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Isolation and characterization of a *Bacillus thuringiensis* strain with potential for epizootics in *Plodia interpunctella*

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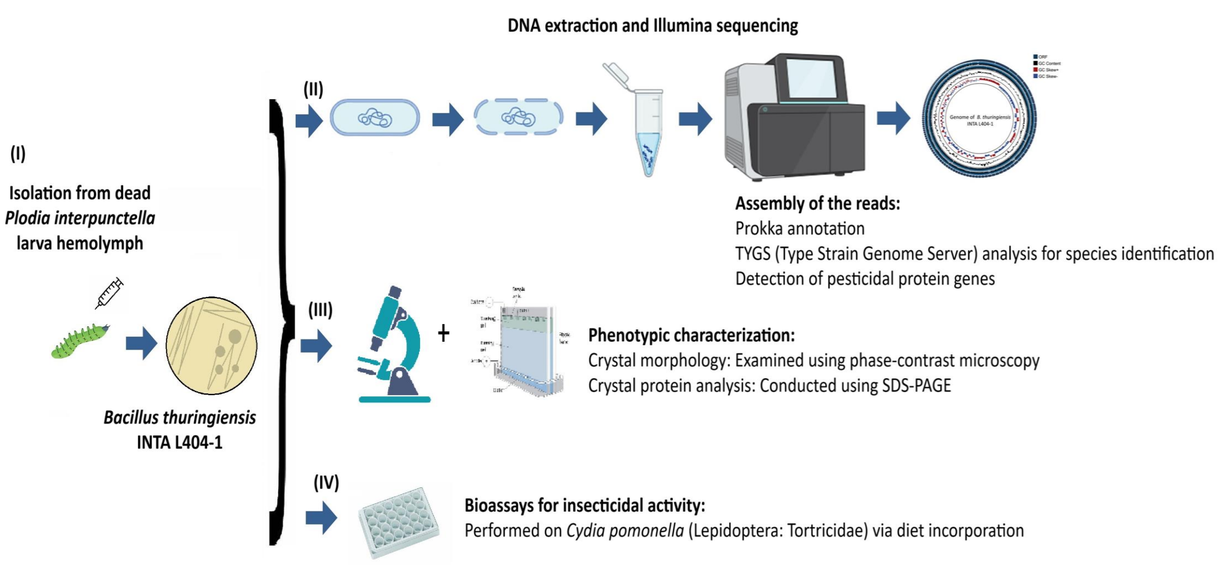
INTRODUCTION & AIM

Bacillus thuringiensis is a gram-positive, spore-forming bacterium renowned for its production of pesticidal proteins, which make it highly effective against a wide range of insect pests. These proteins, primarily Cry and Vip, specifically target the gut epithelium of susceptible insects, causing feeding cessation and eventual death. As a result, *B. thuringiensis* has become a cornerstone of biological pest control, particularly in integrated pest management programs.

Although widely used, *B. thuringiensis* is generally considered an opportunistic entomopathogen due to the rarity of natural epizootics. However, certain strains have been linked to significant pest mortality in specific environments.

This study focused on isolating and characterizing *B. thuringiensis* strain INTA L404-1, which was associated with high mortality in an artificially reared population of *Plodia interpunctella* (Lepidoptera: Pyralidae), a significant pest of stored grains. By investigating its genetic profile, the phenotypic characteristics of its crystals, and its insecticidal properties, we aim to evaluate its potential as an effective biocontrol agent.

METHOD



The workflow started with the isolation of *B. thuringiensis* INTA L404-1 from dead *P. interpunctella* larvae (I). DNA was extracted from the isolated strain and subjected to Illumina sequencing for genome analysis (II). The assembled reads underwent Prokka annotation and TYGS analysis for species identification (www.tygs.dsmz.de), and the presence of pesticidal protein genes were confirmed (II). In parallel, the phenotypic characterization of INTA L404-1 was conducted by examining crystal morphology using phase-contrast microscopy and analyzing crystal proteins via SDS-PAGE (III). Finally, bioassays were performed using neonate *C. pomonella* larvae, chosen as a Lepidoptera model for their availability. The spore-crystal complex of INTA L404-1 was incorporated into their diet at a concentration of 100 μg/ml to evaluate its insecticidal effects (IV).

RESULTS & DISCUSSION

The genome assembly of *B. thuringiensis* INTA L404-1 is summarized in Table 1.

Table 1. Key statistics of the genome assembly for INTA L404-1

Genome size (Mb)	6.2
GC content (%)	34.76
Coverage depth	347.7 X
Total conting count	232
Largest contig (nucleotide count)	286,296
N50 value	55,157
N75 value	33,117
L50 value	29
L75 value	65
Predicted genes	1814

A phylogenetic investigation performed with the TYGS revealed that INTA L404-1 formed a distinct cluster alongside strain types *B. thuringiensis* ATCC 10792 and *B. cereus* ATCC 14579 (Fig. 1). In accordance with a recently suggested classification, this cluster, referred to as *Bacillus cereus sensu stricto*, includes the majority of biovar Thuringiensis strains.

