, 2 mM $MgCl_2$, 2U Taq DNA polymerase, 1 µM forward and reverse primers, and 100 ng of DNA. PCR conditions were: melting temperature of 94 °C for 1 min, followed by 35 cycles of melting at 94 °C for 1 min, annealing at 53 °C for 30 s, and extension at 72 °C for 1 min, with additional step of extension of 72 °C for 5 min. Water was used as negative control (data not shown). Crystal extracts were prepared and solubilized in 50 mM sodium carbonate buffer (pH 10.0). The concentration of solubilized protoxin was determined by Bradford assay [17]. Protoxin was analyzed by SDS-PAGE.

Bioassays. Biological activity of BtkHD1 was monitored on the first instar larvae of Ts from a laboratory colony and a rearing diet [6]. An easy infrastructure is needed for hatching, rearing, keeping in cages, and finally for the life cycle development of these insects (egg, larva, pupae, and adult). Eggs were put into small rearing cages containing potato tubers where larvae are fed until they are mature enough to lay new eggs. For toxicity tests, autoclaved potato sheets of 0.2 mm in Petri dishes impregnated with 0.7% ascorbic acid and 0.5% methylparaben were surface inoculated with a protein concentration of 0–400 ng/cm^2 diluted in 50 µL deionized water. The potato sheets were infested with one Ts first instar larvae. Plates were sealed and incubated into 18 °C, 60 ± 5% relative humidity, and 12:12 h light: dark photoperiod room. For insect bioassay, 20 neonate larvae with four replicates per treatment were used, and mortality was recorded after 5 days.

Potato growth stimulation activity of BtkHD1. BtkHD1 strain was characterized for plant growth promotion. Nitrogen fixation was evaluated by inoculation of bacterial colony into Nitrogen free bromothymol blue (NFb) agar (0.5% malic acid; 0.4% KOH; 0.5% K_2HPO_4; 0.005% $FeSO_4 \cdot 7H_2O$; 0.001% $MnSO_4 \cdot H_2O$; 0.001% $MgSO_4 \cdot 7H_2O$; 0.002% NaCl; 0.001% $CaCl_2$; 0.0002% Na_2MoO40; 2 mL Bromothymol blue (0.5% ethanol); 0.005% yeast extract; 2% agar, pH 6.6–7.0). Phosphate solubilization activities were measured through SRS agar (0.05% $(NH_4)_2SO_4$, 0.02% KCl, 0.03% $MgSO_4 \cdot 7H_2O$, 0.0004% $MnSO_4 \cdot H_2O$, 0.0002% $FeSO_4 \cdot 7H_2O$, 0.02% NaCl, 1% glucose, 0.05% yeast extract, 0.01% Bromocresol purple, 0.5% $Ca_3(PO_4)_2$, 1.8% agar, pH 7.0–7.2). Siderophore production was verified by Chrome Azurol sulphonate (CAS) agar by means of pre-CAS solution (0.065 g CAS in 50 mL of HPLC water + 10 mL of 0.0027 g $FeCl_3 \cdot 6H_2O$ in 10 mL of 10 mM HCl + 0.073 g Hexa-decyl Trimethyl Ammonium bromide (HDTMA) in 40 mL of HPLC water) and added

to 0.9% agar (CAS solution). Then the CAS solution was added to a inoculated nutritive agar and incubated at 28 °C (darkness) for 3 h. IAAsynthesis was identified by inoculation of 0.3 mM L-tryptophan in nutritive agar (30 °C, 48 h), then Salkowski reagent (35% perchloric acid plus 10 mM $FeCl_3$) was added to crude bacterial extracts and evaluated after 2 h at 30 °C. BtkHD1 strain was also used to evaluate the growth promotion effect on potato. Creole potato (*S. tuberosum* Group *phureja*) was host for the bioassay, which required up to 35 days to complete. All soil and materials used in each treatment were sterile. BtkHD1 treatment contained vegetative cells ($1 \cdot 10^{10}$ UFC/mL) plus two positive (*Pseudomonas aeruginosa* strain PAO1 (Pa PAO1) at $1 \cdot 10^{10}$ UFC/ml and urea fertilizer (0.09 g)) and one negative controls. A total of 371 individuals in intervention and control groups, respectively, were analyzed. Analytical phenotypes in this process were root and stem length, root and stem wet weight, root and stem dry weight, foliar area, and stem and leaf number. Data were analyzed using ANOVA and Bonferroni tests at 5% level of probability.

In silico *analysis of Cry proteins.* Amino-acid sequences of Cry1Ac1 and Cry1Ba1 proteins were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nih.gov) (accession numbers: AAA22331 and CAA29898 respectively). Structural domain identification was realized with the Conserved domain database (CDD) from NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). Primary sequence alignment between Cry1Ac1 and Cry1Ba1 domains II was carrying out with the ClustalOmega program [18] (https://www.ebi.ac.uk/Tools/msa/clustalo/). Kyte and Doolittle hydrophobicity, flexibility, and accessible residues plots were generated using ProtScale [19] (http://www.expasy.ch/cgi-bin/protscale.pI) and Horizontal Protein Comparison Tool (HePCaT) Portal [20] (http://best.bio.jhu.edu/HePCaT/). The theoretical isoelectric point (pI) and molecular weight (Mw) were calculated with Compute pI/Mw [21] (http://www.expasy.ch/tools/pi_tool.html). The Antigenicity Plot was realized with EMBOSS Antigenic (EMBOSS package) [22, 23] (http://www.bioinformatics.nl/cgi-bin/emboss/antigenic). Experimental structure templates for model construction were Cry1Ac (PDB: 4ARX) and Cry8Ea1 (PDB: 3EB7) proteins. By means of DeepView-Swiss-PdbViewer 4.1 [24] (https://spdbv.vital-it.ch/), a 3D model for a hybrid toxin Cry1Ac1/Cry1Ba1/Cry1Ac was retrieved. It was also refined using 3Drefine [25] (http://sysbio.rnet.missouri.edu/3Drefine/).

In silico *identification of BtkHD1 — potato proteins related to pest infestation.* Identification of BtkHD1-potato proteins and their relationship with insect aggression was performed on the basis of particular genomes (JMHW00000000 and AEWC01000000 respectively). The strategy required a comparative analysis against existing case studies concerning to induction of plant ISR against insects by use of bacteria and protein identification using tBLASTn with a default Blosum62 matrix.

Results. *BtkHD1 quality control.* The starting points for the BtkHD1 quality control were microscopic, molecular, and biochemical characterizations. BtkHD1 strain was observed using a phase-contrast microscopy and taking into account bipyramidal crystals synthesis with their distinctive refracting surfaces, which confirmed the production of Cry1 proteins (Fig. 1, *a*). For molecular characterization, it was necessary to prorate variables of PCR into individual levels. Setting the number of experiments to 81 (3^4) might identify a combination of DNA, $MgCl_2$, and Taq DNA polymerase and hybridization temperature of primers as the most important four-factor combination of inputs of results. The aim of this part of the project was to identify an experimental arrangement that would engage DNA, $MgCl_2$ and Taq DNA polymerase concentrations and hybridization temperature

