Microbiological Research 186–187 (2016) 9–15

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Isolation and characterization of a new *Bacillus thuringiensis* strain with a promising toxicity against Lepidopteran pests

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ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form 14 February 2016
Accepted 18 February 2016
Available online 22 February 2016

Keywords:
Bacillus thuringiensis
PFGE
cry-type gene
PCR-RFLP
Ephestia kuehniella
Spodoptera littoralis

ABSTRACT

Insecticides derived from *Bacillus thuringiensis* are gaining worldwide importance as environmentally desirable alternatives to chemicals for the control of pests in public health and agriculture. Isolation and characterization of new strains with higher and broader spectrum of activity is an ever growing field. In the present work, a novel Tunisian *B. thuringiensis* isolate named BLB459 was characterized and electrophoresis assay showed that among a collection of 200 *B. thuringiensis* strains, the plasmid profile of BLB459 was distinctive. *SmaI*-PFGE typing confirmed the uniqueness of the DNA pattern of this strain, compared with BUPM95 and HD1 reference strains. PCR and sequencing assays revealed that BLB459 harbored three *cry* genes (*cry30*, *cry40* and *cry54*) corresponding to the obtained molecular sizes in the protein pattern. Interestingly, PCR-RFLP assay demonstrated the originality of the BLB459 *cry30*-type gene compared to the other published *cry30* genes. Insecticidal bioassays showed that BLB459 spore-crystal suspension was highly toxic to both *Ephestia kuehniella* and *Spodoptera littoralis* with LC_{50} values of about 64 (53–75) and 80 (69–91) μg of toxin cm^{-2}, respectively, comparing with that of the commercial strain HD1 used as reference. Important histopathological effects of BLB459 δ-endotoxins on the two tested larvae midguts were detected, traduced by the vacuolization of the apical cells, the damage of microvilli, and the disruption of epithelial cells. These results proved that BLB459 strain could be of a great interest for lepidopteran biocontrol.

1. Introduction

Bacillus thuringiensis is a Gram-positive and aerobic bacterium. Found in soil, on plant surfaces and in grain storage dust (de Maagd et al., 1999), this entomopathogenic bacterium is the most widely used biopesticide. In fact, *B. thuringiensis* can be distinguished from other bacilli by its capacity to produce, during sporulation, crystalline protein inclusions having a broad host spectrum against insects, nematodes, mites and protozoa (Schnepf et al., 1998).

First, crystalline inclusions must be ingested by susceptible larvae then the environment of the midgut promotes crystal solubilization and the consequential release of protoxins. Cleavage sites on the protoxin are recognized and cleaved by host proteases to produce active toxin that subsequently binds to specific receptors on the midgut epithelium. This binding leads to the formation of pores allowing ions and water to pass freely into the cells, resulting in swelling, lysis, and the eventual death of the host (Knowles and Ellar, 1987). *B. thuringiensis* can also serve as a source of toxic genes that can be expressed in plants and thus confer toxic property against different species of insect pests. Genetically modified plants that express the *B. thuringiensis* genes, such as rice, cotton, corn, potato, and soybean are associated with the control of pests, especially *Lepidoptera*. The resistant cultivars caused an increased productivity, greater economic value, reduction in the use of chemical pesticides, and benefits in the selectivity of the target pest (Shelton et al., 2002).

These inclusions contain mainly the δ-endotoxins proteins or Cry toxins encoded by *cry* genes. Until now, more than 500 genes have been identified and classified to 72 classes based on the similarity of their amino acid sequences and their insecticidal specificity (Crickmore et al., 2014). Among them, only a few number of Cry

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http://dx.doi.org/10.1016/j.micres.2016.02.004
0944-5013/

