CLONING AND EXPRESSION OF A BACTERIOCIN GENE OF *Bacillus thuringiensis* subsp. *morrisoni* (LBIT 269)

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Introduction. Bacteriocins (Bac) are antimicrobial peptides ribosomally synthesized and produced by bacteria (1). *Bacillus thuringiensis,* the most successful bioinsecticide worldwide, produces bacteriocins with wide range of inhibitory activity and stability at different values of temperature and pH. *B. thuringiensis* subsp. *morrisoni* (LBIT 269) is a Mexican strain that produces Bac with activity against gram-positive and gram-negative bacteria (2). In spite of the amino acid sequence of some bacteriocins of *B. thuringiensis* have been determined, only the putative operons of Thurincin H and Thuricin CD has been reported (3,4). Currently, it has been not cloned a *bac* gene from a Mexican strain of *B. thuringiensis.* Here, we report by first time, the cloning of a structural bacteriocin gene (*bac*) and also of a putative Fe-S oxidoreductase (FeS Ox) of LBIT 269. Advances in the characterization and expression of Bac in *Escherichia coli* and *B. thuringiensis* will be shown.

Methods. Genomic/plasmid DNA and RNA were purified from LBIT 269. The first strand was synthetized using oligo dT. Oligonucleotides used to amplify *bac* and FeS Ox were designed by the alignment of Bac of *B. thuringiensis* and using the sequence reported for *B. thuringiensis* SF361, respectively. Genes (Fig. 1) were amplified by PCR using ssDNA and dsDNA. Amplicons were cloned into pCR 4-TOPO, pColdI and pEt22 *E. coli* vectors and also into pHT3101, a shuttle vector of *B. thuringiensis.*

Results. The thuricin gene was amplified from cDNA as an amplicon of 372 bp. This was sequenced and found in two ORFs (Fig. 1A). Amplicons of ~1380 bp and ~2158 bp were amplified and harbored the FeS Ox and the tandem (T1, T2, T3, FeS Ox), respectively (Fig. 1A y 1B). The Fe-S oxidoreductase (FeS Ox) is a protein related to the formation of bonds Cys-α carbon (3,4). The bacteriocin and the tandem were cloned firstly into pCR 4-TOPO and then into pET22b and pHT3101. FeS Ox protein was cloned in pET28a directly. When *E. coli* BL21 Rosetta 2 was transformed with the constructions harboring the only the bacteriocin gene, expression of the antimicrobial peptide was not detected. It is possible that *E. coli* requires another factor involved in posttranslational modifications. FeS Ox could be the responsible of these modifications.

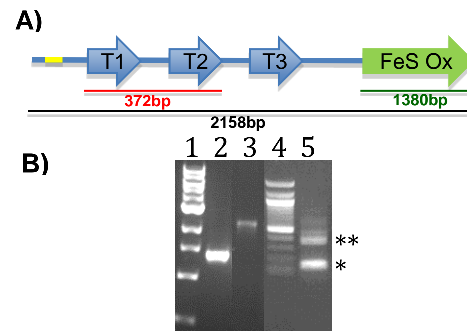

Fig.1. A) Diagram corresponding to tandem construction. T1, T2 and T3 are thuricin genes. FeS Ox is a Fe-S Oxidoreductase enzyme. In yellow is indicated the RBS. Three products were obtained by PCR. Red line: Genes of thuricin, green line: FeS Ox gene and black line: Tandem of four genes. **B)** Gene amplification. 1: 1 kb ladder, 2: FeS Ox, 3: Tandem, 4: 100 bp ladder, 5: Thuricins. * one gen, ** two genes.

Conclusions. *Bacillus thuringiensis* subsp. *morrisoni* (LBIT 269) has thuricin genes in tandem with FeS Ox protein. The thuricin gene requires another factor not determined to be expressed heterologously in *E. coli.*

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References.
1. Tagg JR, Dajani AS and Wannamaker LW. (1976). *Microbiol. Rev.* 40, 722-756.
2. Barboza-Corona JE, Vázquez-Acosta H, Bideshi DK and Salcedo-Hernández R. (2007). *Arch. Microbiol.* 187: 117-126
3. Lee H, Churey JJ and Worobo RW. (2009). *FEMS Microbiol. Lett.* 299: 205-213
4. Rea MC, Sit CS, Clayton E, O´connor PM, Whittal RM, Zheng J, Vederas JC, Ross RP and Hill C. (2010). *P Natl Acad Sci USA* 107: 9352-9357.