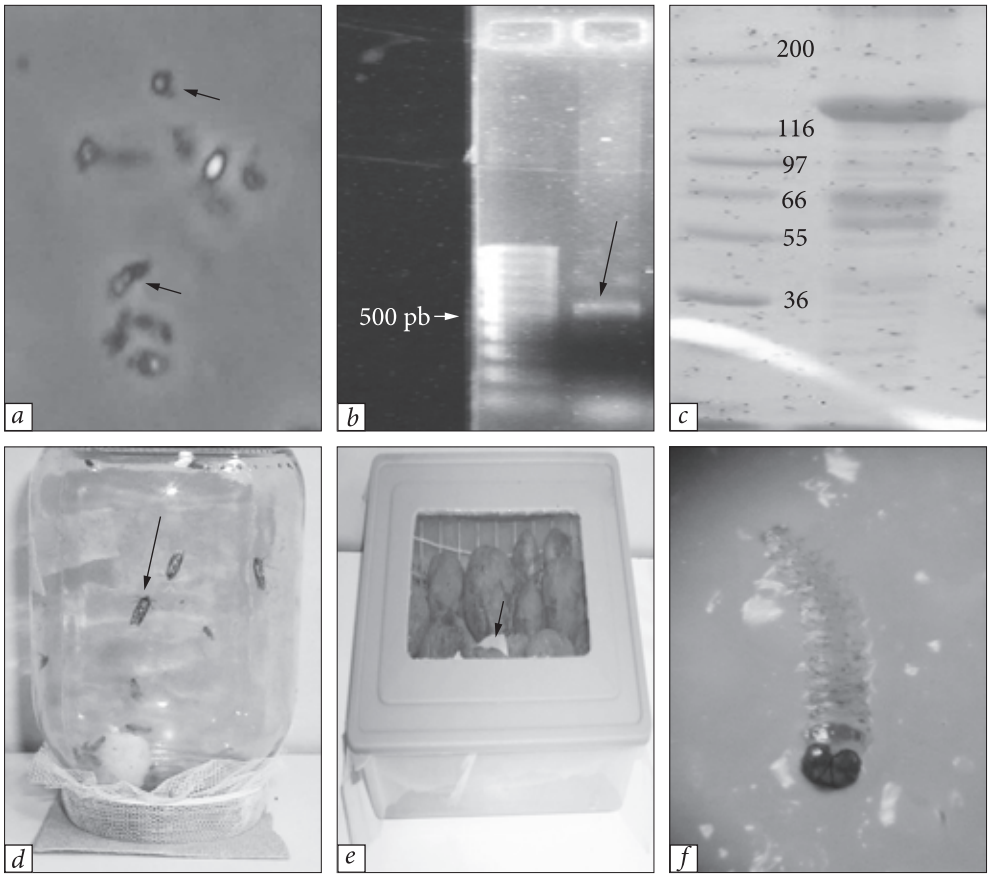


Fig. 1. Microscopic, molecular and protein profile of BtkHD1, and rearing of Ts: *a* — Microscopy shows that spores have refringence and crystals are opaque (arrows); *b* — PCR showing *cry1* band (arrow); *c* — SDS-PAGE shows bands of 130 and 66 kDa related to Cry1 and Cry2 proteins; *d* — Rearing flasks containing Ts adults (arrow); *e* — Rearing container for paper that contains eggs (arrow), larvae, pupae and emerging adults from Ts; *f* — Ts larva on potato diet

of primers, which influence PCR for *cry1* gene identification, using the Taguchi method [15] in order to reach a minimum number of experiments**.** For the *cry1Ac-cry1Ba* genes, the relative size of amplicons regarding the conserved region is 558-564 bp in the case of BtkHD1 in our work (Fig. 1, *b*). SDS-PAGE for BtkHD1 shows two bands with a molecular weight of ~130 kDa and ~70 kDa (Fig. 1, *c*).

Biological assays with Ts. Depending on the temperature, the Ts life cycle could take ~75 days. Ts adults were divided into several glass jars and fed with 30–50% of honey on a cotton pad. Eggs were collected daily on absorbent paper and incubated on healthy potato at room temperature. Larvae that hatch from eggs often develop in potato tuber (Fig. 1, *d*–*e*). Eggs should be collected at frequent intervals at least twice per day and placed in sterile Petri dishes until they hatch. Larvae survival rate on potato diet is 95% with a variation coefficient of 0.01%, which is suitable for bioassays (Fig. 1, *f*) [26]. A preservative solution was used as a diluent or vehicle for Cry toxin extracts. Three biological assays with a total of five concentrations (0-400 ng/cm^2 of protein) were evaluated for effects of BtkHD1 Cry protein on Ts larvae compared to the negative control (water). The lethal concentration 50 (LC_{50}) for this bioas-

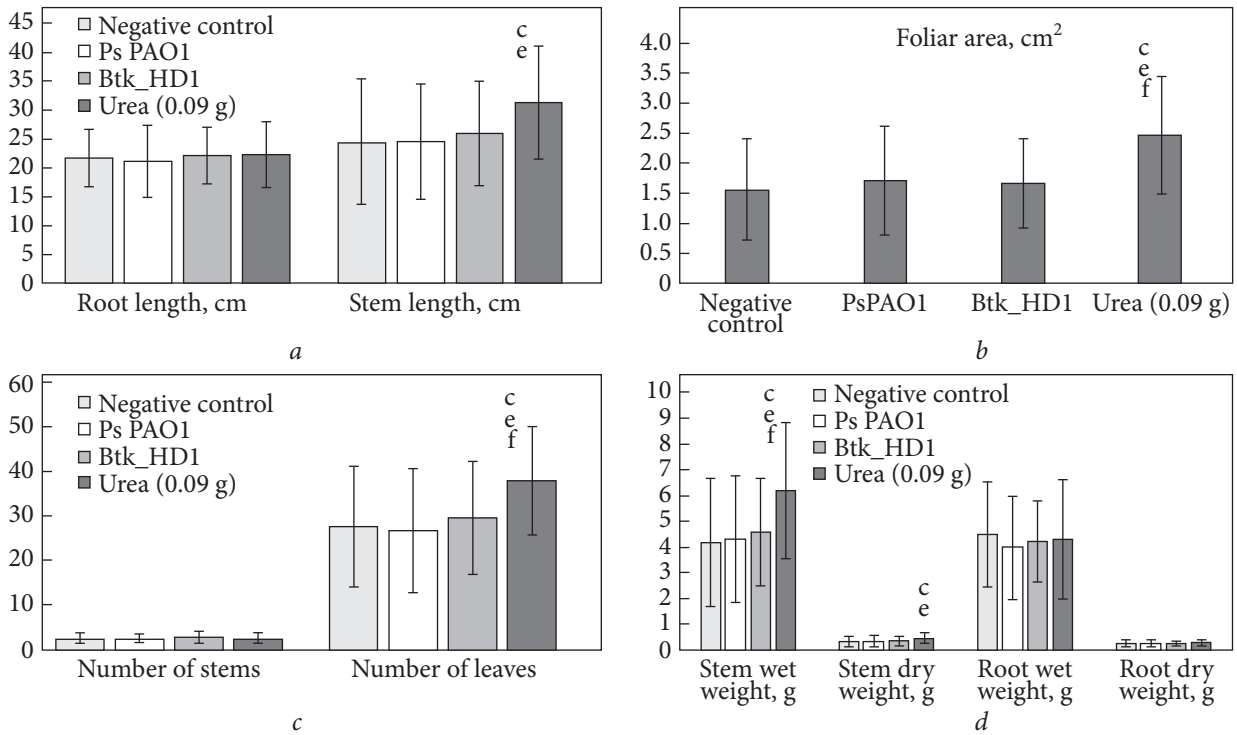


Fig. 2. Biological evaluation of BtkHD1 on potato. A. Evaluation of root and stem length. B. Evaluation of foliar area. C. Evaluation of stem and leaf number. D. Evaluation of root and stem wet weight and root and stem dry weight. Error bars are depicting the variation. The letters above indicate the significance for a comparison ($p<0.05$) of the options: *a* — negative control vs Pa PAO1, *b* — negative control vs BtkHD1, *c* — control vs fertilizer, *d* — Pa PAO1 vs BtkHD1, *e* — Pa PAO1 vs fertilizer, *f* — BtkHD1 vs fertilizer. Letter is located on highest mean

say was 536 ng/cm^2 of diet (Probit analysis, 95% confidence interval: 167, 1725).