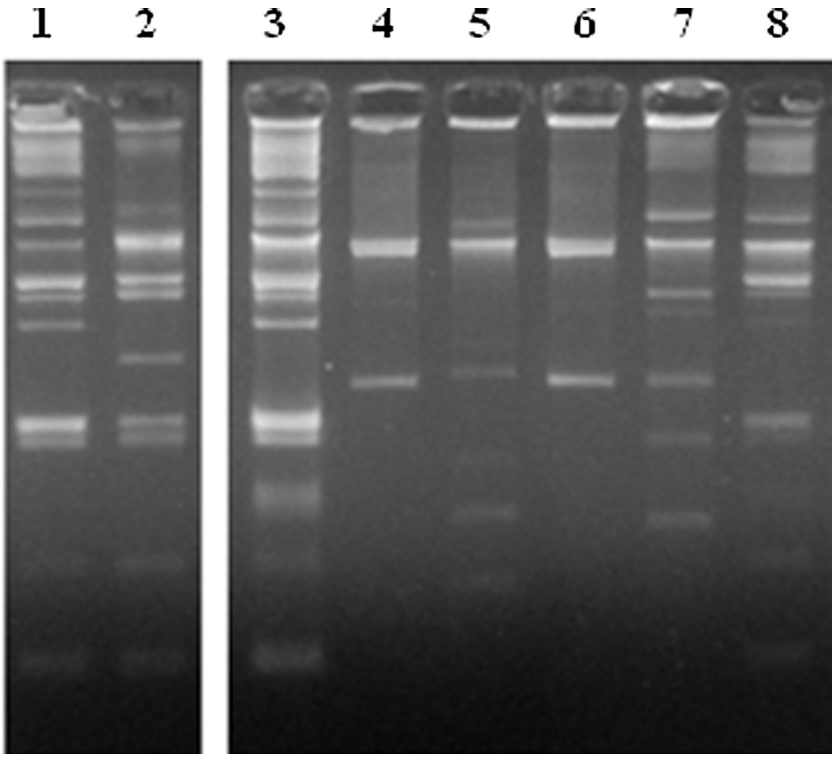

Fig. 1. Plasmid DNA electrophoresis of *B. thuringiensis* strain class representatives. Lanes: 1, BUPM95; 2, BLB384; 3, BLB427; 4, BLB428; 5, BLB448; 6, BLB456; 7, BLB459; 8, BLB471.

toxins are widely used to control pests. Consequently, some cases of insect resistant populations to Cry toxins are emerged like the resistance of *Heliothis virescens* to Cry1Ac and Cry2Aa toxins, and the resistance of the diamondback moth *Plutella xylostella* to Cry1Ac (Jurat-Fuentes et al., 2003; Sayyed et al., 2004). Therefore, isolation and cloning of novel insecticidal crystal protein genes are imperative for increasing the diversity of toxins and overcoming potential problems associated with resistance.

In order to obtain novel *B. thuringiensis* isolates with higher and broader spectrum of activity, different screening programs have been undertaken. Using PCR-RFLP assay, Zhu et al. (2010) characterized a novel *B. thuringiensis* isolate native to China containing three novel *cry* genes (*cry4Cb1*, *cry30Ga1* and *cry54*-type). Moreover, Tan et al. (2010) developed a fast and efficient strategy by combining the PCR-RFLP and the single-oligonucleotide nested-PCR method for isolating a novel *cry30Fa1* gene (77.1 kDa) active to both *Plutella xylostella* and *Aedes aegypti*.

In the present work, we isolated and characterized a novel strain of *B. thuringiensis*, named BLB459, harboring the *cry30*, *cry40*, and *cry54* genes with a promising activity against the lepidopteran pests *Ephestia kuehniella* and *Spodoptera littoralis*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

New *B. thuringiensis* isolates were obtained from bioinsecticide free soil samples collected from different sites in Tunisia. *B. thuringiensis* subsp. *kurstaki* HD1 (Carozzi et al., 1991) and BUPM95 (Abdelkefi-Mesrati et al., 2005) were used as reference strains. For routine use in the laboratory, isolates were grown in Luria-Bertani medium (LB) (Sambrook et al., 1989) at 30 °C with shaking at 200 rpm. T3 medium (Travers et al., 1987) was used for the parasporal crystals formation during *B. thuringiensis* growth.

Table 1
Primers used in PCR. (A/5) indicates forward primers and (B/3) indicates backward primers.

Primer	Sequence (5′→3′)	Gene	Accession number
Lep2A	CCGAGAAAGTCAAACATGCG	*cry1A*	AAA22561
Lep2B	TACATGCCCTTTCACGTTCC		
Cry2A	ACTATTTGTGATGCGTATAATGTA	*cry2*	AAA83516
Cry2B	AATTCCCCATTCATCTGC		
Cry1IaA	ACTATTTGTGATGCGTATAATGT	*cry1I*	AAC36999
CryIIaB	AATTCCCCATTCATCTGC		
Dip1A	CAAGCCGCAAATCTTGTGGA	*cry4*	CAA68485
Dip1B	ATGGCTTGTTTCGCTACATC		
Col1A	GTCCGCTGTATATTCAGGTG	*cry3*	AAA22336
Col1B	CACTTAATCCTGTGACGCCT		
Up30-5	TTGGCTCAATATGTGTCAAAC	*cry30*	EU503140
Up30-3	GCTTTAACAGCAGGAATTTG		
Up39.40-5	TAAGAGGGTTTGTGGGAAGTAG	*cry40*	BAC72648
Up39.40-3	ACTTTCTGGGAATACCTCTACTG		
S54-5	GTGTCAAGAGAACCAACAGTATG	*cry54*	GU446677
S54-3	ACTAAGTCTCCTCCTGTATGACCAG		