The vegetative insecticidal proteins Vip2S-Vip1S of Bacillus thuringiensis

Bacillus thuringiensis is one of the most successful biological control strategies used as an alternative to the harmful chemical insecticides. Besides the crystals proteins, *B. thuringiensis* produces during the vegetative growth phase a second generation of insecticidal proteins, called Vegetative Insecticidal Proteins (Vips). To date, 15 Vip1, 20 Vip2, and 101 Vip3 proteins have been reported. Vip1 and Vip2 act as a binary toxin for some coleoptera and hemiptera, while Vip3 is active against lepidoptera.

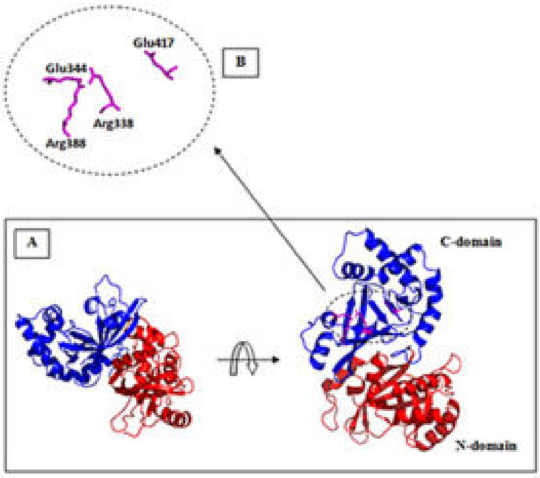

Fig. 1. The 3D-model model of Vip2S showing the presence of two domains: N-domain (in red) and C-domain (in blue) (A). Close-up view of conserved residues in the active site cleft of C-domain (B).

The *Bacillus thuringiensis* S1/4 strain was previously found to harbour *vip1S*, *vip2S*, and *vip3* genes. Its plasmid curing led to the obtaining of several partially cured strains. Using these derivative strains as templates, PCR amplification showed that S1/4-2, S1/4-3, S1/4-7, and S1/4-9 derivative strains harbor the *vip3* gene but not the *vip2S–vip1S* operon. However, the derivative strain S1/4-4 harbors only the *vip2S-vip1S* operon, which indicated that *vip2S*, *vip1S*, and *vip3* were extra-chromosomal genes. This latter finding was confirmed by southern blot assay using two radioactive probes targeting the *vip3* gene and *vip*2S-*vip1S* operon. Interestingly, southern blot analysis showed also that *vip3* gene and *vip2S–vip1S* operon were not localized on the same *B. thuringiensis* plasmid.

To better identify the studied *vip2S-vip1S* operon, bioinformatics studies were conducted. Sequencing results showed that this operon consists of two ORF (open reading frame), *vip2S* (1.356 kb) and *vip1S* (2.637 kb) separated by an intergenic spacer of 4 bp, downstream the promoter region. Using the InterPro tool, Vip2S was found to belong to the family of Binary exotoxin A and Vip1S to bacterial exotoxin B. The three-dimensional structure of Vip2S of *B. thuringiensis* was constructed by homology modeling using the crystal structure of VIP2 of *Bacillus cereus* (PDB code: 1QS1) since both proteins were noted to share 70% identity. It was shown that Vip2S is a mixed α/β protein (Fig. 1A) and is formed by two domains termed the N-domain (residues 51-255) and the C-domain (residues 256-451). The presence of conserved residues in all actin-ribosylating binary toxins namely Arg338, Glu344, Arg388 and Glu417 (Vip2S numbering) was detected in the active site cleft of C-domain of Vip2S (Fig. 1B). These conserved residues are involved in NAD binding and are essential for catalytic mechanism. Furthermore, a conserved sequence (residues 376–378), called the 'STS motif' that lies at the end of C-domain β11 and stabilizes the NAD binding pocket, was also found.

Seeing that, to date, there is no available structure for Vip1 proteins, Phyre2 server was used to generate the 3D-model of Vip1S. The proposed model (Fig. 2A) was build using as template the fold of anthrax protective antigen (PDB code: 1acc) with 35% identity. This sequence identity could be considered as significant, suggesting that the two proteins have similar modes of action. Further analysis showed that Vip1S contains a flexible loop rich of glycin with alternating hydrophobic and hydrophilic residues (Fig. 2B). Similar loops advanced to play the role of channel-forming domain are also conserved in the Vip1Ac of *Bacillus cereus* and the protective antigen (PA) of *Bacillus anthracis*.

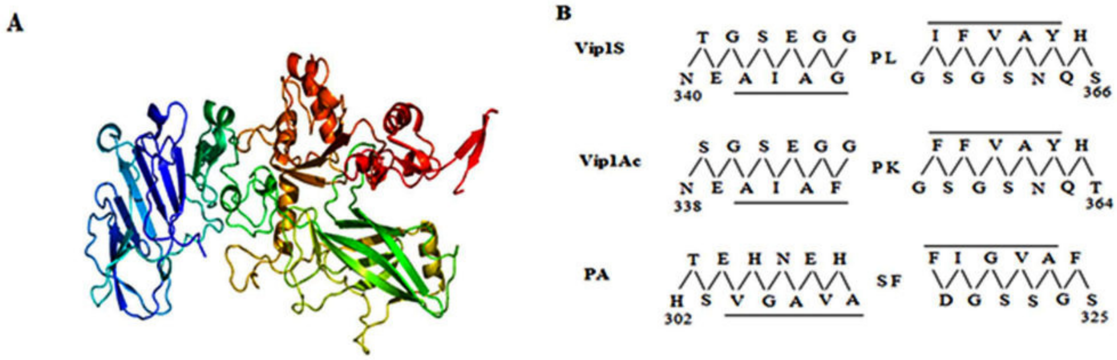

Fig. 2. The 3D-model of Vip1S proposed by Phyre2 server. The N-terminal end is indicated in blue and the C-terminal end in red (A). Identification and comparison of the putative channel-forming domain of Vip1S with those of Vip1Ac and protective antigen "PA". Hydrophobic residues proposed to be oriented toward the lipid bilayer are underlined (B).

Bioassays of the partially cured strains supernatants showed a weak toxicity of S1/4-4 to the lepidopteran *Spodoptera littoralis* comparing to a better effect of S1/4-2, S1/4-3, S1/4-7, and S1/4-9, suggesting its eventual contribution to the toxicity. Nevertheless, the concentrated supernatant of S1/4-4 strain was not toxic against the coleopteran *Tribolium castaneum*.

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Publication

Localization and in silico study of the vegetative insecticidal proteins Vip2S-Vip1S of Bacillus thuringiensis.
Sellami S, Jemli S, Abdelmalek N, Dabbéche E, Jamoussi K
Int J Biol Macromol. 2016 Oct