Biological assay of BtkHD1 and potato for growth promotion. First of all, BtkHD1 was positive for some biochemical tests associated to plant growth promotion: nitrogen fixation, siderophore synthesis, and acid production on SRS medium for phosphate solubilization (results not shown). In a subsequent phase, this study found that potato plants actually were stimulated by urea fertilizer for all phenotypes. It was possible to determine that BtkHD1 has the same effect as the fertilizer based on urea, specifically for the length and dry weight of stems ($p<0.05$) (Fig. 2). Furthermore, it may be observed that BtkHD1 does not differ significantly regarding Pa PAO1 strain.

Hybrid Cry1Ac1-Cry1Ba1 protein. Cry1Ac and Cry1Ba toxins were used as a basis for structure/activity comparison for the purpose of domain swapping. CDD analysis was an important tool for identification of boundaries for domain II from Cry1Ac1 and Cry1Ba1 toxins. Cry1Ac1 domain II is composed of residues T259-E461. Cry1Ba1 binding receptor domain is formed by residues T278-D489. A hybrid protein containing the Cry1Ac1/Cry1Ba1/Cry1Ac1 primary sequence was constructed by exchange of domain II. Cry1Ac1/Cry1Ba1/Cry1Ac1 as a protoxin begins with M1 and ends with E1187. Hybrid protoxin is comprised of a N-terminal fragment susceptible to cleavage (M1-L47), a functional three-domain conformation (S48-T571), and a C-terminal region which is lost

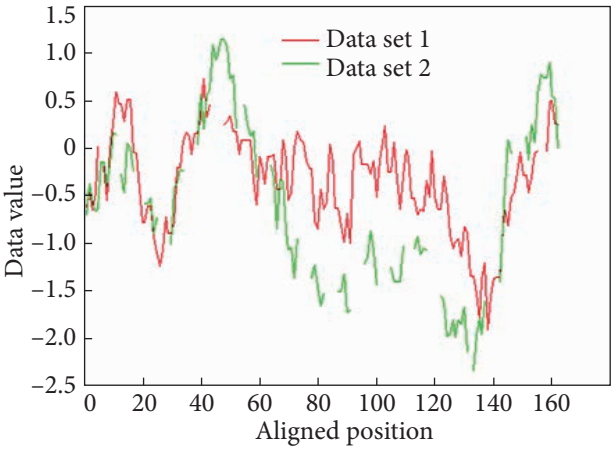
due to proteolytic cleavage to release the active toxin. The sequence alignment in both Cry1Ac1 and Cry1Ba1 highlights 175 differences between two intrinsic domains II (conserved substitutions = 37; semi-conserved substitutions = 23) (Fig. 3, *a*). Cry1Ac1 domain II has a shorter number of residues than Cry1Ba1 domain II (nine residues). Therefore, a comparison between domains II of Cry1Ac1 and Cry1Ba1 had to be established. Domain II of hybrid toxin (corresponding to Cry1Ba1) is moderately hydrophobic, flexible, and very accessible to solvent (Fig. 3, *b*–*d*). Theoretical pI/Mw for hybrid toxin and parental Cry1Ac1 toxin was is 6.15/64210 and 6.51/64839 respectively, there is no important variation between them. The antigenicity plot shows possible determinants for receptor recognition in domain II (Cry1Ba1) (Fig. 3, *e*). Based on structural alignment of the amino acid sequence of Cry1Ac1/Cry1Ba1/Cry1Ac1 hybrid toxin with Cry1Ac and Cry8Ea1 toxins, a theoretical model of the hybrid toxin was obtained, which corresponds to residues S48-T618 of the primary protoxin structure. Hybrid toxin has three domains. Domain I (residues 48-271) consists of a bundle of eight antiparallel α-helices in which helix 6 is encircled by the remaining helices; domain II (corresponding to Cry1Ba1: residues 272-442) is composed of three antiparallel β-sheets united in a termed «Greek key» topology; domain III (residues 443-618) consists of three twisted antiparallel β-sheets (Fig. 3F). Structural comparison of Cry1Ac1 and Cry1Ba1 domains II indicates differences in loops of domains II (Fig. 3, *g*).

BtkHD1-Potato-Ts interaction. These possible BtkHD1 proteins related to triggering plant ISR in response to insects were analyzed on the basis of background studies analyzed in detail and then related on the basis of gene transcription per plant-*Bacillus sp*.-pest insect relationship, with objective criteria that generate certainty for the organisms (potato and BtkHD1) in the interaction model. The main findings, which are based on specific studies reported by Deng et al. [11], Rashid et al. [12], and Valenzuela-Soto et al. [13], may be summarized in Table 1 and 2.

Discussion. The main mission of the Bt program for biological control of pests is identification of the toxic activity of different strains and Cry proteins, and of all the parallel compounds derived from various biological and molecular approaches. The paper then proceeds to discuss BtkHD1 application as a set of diverse interactions that act and impact upon Ts control and potato response to the attack of insects. Quality control of BtkHD1, a relevant microorganism in the insect control, needs to be evaluated with microscopy to determine whether (1) plasmids carrying *cry1* genes are required for Cry+ phenotype not to be lost, and (2) BtkHD1 is free of bacteriological contamination. Cry proteins, however, conserved five blocks separated by sequences with high variability. Slightly different PCR procedures have been described using primers corresponding to highly conserved regions in *cry* genes. Due to the high percentage of homology of Cry1 proteins (45-78%), there is a highly conserved region used for detection of *cry1* genes by means of general primers with an expected size of their PCR products of 543-594 bp (depending on a particular gene, e.g. *cry1Ac* is 564 bp, or *cry1Ba* is 558 bp) [16]. If required, there are sufficient primers designed for PCR amplification of *cry* genes to also be used in characterization of Bt strains, covering wide ranks of Cry proteins and conserved-variable regions or functional domains [27]. PCR is susceptible to essential components (DNA, dNTPs, $MgCl_2$, Buffer, Taq polymerase, and primers) because it depends on concentration and cycle conditions, most of which are difficult to assess in individual evaluations, so experiments that would be needed to implement an optimal PCR seem to be out of proportion to the purpose. That is why optimization for PCR had to be developed through any experimental design so that to consider relevant variables. Taguchi


Cry1Ba1
Cry1Ac1

a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Data set 2
Data value
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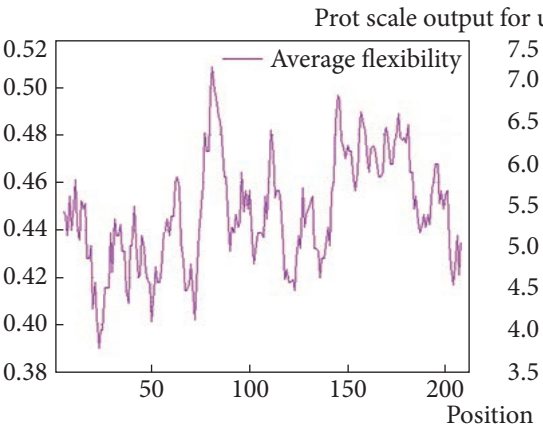
b