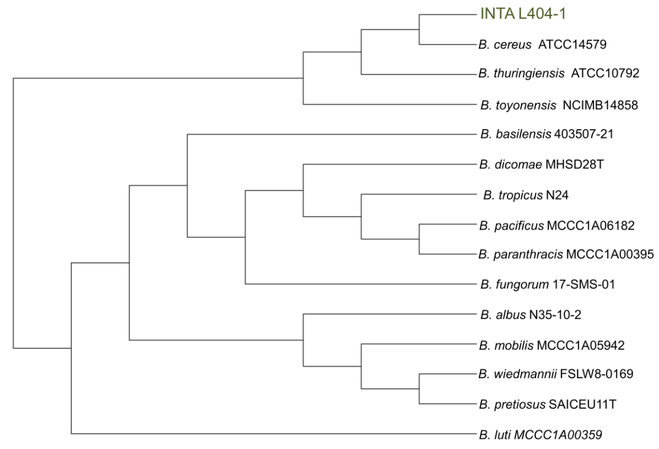


Fig. 1. Phylogenetic analysis of the genome of *B. thuringiensis* INTA L404-1 based on whole-genome data analyzed with the TYGS platform, highlighting its evolutionary position.

The draft genome sequence of INTA L404-1 harbors potential pesticidal proteins. Nine coding sequences showed significant BlastX similarity to known pesticidal proteins, which have demonstrated efficacy against a broad spectrum of insects, spanning four different orders (Table 2).

Table 2. Pesticidal proteins repertoire identified in the INTA L404-1 genome. Experimentally derived data from the Bacterial Pesticidal Protein Resource Center (BPPRC) specificity database for the target species (www.bpprc-db.org)

Predicted protein	Length (aa)	Predicted Molecular Mass (kDa)	Identity (%)	Target order
Cry1Aa	1145	133.13	99.77	Lepidoptera
Cry1Ab	1148	130.62	100.00	Lepidoptera
Cry1Ca	1189	99.74	95.94	Lepidoptera
Cry1Da	1165	132.48	91.59	Lepidoptera
Cry1Ia	719	81.26	100.00	Coleoptera / Lepidoptera
Cry2Ab	633	70.74	95.18	Lepidoptera / Diptera
Cry9Ea	1150	129.89	100.00	Lepidoptera
Spp1Aa	506	56.14	80.67	Lepidoptera / Blattodea
Vip3Aa	789	88.67	100.00	Lepidoptera

Additionally, the deduced proteins from the genes similar to *cry9Ec*, *mpp3Aa*, and *tpp80Aa* exhibited sequence identities below 34%.

Bipyramidal crystals were observed in INTA L404-1, and SDS-PAGE analysis of parasporal crystals revealed a unique *ca*. 130 kDa protein (Fig. 2). This strain demonstrated significant insecticidal activity, causing 100% mortality in *C. pomonella*.

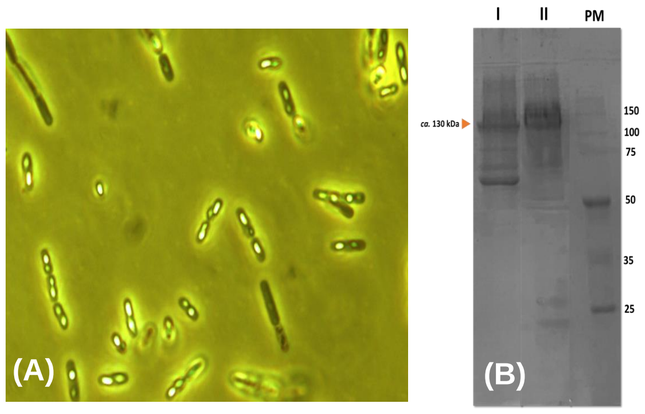


Fig. 1. (A) Bacterial culture at 48 hours post-inoculation, observed through phase-contrast microscopy at 1000x magnification. (B) Electrophoretic analysis of cristal proteins. Lanes: I, *kurstaki* HD-1; II, INTA L404-1. MW with sizes indicated on the right (kDa) ("Broad Range Protein Molecular Weight Markers," Promega).

CONCLUSION

- The natural origin of INTA L404-1, isolated from a dead *P. interpunctella* larva indicates a close link between this strain and pest mortality.
- The genotypic and phenotypic characteristics of *B. thuringiensis* INTA L404-1, along with bioassays using *C. pomonella*, suggest a potential causal link between INTA L404-1 and an epizootic event affecting an artificially reared population of *P. interpunctella.*
- These findings highlight the potential of INTA L404-1 as a promising biocontrol agent for controlling Lepidoptera pests.

FUTURE PERSPECTIVES

- Perform whole-genome sequencing of *B. thuringiensis* INTA L404-1 using Nanopore technology for a more precise identification of insecticidal protein-coding genes.
- Bioassays with *P. interpunctella* are required to confirm that INTA L404-1 causes mortality in this species.