Prot scale output for user sequence
Average flexibility
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0.48
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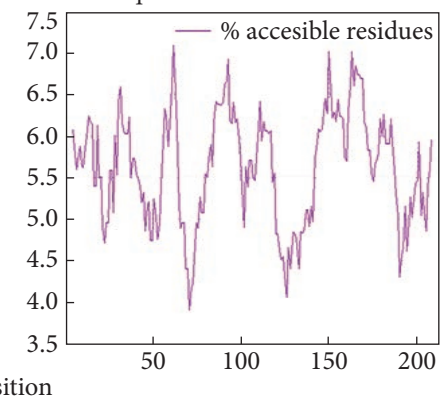
c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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
50
100
150
200

d


Cry1Ba1 domain II
Sequence
ESYSHRLSHIGIILQSRVNVPVYSW
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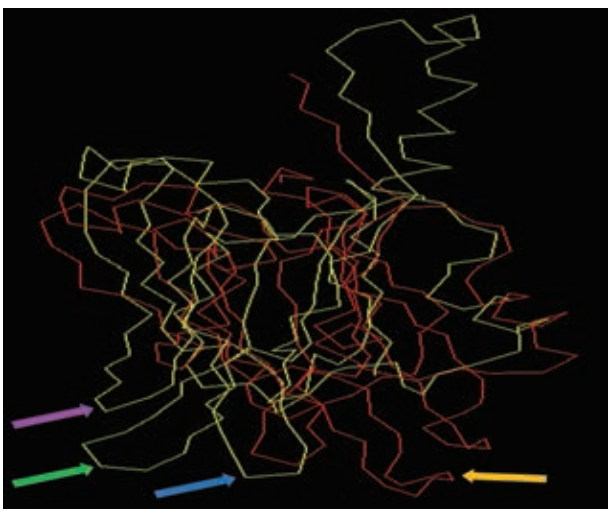
SFSAIEAAAIRSPHLLDFLEQLTIFSA
INPVTLRFASRDV
YSQPYESPGLQLK

e

f



g

◄ ***Fig. 3.*** *In silico* analysis of Cry1Ac1/Cry1Ba1/Cry1Ac1 hybrid toxin: *a* — Cry1Ba1 and Cry1Ac1 domains II alignment. Symbols: «*» means that the residues or nucleotides in that column are identical in all sequences in the alignment; «:» means that conserved substitutions have been observed, according to the colors; «.» means that semi-conserved substitutions are observed. Colors: Red (AVFPMILW) means small (small + hydrophobic (including aromatic -Y)); Blue (DE) means acidic; Magenta (RK) means basic; Green (STYHCNGQ) means Hydroxyl + Amine + Basic — Q; *b* — Hydrophobic surface comparison of Domain II between Cry1Ac1 and Cry1Ba1 proteins. Red is Cry1Ac1 (DILNSITIYTDAHRGYYYWS) and green is Cry1Ba1 (AAIRSPHLLDFLEQLTIFSA), hydrophobic residues are A, L, I, P, F, and M; *c* — Average flexibility of Cry1Ba1 Domain II, peaks correspond to higher flexible regions; *d* — Solvent accessible residues in Cry1Ba1, peaks correspond to exposed regions; *e* — Probable antigenic residues in Cry1Ba1 domain II; *f* — Structure of Cry1Ac1/Cry1Ba1/Cry1Ac1 hybrid toxin showing a Domain I (red), Domain II (yellow) and Domain III (green); *g* — Superposition of the domain II Cα traces of Cry1Ac1 (red) and Cry1Ba1 (yellow), arrows indicate the exposed loops. Cry1Ba1: blue arrow is 354QN355, green arrow corresponds to 293IGG295, and purple arrow is related to 382LPPETTE388. Cry1Ac1: yellow arrow corresponds with 369RPFNIGINNQ378

method [15] was used to predict an optimal combination of factors and their corresponding levels, which may lead to improvement of PCR with few experiments. For this, an experiment was designed, taking into account factors that have the greatest effect on PCR and are adjusted in an orthogonal array where relations between two columns are adapted so that each level is present in the equal number of occasions. Every array can be recognized through the formula $L_A(B^C)$, where L is represented by A which shows a necessary number of experiments, term B simply signals a number of levels or concentrations included in an appropriate column, C identifies the most important variables to be included into the experiment. These arrays consider the effect of each factor and their interactions. Finally, protein analysis indicates the presence of bands that demonstrate expression of *cry* genes, being identified with two bands of ~130 kDa and ~70 kDa, which relate to BtkHD1 and their Cry1 (Cry1Aa, Cry1Ab, and Cry1Ac) and Cry2 proteins respectively [28].

Potato diets containing low concentrations of ascorbic acid and methyl paraben were used because larvae can miss rearing if food preservative is found in high concentration [29]. An autoclave cycle should be used to avoid the risk of enzymatic potato oxidation. Petri dishes were covered with potato foil which is 0.2 mm thick to allow larvae to construct caves for feeding and easy observation with a stereoscope (Fig. 1, *f*). A drying process of seven hours immediately after placing preservative + Cry toxin solution was needed to adequate humidity before laying Ts larvae. The overall diet survival was high (>95%) and similar for each repetition whether the preservative solution was used or not. Our bioassay results are consistent with the previous data [6], and with an LC_{50} of ~536 ng/cm^2 of diet, it is feasible to think that potato field application can be sustainable.

The effectiveness and impact of Bt as a plant growth-promoting bacterium have to be comprehensively analyzed. Plant growth-promoting bacteria have unique properties that can help the plant development including the synthesis of IAA, 1-aminocyclopropane-1-carboxylate-deaminase (ACC-deaminase), phosphate solubilizing enzymes, siderophores, proteases, catalase enzyme, and antimicrobial factors among others. Assessment of plant growth promotion features of different strains of Bt has been poorly studied. Some strains of Bt produce IAA, ACC-deaminase, phosphate solubilizing enzymes, and siderophores as direct mechanisms. However, there are Bt indirect mechanisms (antagonist outcome) such as bacteriocin, zwittermicin, fengycin, chitinase, and cell wall-degrading enzyme production, which provides for protection of crops. In addition, some Bt strains are active producers of VOCs. Bt strains have

displayed effect on wheat, corn, lettuce, pea, and lentil (co-inoculated with *Rhizobium leguminosarum*), and soybean (co-inoculated with *Bradyrhizobium japonicum*) [9, 30]. Our study indicates that BtkHD1 is able to nitrogen fixation, siderophore production, and phosphate solubilization, it also has a beneficial effect on the number of leaves and dry weight of stems for potato plantlets. Besides, it must be taken into account that in the case of potato growth-promoting bacterium *P. aeruginosa* [31], BtkHD1 had a similar degree of achievement for all the potato features of this study with regard to Pa PAO1.

Table 1. **Identification of BtkHD1 proteins connected with the potato — ISR against insects**

Genes involved in lipopeptide synthesis from *Bacillus sp.*	Proteins associated to BtkHD1 lipopeptide synthesis (tBLASTn)	Query cover, %	E value	Identity, %	Accession
>ABS72505.1_SpoVT_Bacillus_velezensis_FZB42	AbrB/MazE/SpoVT family DNA-binding domain-containing protein	58	4E-17	37.14	WP_000648325.1
>ABS75594.1_DegU_Bacillus_velezensis_FZB42	response regulator transcription factor	96	2E-55	40.99	WP_000694629.1
>ABS75207.1_ComA_Bacillus_velezensis_FZB42	response regulator transcription factor	97	4E-35	36.02	WP_000590673.1
>ABS72800.1_RapC_Bacillus_velezensis_FZB42	hypothetical protein	97	3E-38	28.00	WP_001102658.1
>ABS72486.1_AbrB_Bacillus_velezensis FZB42	AbrB/MazE/SpoVT family DNA-binding domain-containing protein	100	2E-54	85.11	WP_000843036.1
>ABS72774.1_YczE_Bacillus_velezensis_FZB42	membrane protein	90	5E-25	27.50	WP_000943428.1
>ABS75211.1_DegQ_Bacillus_velezensis_FZB42	phage tail protein	78	0.028	30.56	WP_001173498.1
>AFZ90901.1_Iturin_A_synthetase_A_Bacillus_velezensis_AS43.3	non-ribosomal peptide synthetase	67	0.0	35.59	WP_042969737.1
>ABS74972.1_PhoR_Bacillus_velezensis_FZB42	sensory box histidine kinase PhoR	99	0.0	49.41	WP_042969826.1
>ABS74973.1_PhoP_Bacillus_velezensis_FZB42	transcriptional regulatory protein YycF	98	6E-85	52.97	AIE36796.1
>ABS74206.1_FenD_Bacillus_velezensis_FZB42	non-ribosomal peptide synthetase	99	0.0	34.86	WP_042969737.1
>CAE11276.1_FenB_Bacillus_velezensis_FZB42	non-ribosomal peptide synthetase	99	0.0	34.93	WP_001255746.1
>ABS72764.1_surfactin_non_ribosomal_peptide_synthetase_SrfAA_Bacillus_velezensis_FZB42	bacitracin synthetase 1	98	0.0	35	AIE33772.1
>ABS72765.1_surfactin_non_ribosomal_peptide_synthetase_SrfAB_Bacillus_velezensis_FZB42	tyrocidine synthetase III (plasmid)	99	0.0	34.73	AIE37512.1
>ABS72767.1_surfactin_non_ribosomal_peptide_synthetase_SrfAC_Bacillus_velezensis_FZB42	amino acid adenylation enzyme/thioester reductase family protein (plasmid)	97	5E-157	31.21	AIE37506.1

Table 2. **Identification of potato proteins associated with ISR by BtkHD1 against insects**

Proteins involved in interaction *Bacillus sp.* — plant — insect	Proteins related to potato ISR (tBLASTn)	Query cover, %	E value	Identity, %	Accession
arginase_2_Solanum_lycopersicum	Solanum tuberosum arginase 1, mitochondrial-like	100	0.0	95.86	XM_006347818.2
P04284_Pathogenesis_related_leaf_protein_6_Solanum_lycopersicum	Solanum tuberosum mRNA for pathogenesis related protein PR-1 (pr1-1 gene)	100	4E-107	91.19	AJ250136.1
AA34142.1_chlorophyll_a/b_binding_protein_precursor_partial_Solanum_lycopersicum_M17559.1	Solanum tuberosum chlorophyll a-b binding protein 5, chloroplastic	100	6E-176	100.00	XM_006351951.2
AAZ94182.1_proteinase_inhibitor_I_precursor_Solanum_tuberosum	Solanum tuberosum clone StDT82 proteinase inhibitor I mRNA	100	7E-79	97.44	JX683427.1
CAA41439.1_pathogenesis_related_protein_P2_Solanum_lycopersicum	Solanum tuberosum pathogenesis-related protein P2-like	100	4E-94	95.10	NM_001288679.1
AAZ08249.1_proteinase_inhibitor_1_PPI3A4_Solanum_tuberosum	Solanum tuberosum proteinase inhibitor 1 PPI3A4 (PPI3A4) mRNA	100	5E-76	100.00	DQ087225.1
AAA34147.1_chlorophyll_ab_binding_protein_Cab_1B_Solanum_lycopersicum_M14443.1	Solanum tuberosum chlorophyll a/b binding protein (Lhcb1-1) gene, nuclear gene encoding chloroplast protein	100	0.0	95.09	U21111.1
AAF34802.1_putative_flavonol_synthase_like_protein_Euphorbia_esula_AF228663_1	Solanum tuberosum uncharacterized LOC102577668	99	1E-165	72.79	NM_001287849.1
AAZ83363.1_cytosolic_ascorbate_peroxidase_1_Solanum_lycopersicum_dq096286.1	Solanum tuberosum L-ascorbate peroxidase 1, cytosolic (APX)	100	3E-166	91.20	XM_006366063.2
NP_566518.1_Ubiquitin_like_superfamily_protein_Arabidopsis_thaliana	Solanum tuberosum autophagy-related protein 8i-like	99	6E-65	76.32	NM_001288500.1
AAK58857.1_EIL1_Solanum_lycopersicum_AF328784	Solanum tuberosum protein ETHYLENE INSENSITIVE 3-like	100	0.0	93.18	XM_006361067.2
AAA34152.1_chlorophyll_ab_binding_protein_Cab_1C_partial_Solanum_lycopersicum_M30618.1	Solanum tuberosum chlorophyll a/b binding protein (Lhcb1-1) gene, nuclear gene encoding chloroplast protein	100	4E-80	100.00	U21111.1
CAA54561.1_cell_wall_protein_Solanum_lycopersicum	Solanum tuberosum tyrosine and lysine rich cell wall protein (Trlp)	44	1E-06	58.97	GU233535.1
CAA78403.1_pre_pro_cysteine_proteinase_partial_Solanum_lycopersicum	Solanum tuberosum cysteine proteinase 15A-like	94	0.0	95.89	XM_006341935.2
AAB35432.1_LeAux_Arabidopsis_auxin_regulated_protein_homolog_Lycopersicon_esculentum_tomatoes_VFN8_Peptide_Partial_150 aa	Solanum tuberosum auxin-responsive protein IAA4-like	100	3E-99	95.33	XM_006350599.2

Continuation of Table 2.

Proteins involved in interaction *Bacillus sp.* — plant — insect	Proteins related to potato ISR (tBLASTn)	Query cover, %	E value	Identity, %	Accession
ABC69046.1_cinnamic_acid_4_hydroxylase_Solanum_tuberosum	Solanum tuberosum cinnamic acid 4-hydroxylase gene	100	2E-170	95.04	DQ341174.1
AAS58469.1_ultraviolet_B_repressible_protein_Gossypium_hirsutum_AY551823.1	Solanum tuberosum clone St-TNT48 ultraviolet-B-repressible protein	98	2E-28	47.93	JX576215.1
P26600.1_PAL5_SOLLC_RecName_Full_Phenylalanine_ammonia_lyase_Short_PAL	Solanum tuberosum phenylalanine ammonia-lyase-like	100	0.0	95.44	NM_001318638.1
pir_A24727_phenylalanine_ammonia_lyase_EC_4.3.1.5_kidney_bean_fragment	Solanum tuberosum phenylalanine ammonia-lyase	100	0.0	83.37	MH636300.1
AAP59427.1_phospholipid_hydroperoxide_glutathione_peroxidase_Solanum_lycopersicum_AY301280.1	Solanum tuberosum probable glutathione peroxidase 2	100	5E-118	97.60	XM_006347213.2
ABB29467.1_salt_tolerance_protein_Glycine_max	Solanum tuberosum B-box zinc finger protein 20 (BBX20)	100	1E-99	65.84	KX576530.1
AAA66308.1_thiol_protease_partial_Solanum_lycopersicum_M21444.1	Solanum tuberosum low-temperature-induced cysteine proteinase-like	100	0.0	87.57	NM_001287948.1
LOC_Os12g14440.1_Jacalin_like_lectin_domain_containing_protein	Solanum tuberosum agglutinin-like	61	5E-21	40.32	XM_006342156.2
LOC_Os11g32650.1_chalcone_synthase	Solanum tuberosum chalcone synthase 1b	93	0.0	84.99	U47740.1
LOC_Os06g01250.1_cytochrome_P450	Solanum tuberosum cytochrome P450 93A2	92	8E-143	43.27	XM_006366196.2
LOC_Os12g37260.1 lipoxygenase 2.1, chloroplast precursor, putative, expressed	Solanum tuberosum linoleate 13S-lipoxygenase 2-1, chloroplastic-like	87	0.0	49.88	NM_001287914.1
LOC_Os12g04500.1 response regulator receiver domain containing protein, expressed	Solanum tuberosum two-component response regulator ORR9-like	75	2E-77	74.19	XM_006351214.2
LOC_Os12g37320.1 lipoxygenase 2.2, chloroplast precursor, putative, expressed	Solanum tuberosum linoleate 13S-lipoxygenase 2-1, chloroplastic-like	97	2E-48	33.42	NM_001287914.1
LOC_Os12g37350.1 lipozygenase protein, putative, expressed	Solanum tuberosum linoleate 13S-lipoxygenase 2-1, chloroplastic-like	100	7E-177	56.26	NM_001287914.1
LOC_Os01g63190.1 laccase precursor protein, putative, expressed	Solanum tuberosum laccase-7-like	95	0.0	50.89	XM_006342223.2
LOC_Os02g41650.3 phenylalanine ammonia-lyase, putative, expressed	Solanum tuberosum phenylalanine ammonia-lyase	97	0.0	74.12	MH636300.1

Continuation of Table 2.

Proteins involved in interaction *Bacillus sp.* — plant — insect	Proteins related to potato ISR (tBLASTn)	Query cover, %	E value	Identity, %	Accession
LOC_Os05g35290.1 phenylalanine ammonia-lyase, putative, expressed	Solanum tuberosum phenylalanine ammonia-lyase	96	0.0	71.80	MH636300.1
LOC_Os12g01530.1 ferritin-1, chloroplast precursor, putative, expressed	Solanum tuberosum ferritin-2, chloroplastic-like	79	9E-93	67.79	NM_001288475.1
LOC_Os05g06920.1 relA-SpoT like protein RSH4, putative, expressed	Solanum tuberosum probable GTP diphosphokinase CRSH, chloroplastic	94	8E-126	44.88	XM_006349806.2
LOC_Os11g01530.1 ferritin-1, chloroplast precursor, putative, expressed	Solanum tuberosum ferritin	78	5E-64	66.43	NM_001288074.1
LOC_Os10g40720.1 expansin precursor, putative, expressed	Solanum tuberosum beta expansin 1 precursor	86	2E-79	49.79	NM_001288462.1
LOC_Os07g42960.1 phospho-2-dehydro-3-deoxyheptonate aldolase, chloroplast precursor, putative, expressed	Solanum tuberosum Phospho-2-dehydro-3-deoxyheptonate aldolase 1, chloroplastic (SHKA)	89	0.0	84.02	NM_001288432.1
LOC_Os07g24190.1 CESA3 - cellulose synthase, expressed	Solanum tuberosum cellulose synthase A catalytic subunit 2 [UDP-forming]	100	0.0	67.58	XM_006367741.2
LOC_Os07g48040.1 peroxidase precursor, putative, expressed	Solanum tuberosum cationic peroxidase 1-like	92	1E-119	60.14	XM_006349506.2
LOC_Os04g43800.1 phenylalanine ammonia-lyase, putative, expressed	Solanum tuberosum phenylalanine ammonia-lyase-like	99	0.0	72.80	NM_001318638.1
LOC_Os02g41630.2 phenylalanine ammonia-lyase, putative, expressed	Solanum tuberosum phenylalanine ammonia-lyase-like	99	0.0	70.34	XM_006354277.2
LOC_Os03g53800.3 periplasmic beta-glucosidase precursor, putative, expressed	Solanum tuberosum beta-glucosidase BoGH3B-like	98	0.0	73.62	XM_015315133.1
LOC_Os07g49310.1 omega-3 fatty acid desaturase, chloroplast precursor, putative, expressed	Solanum tuberosum omega-3 fatty acid desaturase, chloroplastic-like	91	0.0	70.03	NM_001287954.1
LOC_Os08g30020.2 membrane protein, putative, expressed	Solanum tuberosum uncharacterized	92	9E-150	51.26	XM_006340206.1
LOC_Os01g64960.1 chlorophyll A-B binding protein, putative, expressed	Solanum tuberosum photosystem II 22 kDa protein, chloroplastic	100	5E-86	61.43	XM_006363831.2
LOC_Os04g38600.1 glyceraldehyde-3-phosphate dehydrogenase, putative, expressed	Solanum tuberosum glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	91	0.0	77.48	XM_006359217.2

Continuation of Table 2.

Proteins involved in interaction *Bacillus sp.* — plant — insect	Proteins related to potato ISR (tBLASTn)	Query cover, %	E value	Identity, %	Accession
LOC_Os04g43760.1 phenylalanine ammonia-lyase, putative, expressed	phenylalanine ammonia-lyase-like [Solanum tuberosum]	98	0.0	71.65	NP_001305567.1
LOC_Os08g01380.1 2Fe-2S iron-sulfur cluster binding domain containing protein, expressed	ferredoxin-1, chloroplastic [Solanum tuberosum]	71	1E-52	75.00	XP_006339919.1
LOC_Os02g15750.1 expressed protein	rhodanese-like domain-containing protein 4, chloroplastic [Solanum tuberosum]	92	5E-125	51.33	XP_006339688.1
LOC_Os06g06290.1 GDSL-like lipase/acylhydrolase, putative, expressed	GDSL esterase/lipase At5g45910-like [Solanum tuberosum]	95	2E-95	43.22	XP_006367777.2
LOC_Os09g36680.1 ribonuclease T2 family domain containing protein, expressed	extracellular ribonuclease LE [Solanum tuberosum]	87	1E-42	31.70	XP_006355102.1
LOC_Os08g06100.1 O-methyltransferase, putative, expressed	caffeic acid 3-O-methyltransferase [Solanum tuberosum]	100	3E-170	59.57	XP_015164331.1
LOC_Os06g36090.1 ABC-2 type transporter, putative, expressed	ABC transporter G family member 35-like [Solanum tuberosum]	99	0.0	72.54	XP_006343042.1
LOC_Os12g17600.1 ribulose bisphosphate carboxylase small chain, chloroplast precursor, putative, expressed	ribulose bisphosphate carboxylase small chain 2C, chloroplastic [Solanum tuberosum]	96	9E-86	68.16	XP_006363111.1
LOC_Os09g36450.1 triosephosphate isomerase, chloroplast precursor, putative, expressed	triosephosphate isomerase, chloroplastic-like [Solanum tuberosum]	89	4E-168	82.35	XP_006367334.1
LOC_Os11g06720.1 abscisic stress-ripening, putative, expressed	cold inducible product; similar to other osmotic stress induced gene products	63	7E-16	64.37	AAD00254.1
LOC_Os06g09610.1 peroxiredoxin, putative, expressed	peroxiredoxin Q, chloroplastic [Solanum tuberosum]	56	3E-95	85.91	XP_006353774.1
LOC_Os02g18450.1 GTP-binding protein typA/bipA, putative, expressed	GTP-binding protein TypA/BipA homolog [Solanum tuberosum]	90	0.0	82.70	XP_006352137.1
LOC_Os11g47970.1 AAA-type ATPase family protein, putative, expressed	ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic-like isoform X2 [Solanum tuberosum]	89	0.0	79.52	XP_006339015.1
LOC_Os07g34006.1 transporter family protein, putative, expressed	glucose-6-phosphate/phosphate translocator 2, chloroplastic-like [Solanum tuberosum]	86	0.0	73.53	XP_006343413.1
LOC_Os06g18010.1 UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed	UDP-glycosyltransferase 708A6-like [Solanum tuberosum]	95	2E-109	41.00	XP_015162319.1

Continuation of Table 2.

Proteins involved in interaction *Bacillus sp.* — plant — insect	Proteins related to potato ISR (tBLASTn)	Query cover, %	E value	Identity, %	Accession
LOC_Os07g32570.1 OsAPRL1 adenosine 5'-phosphosulfate reductase-like OsAPRL1, expressed	5'-adenylylsulfate reductase 1, chloroplastic [Solanum tuberosum]	90	0.0	74.94	XP_006348128.1
LOC_Os06g18670.1 anthocyanidin 3-O-glucosyltransferase, putative, expressed	UDP-glycosyltransferase 708A6-like [Solanum tuberosum]	95	6E-101	37.58	XP_015162319.1
LOC_Os07g33910.2 transporter family protein, putative, expressed	glucose-6-phosphate/phosphate translocator 1, chloroplastic [Solanum tuberosum]	59	3E-90	78.57	XP_006366041.1
LOC_Os02g46260.1 OsSCP9 — Putative Serine Carboxypeptidase homologue, expressed	serine carboxypeptidase-like 12 isoform X2 [Solanum tuberosum]	95	8E-150	47.54	XP_015168617.1
LOC_Os08g04500.1 terpene synthase, putative, expressed	(-)-germacrene D synthase-like [Solanum tuberosum]	96	8E-126	39.36	XP_006364906.1
LOC_Os05g12640.1 expressed protein	BURP domain-containing protein precursor [Solanum tuberosum]	93	7E-55	44.39	NP_001275413.1
LOC_Os11g47980.1 expressed protein	ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic-like isoform X1 [Solanum tuberosum]	75	1E-69	40.43	XP_006339014.1

Toxin-receptor interaction is a prerequisite for pore formation and thus dictates toxin specificity [32]. Folding of Cry toxins is relevant to achieve a specific interaction with insect receptors [33]. Strict specificity of receptor-toxin interaction has promoted development of hybrid *cry* genes by exchange of domains II and III, as very promising tools to construct insecticidal toxins and transgenic plants with specific insect resistance or increased Ts lethality [34]. Cry1Ac and Cry1Ba are highly related proteins [5]. They are lethal for Ts [6]. In addition, it was further established that Cry1Ac protoxin obtained from Bt *kurstaki* HD-73 has lethality against Ts (LC_{50} = 295 ng/cm^2 of diet) (data not shown). For all these reasons, the two toxins were chosen for an *in silico* domain II exchange as an opportunity to focus on this region. It was suggested that receptor binding sites for Cry1 proteins must have two basic characteristics: a highly conserved structure because Cry1 proteins have similar primary sequences and 3D structures and can recognize similar receptors found in midguts of several lepidopteran insects, as well as a non-conserved structure because Cry1 proteins also exhibit highly specific insecticidal activity and can distinguish host species in the lepidopteran range [35].

It was observed that site-specific changes in loop II of the receptor binding domain of Cry1Ac toxin (365TLYRRPFN372) altered toxicity of this protein in lepidopteran larvae, herein of particular interest were mutant toxins 368AR369 (not stable) and double mutants (368AA369, 368EE369, 368FF369, 368HH369) which all led to reduction in toxicity. These residues were chosen in view of their lower volume, less hydrogen bonding capability, and hydrophobicity with regard to arginine (relative solvent accessibility in Å^2 of 265, 174.2 g/mol, basic polar), which has an amphipathic side chain, is located in protein

surfaces exposed to solvent, and can interact forming hydrogen bonds and salt bridges. To sum up, A (relative solvent accessibility in Å^2 of 121.0, 89.094 g/mol, nonpolar) is a non-reactive residue because of their methyl side-chain, E (relative solvent accessibility in Å^2 of 214, 147.130 g/mol, acidic polar) has reactivity by carbons 2, 4 and 5 and is abundant in α-helices, F (relative solvent accessibility in Å^2 of 228, 165.192 g/mol, aromatic nonpolar) is neutral due to its benzyl side chain, H (relative solvent accessibility in Å^2 of 216, 155.157 g/mol, basic aromatic polar) is related to catalytic sites with their basic nitrogen in the imidazole sidechain. Furthermore, residue substitutions for Cry1Ac loop II mostly affect reactivity, apparently accounting for detraction of toxicity and probable hydrophobic interactions between receptors and Cry1Ac toxin [36, 37]. Aromatic amino acids (Y, F, H and W) are determinants of binding affinity in several proteins such as human lysozyme, diphtheria toxin, human growth hormone, and extracellular binding domain of their receptor and other insecticidal Cry toxins. The more hydrophobic F rings or removal of phenolic hydroxyl groups at residues of Y, the higher binding affinity and activity [38]. Hybrid Cry toxin has three exposed solvent loops in domain II (1: 340IGG342; 2: 401QN402; 3: 429LPPETTE435), however, none of them contains aromatic residues, but some aromatic residues are located inside the beta sheet (387IHGVPTVRFNFTNP400). What can we learn about 3D structure when we revise these loops as a model? Specific changes would be an important determinant of receptor binding. G has no sidechain (no beta carbon atom), consequently, it can be very flexible, which enables it to adopt conformations that are prohibited to other amino acids, such as 3D structure loops where the protein has to create harsh turns [39]. Q is a twice-charged zwitterion because of both intramolecular backbone interactions and compact intermolecular connections. In addition, the study focused on the production of 3–14 GHz spectra, applying laser pulses in conjunction with two distinct chirped-excitation Fourier transform microwave spectrometers. It was determined that Q conformers contained no intramolecular hydrogen bonding with the backbone, perhaps the Nc-H···O=C interaction may be considered weak. The report also noted that an extra CH_2 unit in the carbon chain when going from N to Q intensifies flexibility of the residue, which can form a basis for recognition of specific sequences [40], as observed in the second exposed solvent loop of domain II. N has a polar amide side chain (-CH_2-CONH, -CH2-COO^-) with donor and acceptor H-bonds; it is profuse in γ- and β-turns with an attaching connecting the side chain to its adjacent environment (involving CO and NH sites of the core chain) resulting in stabilization of those turns. The approach, focused on gas phase quantum chemistry and IR/UV double resonance, established that N is a turn inducer, mostly for β-turns, which could be attributed to an attaching process referring to its side and core chains. So, N in position i + 2 is approximately three times more frequent suggesting a stabilization role. N is stable owing to 5 or 7 H-bonds concerning an NH donor to a CO acceptor on backbone similar to apolar A or F [41]. T has a small and aliphatic side chain with a slow-reacting polar hydroxyl group, which is not often involved in chemical reactions except for acetylation with acetyl chloride. Even polar groups can bind hydrogen to backbone peptide groups and inhibit hydrogen bonding of α-helices; these connections are established at the end of α-helices due to the presence of carbonyl oxygens or —NH groups, which are not related to hydrogen bonding of helix [39]. Recently, a protein—protein interaction mediated by beta barrel-based Ts has been described as follows: these Ts are packaged as a one-piece component, and the T-T H-bonds have relevance to structure stabilization [42]. E has various torsion angles whose stability is affected by the environment, and the zwitterionic

form is stable; the carboxyl group of its side chain is ionized and polar (pKa = 4.3). E side chain is a chelator for some metals. E is most common in the N-terminus of α-helices [39]. P has stabilization, helix breaker, and aromatic-aromatic interaction roles in globular proteins, so, the Møller—Plesset perturbation method (MP), the density functional theory augmented with empirical dispersion term (DFT-D), and the symmetry adapted perturbation treatment based on the density functional theory (DFT-SAPT) applied to P-W residue interaction (P-aromatic residue interaction) show that there is a strong interaction (-7.8 kcal mol^{-1} at MP2 level; -7.6 kcal mol^{-1} at DFT-D level) due to the hydrogen bond formation between P backbone C=O group and W ring NH group [43]. But the opposite situation also exists, I, L and V are hydrophobic residues that do not raise solid electrostatic interactions between their sidechains and polar atoms in the protein backbone, which depends on the phi/psi torsion angles [44]. In this respect, there are relevant amino acids to intermolecular interactions (e.g. Q or N) and for effective stabilization of the protein (such as T or P). The computational model emphasizes that a new domain combination may result in a higher activity against Ts according to experimental data. Although molecular mechanisms underlying this phenomenon are not well understood, the reports suggested that domains II and III confer two separate steps in binding to Cry protein in a two-phase step model and that the first phase step may be rate-limiting for that binding. For this reason, a proper combination of domains II and III may optimize binding and toxicity [45]. The Cry1Ac/Cry1Ba model provides a guide for the design of domain exchange manipulations aimed at elucidating the action mechanism and improving Cry toxicity Plant ISR may be triggered by beneficial bacteria to take the form of a signaling cascade regulated by jasmonic acid/ethylene responsive genes without involvement of pathogenesis-related proteins or salicylic acid, distinguishing it from systemic acquired resistance (SAR) [46]. Additionally, it has been reported that Bt promotes, directly and indirectly, plant growth because it produces 1-aminocyclopropane-1-carboxylate deaminase, indole-3-acetic acid, thuricin, phosphate solubilization enzymes or volatile compounds among others [30]. Scientific studies have shown that there is a strong interplay between *Bacillus sp*., plant ISR, and insect infestation. Basically, *Bacillus sp*. often produce a series of cyclic lipopeptides that correspond to surfactin, iturin and fengycin, and are synthesized by non-ribosomal peptide synthetases (NRPS) or hybrid polyketide synthases (PKS) and NRPS. Lipopeptides can stimulate ISR mechanisms including lipopeptide recognizing by a putative receptor and triggering the calcium-dependent signal transduction pathway controlled by jasmonic acid/ethylene and interaction with regulatory proteins named non-expressor of pathogenesis-related genes, kinase receptor, and Mitogen-Activated Protein kinases leading to transcriptional response tocell wall reinforcement, antimicrobial proteins or phytoalexins/flavonoids, phenolic compounds, lectins and Hsp90 chaperonin synthesis among others. Fengycin and surfactin are important activators for defense plant response. Fengycin (a specific solanaceae elicitor) application on potato tuber cells with purified lead to the production of potato phenolic compounds from the phenylpropanoid pathway, which elicits the plant defense response [47]. *B. velezensis* genome revealed genes belonging to lipopeptide synthesis such as spoVT and comA (surfactin), spoVT, abrB, yczE, comA and degQ (iturin) or phoR and phoP (fengycin) among others, with a clear interdependence among various genes relating to BtkHD1. Furthermore, it was found that potato has proteins strongly related to ISR, which enables interaction among BtkHD1, potato, and pest insect, including those related to the calcium-dependent transduction pathway (e.g. L-rich repeat receptors: protein kinase EXS precursor,

protein kinase 5 receptor, and brassinosteroid-insensitive 1-associated receptor kinase 1 precursor), calcium signaling (e.g. calmodulin binding protein or calmodulin-dependent protein kinases), jasmonic acid/ethylene synthesis (lipoxygenase), oxidative stress (peroxiredoxin, catalase, and peroxidase), flavonoids, phenolics compounds and lignin biosynthesis, protease inhibitors, and pathogen defense (Table 1, 2) [11–13].

Conclusions. It can therefore be concluded that the success of BtkHD1 as an insect pathogen may also be related to potato's ISR triggering and growth promotion. The results suggest a differential molecular dynamics regarding to specific residues inside domains II of Cry1Ba and Cry1Ac proteins. BtkHD1 has a beneficial potential with reference to potato protection under Ts attacks. BtkHD1 can affect the development of field potato plants through nitrogen fixation, siderophore production, and phosphate solubilization, including protection against phytopathogens by means of ISR; this implies an evolving interaction between Bt and plants.

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ІНСЕКТИЦИДНА ТА РІСТСТИМУЛЮЮЧА АКТИВНІСТЬ *BACILLUS THURINGIENSIS KURSTAKI* HD-1

Bacillus thuringiensis (Bt) продукує токсини Cry проти комах-шкідників. Білки Cry формуються трьома доменами, пов'язаними з утворенням пор і розпізнаванням білкових рецепторів. Домен I пов'язаний з утворенням пор, тоді як домени II та III відіграють важливу роль у розпізнаванні рецепторів, особливо петлі домену II. Відомо, що різні види *Bacillus* здатні стимулювати ріст рослин шляхом фіксації азоту, виробництва сидерофорів або розчинення фосфатів. *Tecia solanivora* (Ts) (Lepidoptera: Gelechiidae) є важливим шкідником картоплі в Америці, який чутливий до білків Cry1Ac та Cry1B з Bt. Системна стійкість рослин (ISR) викликається атакою шкідників. Водночас її може активувати *Bacillus* sp., наприклад, *B. velezensis* має гени, пов'язані зі синтезом ліпопептидів spoVT, та comA, spoVT, abrB, yczE, comA та degQ, phoR та phoP. Що, в свою чергу, пов'язані із кальцій-залежним шляхом трансдукції (L-багаті повторювані рецептори як попередники протеїнкінази EXS, рецептор протеїнкінази 5 та нечутливий до брасиностероїдів 1-асоційований рецептор кінази 1), а також кальмодулін-залежні протеїнкінази або синтез жасмонової кислоти / етилену, окислювальний стрес, флавоноїди, феноли, інгібітори та захисні білки патогенів. **Мета.** Дослідити Bt

kurstaki HD-1 (BtkHD1) стосовно контролю Ts (включаючи аналіз доменів Cry1Ac та Cry1B II), стимулювання росту картоплі та ISR рослин під дією шкідників до BtkHD1. **Методи.** Для забезпечення постійного контролю якості BtkHD1 підтримувався синтез кристалів (оцінювали за допомогою мікроскопії), наявність генів cry1 (оцінювали за ПЛР) та продукція протеїну Cry (оцінювали за допомогою SDS-PAGE). Bt вивчали для фенотипу стимулювання росту рослин (фіксація азоту, виробництво сидерофору, солюбілізація фосфатів та синтез індолилоцтової кислоти). Біоаналізи проводили із застосуванням кристалів Bt на личинках першого віку Ts (для боротьби з комахами), потім саджанці картоплі (сорт Креол) інокулювали $1 \cdot 10^{10}$ КУО/мл BtkHD1. Проведено аналіз *in silico* гібридного білка Cry1Ac-Cry1Ba для ідентифікації відповідних залишків у розпізнаванні рецепторів ISR картоплі, пов'язаних із впливом BtkHD1, який визначали за допомогою геномного аналізу Bt та картоплі. **Результати.** Показано характеристику BtkHD1 та хорошу якість штаму, включаючи біпірамідальні кристали, наявність генів cry1 та синтез Cry1-подібних білків 130 кДа. Змінні ПЛР (концентрація ДНК, $MgCl_2$, Taq ДНК-полімераза та температура гібридизації праймерів) оцінювали за допомогою конструкції Taguchi дев'ятьма експериментами (E = 2k + 1 (E = кількість експериментів, k = кількість факторів)). Біоаналіз білка BtkHD1 на личинках Ts показує LC_{50} в раціоні 536 нг/см^2, а також фенотипи, пов'язані зі стимулюванням росту рослин: фіксація азоту, вироблення сидерофору та розчинення фосфатів (шляхом продукування кислот). Аналіз стимулювання росту картоплі виявив вплив BtkHD1 на картоплю — довжину і суху масу стебел. Проспективний аналіз враховував відповідні фактори, що впливають на біологічну функцію гібридного білка, орієнтованого на домен II, пов'язаний з трьома відкритими петлями розчинника (1: 340IGG342; 2: 401QN402; 3: 429LPPETTE435). Ідентифіковано 15 білків BtkHD1 та 68 білків картоплі, пов'язаних із шкідниками рослин. Це дослідження показує, що гени, пов'язані з циклічними ліпопептидами (сурфактин, ітурин та фенгіцин), які продукуються нерибосомними пептид-синтетазами або гібридними полікетид-синтазами. Ліпопептиди стимулюють кальцій-залежний шлях передачі сигналу, що контролюється жасмоновою кислотою/етиленом, взаємодію з регуляторними білками як неекспресором генів, пов'язаних з патогенезом, рецепторами кінази та протеїнкіназами, активованими мітогеном, що приводить до формування в рослин системної стійкості. **Висновки.** Цей проект спрямований на валідацію токсичності для личинок Ts та ефекту росту картоплі на основі BtkHD1, включаючи попередній аналіз гібридного білка Cry1Ac1-Cry1Ba1 та білків, пов'язаних із цим штамом та картоплею, для виявлення системної стійкості, активованої шкідниками.

Ключові слова: *Bacillus thuringiensis ABTS-351, білок Cry, Tecia solanivora, сприяння розвитку Solanum tuberosum, індукована системна резистентність.*